Evaluation of MicroScan WalkAway and Vitek 2 for determination of the susceptibility of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates to cefepime, cefotaxime and ceftazidime

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Received 29 January 2013; returned 2 March 2013; revised 1 April 2013; accepted 4 April 2013

Objectives: The aim of this study was to evaluate the accuracies of these automated susceptibility test systems with cefepime, cefotaxime and ceftazidime using the new CLSI and EUCAST guidelines in extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae.

Methods: A total of 220 ESBL-producing clinical isolates were collected from 12 hospitals in Korea. Susceptibility testing for cefepime, cefotaxime and ceftazidime was performed by MicroScan WalkAway, Vitek 2 and the CLSI broth microdilution test. ESBL genotypes were determined by PCR amplification.

Results: The proportion of isolates classified as susceptible to cefepime and ceftazidime with the CLSI and EUCAST guidelines was 35.0% versus 2.3% for cefepime (P, 0.001) and 21.8% versus 8.2% for ceftazidime (P, 0.001), respectively, and the susceptible isolates were mainly the CTX-M-9 group or SHV-type ESBL producers. All of the isolates were resistant to cefotaxime. Against the total of 220 ESBL-producing isolates, using the CLSI (EUCAST) criteria, very major/major error rates of MicroScan and Vitek 2 were as follows: 1.9%/20.8% (1.8%/20.0%) and 27.4%/0% (12.2%/0%) for cefepime and 2.6%/8.3% (1.2%/0%) and 4.5%/0% (2.3%/0%) for ceftazidime, respectively. The very major error rates of MicroScan and Vitek 2 with cefotaxime were 0.9% and 1.4%, respectively. The errors were mainly major errors for MicroScan and very major errors for Vitek 2.

Conclusions: A substantial portion of ESBL-producing isolates were susceptible to cefepime and ceftazidime by using the CLSI and EUCAST breakpoints. Unfortunately, the error rates of the two automated susceptibility systems were not acceptable for cefepime and ceftazidime.

Keywords: CLSI, EUCAST, ESBLs, cephalosporins, antimicrobial susceptibility testing

Introduction

In 2010, the CLSI and EUCAST revised their guidelines concerning extended-spectrum β-lactamase (ESBL) detection and the interpretation for the third- and fourth-generation cephalosporins should be reported according to the results of susceptibility tests.1,2 This was done based on some clinical studies that demonstrated the success of cephalosporin therapy is better correlated with MIC values than with the presence of an ESBL and is also correlated with MIC values within isolates producing ESBLs.3–5 Therefore, the susceptibility results based on MICs may have a substantial impact on antibiotic use and routine susceptibility testing should be precise. In addition, there are differences in the susceptibility breakpoints for cefepime and ceftazidime between CLSI and EUCAST: with CLSI, the susceptible breakpoints for cefepime and ceftazidime are ≤8 and ≤4 mg/L, respectively, and with EUCAST, the breakpoints for the two antibiotics are ≤1 mg/L; however, the susceptible breakpoints for cefotaxime in both guidelines are ≤1 mg/L.

In this study, we evaluated the accuracies of the automated susceptibility test systems with cefepime, cefotaxime and ceftazidime compared with broth microdilution (BMD) according to the CLSI6 in ESBL-producing Escherichia coli and Klebsiella pneumoniae using the new CLSI and EUCAST breakpoints.1,2

Materials and methods

A total of 220 ESBL-producing clinical isolates (103 E. coli and 117 K. pneumoniae) were collected in 2009 from 12 hospitals in various regions of Korea.
Determination of MICs for ESBL-producing E. coli and K. pneumoniae

and all the isolates were centralized in a single centre for MIC determination and evaluation of automated systems.

The MICs were determined by the CLSI BMD method,6 MicroScan Walk-Away (Siemens Healthcare Diagnostics Inc., West Sacramento, CA, USA) using the NEG-MIC Type 37 panel and the Vitek 2 (bioMérieux, Hazelwood, MO, USA) using the AST-N224 card according to the manufacturer’s instructions. Quality control was assured via concurrent testing of four quality control strains, including Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853.

ESBL production was detected by an inhibitor-potentiated disc diffusion method, as described previously.7 ESBL types (blaCTX-M-1, -2, -9, blaoxM and blaoxa) were determined by PCR amplification8,9 and the amplified products of SHV-specific genes were digested with NheI for detection of SHV ESBLs.10 The PCR products of TEM-specific genes were sequenced. The nucleotide sequences were analysed with the BLAST program (http://www.ncbi.nlm.nih.gov/blast). TEM-type β-lactamase genes were sequenced only when the other PCR results were negative.

Isolates were considered susceptible, intermediate or resistant in accordance with both the CLSI 2012 and EUCAST 2012 guidelines1,2 and the results from each automated system were compared with those from the reference BMD. The results were analysed for categorical agreement and discrepancies (very major and major errors)11 and the acceptability criteria of >89.9% for categorical agreement, ≤1.5% for very major error (susceptible by tested systems/resistant by BMD) and ≤3% for major error (resistant by tested systems/susceptible by BMD) were applied.12,13 For isolates showing discrepant results between BMD and automated systems, both the automated systems and BMD tests were repeated. If initial and repeated test results were not the same, we repeated the test once more and the results that were obtained two out of three times were recorded as the final results.

The results were also evaluated by the editing of susceptibility results to resistant when the isolates were considered ESBL positive using expert systems of the two automated systems (MicroScan LabPro System version 3.01 and Vitek 2 Systems software version 05.04, respectively).

The comparison of occurrence rates between guidelines applied and between isolates harbouring different resistance determinants was performed by Pearson’s χ² test or Fisher’s exact test using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). A P value <0.05 was considered to be statistically significant.

Table 1. Evaluation of accuracies of automated systems for susceptibility testing of 220 ESBL-producing isolates against cephalosporins

<table>
<thead>
<tr>
<th>Agents and assays</th>
<th>CLSI 2012</th>
<th>EUCAST 2012</th>
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<tbody>
<tr>
<td></td>
<td>no. (%) of isolates with the indicated errors</td>
<td>no. (%) of isolates with the indicated errors</td>
</tr>
<tr>
<td></td>
<td>Categorical agreement (%)</td>
<td>Very majora/ resistant isolates</td>
</tr>
<tr>
<td>E. coli (n = 103)</td>
<td></td>
<td></td>
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<tr>
<td>Cefepime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroScan WalkAway</td>
<td>66.0</td>
<td>0/56 (0)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>52.4</td>
<td>20/56 (35.7)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroScan WalkAway</td>
<td>100.0</td>
<td>0/103 (0)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>99.0</td>
<td>1/103 (1.0)</td>
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<tr>
<td>Ceftazidime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroScan WalkAway</td>
<td>88.3</td>
<td>1/50 (2.0)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>86.4</td>
<td>4/50 (8.0)</td>
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<tr>
<td>K. pneumoniae (n = 117)</td>
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<td></td>
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<tr>
<td>Cefepime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MicroScan WalkAway</td>
<td>79.5</td>
<td>2/50 (4.0)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>69.2</td>
<td>9/50 (18.0)</td>
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<tr>
<td>Cefotaxime</td>
<td></td>
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<tr>
<td>MicroScan WalkAway</td>
<td>97.4</td>
<td>2/117 (1.7)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>91.5</td>
<td>2/117 (1.7)</td>
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<tr>
<td>Ceftazidime</td>
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<tr>
<td>MicroScan WalkAway</td>
<td>87.2</td>
<td>3/104 (2.9)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>88.0</td>
<td>3/104 (2.9)</td>
</tr>
</tbody>
</table>

aThe percentage of very major discrepancies was determined by dividing the number of isolates classified as susceptible using the test method by the number of isolates classified as resistant using the reference method.

bThe percentage of major discrepancies was determined by dividing the number of isolates classified as resistant using the test method by the number of isolates classified as susceptible using the reference method.

NA, not available. The major error rate could not be established due to the absence of cefotaxime-susceptible isolates in accordance with CLSI and EUCAST breakpoints.
In this study, all the 220 ESBL-producing isolates were resistant to cefotaxime, but a substantial number of isolates were classified as susceptible to cefepime and ceftazidime in accordance with the CLSI criteria for cefepime in E. coli (3.7% versus 35.7%).

We also assessed the results from the two systems applying their expert systems; Vitek 2 detected 95.9% of the ESBL producers and the MicroScan system detected 80.5%. All isolates of the CTX-M-1 group (n=77) were detected by Vitek 2, but MicroScan failed to detect 12 isolates. Among the 58 isolates of the CTX-M-9 group, 6 and 2 isolates were missed by MicroScan and Vitek 2, respectively. The most challenging mechanism to detect was SHV-type ESBLs. Of the 51 SHV-producing isolates, 34 and 47 isolates were detected by MicroScan and Vitek 2, respectively. If the expert systems are applied, the very major error rates, especially of Vitek 2, will decrease. However, most of the ESBL producers that are susceptible to cefepime or ceftazidime will be edited as resistant (Table 2).

### Results

Against the total of 220 ESBL-producing isolates, the proportions of isolates classified as susceptible to cefepime and ceftazidime with the CLSI and EUCAST guidelines were 35.0% versus 2.3% for cefepime (P<0.001) and 21.8% versus 8.2% for ceftazidime (P<0.001), respectively, and the susceptible isolates were mainly the CTX-M-9 group or SHV-type ESBL producers. For cefotaxime, none of the ESBL-producing isolates was susceptible using both guidelines. Of the 220 ESBL-producing isolates, genotypes could be determined for 215 isolates (101 E. coli and 114 K. pneumoniae). The predominant type for E. coli was CTX-M (97.0%, 98/101). In K. pneumoniae both CTX-M and SHV ESBL genes were detected: CTX-M alone in 36.8% (42/114), SHV alone in 43.9% (50/114), a combination of CTX-M and SHV in 16.7% (19/114) and TEM-52 in 2.6% (3/114).

The rates of categorical agreements and errors for the MicroScan and Vitek 2 systems are listed in Table 1. First, when the CLSI guidelines were applied, the rate of categorical agreement for cefepime was the lowest, ranging from 52.4% to 79.5%. MicroScan showed high values of major error (E. coli, 40.0%; K. pneumoniae, 8.5%), while Vitek 2 had high values of very major error (E. coli, 35.7%; K. pneumoniae, 18.0%). For ceftazidime, the categorical agreement rates of both systems were acceptable (>89.9%). For ceftazidime, the categorical agreement rates ranged from 86.4% to 88.3%. Very major error rates were unacceptable for both MicroScan (2.0% for E. coli and 2.9% for K. pneumoniae) and Vitek 2 (8.0% for E. coli and 2.9% for K. pneumoniae), although high major error levels were observed for only for MicroScan (6.8% for E. coli and 25.0% for K. pneumoniae).

Second, when the EUCAST breakpoints were applied, the categorical agreements for cefepime and ceftazidime were unacceptable, ranging from 42.7% to 88.9%, mainly due to minor errors (data not shown). The very major error rate of Vitek 2 was much lower than when using CLSI criteria for cefepime in E. coli (3.7% versus 35.7%).

### Discussion

In this study, all the 220 ESBL-producing isolates were resistant to cefotaxime, but a substantial number of isolates were classified as susceptible to cefepime and ceftazidime in accordance with the CLSI recommendations, as reported previously.\(^\text{14,15}\) To our knowledge, this is the first study evaluating the accuracy of these automated systems for susceptibility testing of ESBL producers against cephalosporins, compared with CLSI 2012 and EUCAST 2012 breakpoints. Large discrepancies were found between BMD and the two automated antimicrobial susceptibility systems for cefepime and ceftazidime, regardless of the breakpoints applied. The Vitek 2 MIC results tended to be several dilutions lower than those of BMD, resulting in high rates of very major error.
for cefepime and ceftazidime. This is consistent with a previous report that demonstrated high rates of very major error for cefepime tested with KPC-producing K. pneumoniae. However, the MicroScan system showed high rates of major error and low rates of very major error by application of the CLSI breakpoints. This is in contrast with the previous study by Bulik et al. where no major error was found with meropenem for KPC-producing K. pneumoniae. However, the accuracy of the automated method for susceptibility testing varies among antibiotics and cephalosporins were not tested in the study by Bulik et al. Application of the EUCAST breakpoints lowered the very major error rates of the two automated systems, particularly for cefepime and ceftazidime. However, when the EUCAST breakpoints were applied to K. pneumoniae, slight increases in the very major error rate with cefepime were observed for Vitek 2. These results were attributable to isolates with MICs of 8 or 16 mg/L, classified as susceptible or intermediate by CLSI breakpoints, but which were categorized as resistant by EUCAST breakpoints.

The sensitivity of the expert systems for detecting ESBL was higher with Vitek 2 (95.9%) than with MicroScan (80.5%). However, Vitek 2 also exhibited a number of false-positive results as compared with MicroScan WalkAway. These results were attributable to isolates with MICs of 8 or 16 mg/L, classified as susceptible or intermediate by CLSI breakpoints, but which were categorized as resistant by EUCAST breakpoints.

In conclusion, a substantial portion of ESBL-producing E. coli and K. pneumoniae in Korea were susceptible to cefepime and ceftazidime, especially with CLSI breakpoints. Unfortunately, the categorical agreement of the automated susceptibility test systems was not acceptable for cefepime and ceftazidime. It might be helpful to report ESBL production along with routine susceptibility results, as described here and by others, in order to relieve high false susceptible rates, especially in countries with a high burden of infections caused by ESBL producers. Although this is a local study from Korea, considering that the ESBL genotypes, mainly CTX-M, are ubiquitous, these results could be easily extrapolated to the European and US situation.

Acknowledgements
Part of this work was presented at the Fifty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, USA, 2012 (abstract D-755). The materials for MicroScan WalkAway were supplied by Siemens Healthcare Diagnostics.

Funding
This work was supported by a research grant from the Korea Center for Disease Control (2012-E44007-00).

Transparency declarations
None to declare.

References


