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Multicenter Laboratory Evaluation of the bioMerieux Vitek Antimicrobial Susceptibility Testing System with 11 Antimicrobial Agents versus Members of the Family Enterobacteriaceae and Pseudomonas aeruginosa

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A four-center study in which a total of 1,082 recent clinical isolates of members of the family Enterobacteriaceae and Pseudomonas aeruginosa were examined versus 11 antimicrobial agents with the bioMerieux Vitek susceptibility test system (Hazelwood, Mo.) and the GNS-F6 card was conducted. In addition, a challenge set consisting of the same 200 organisms was examined in each of the four participating laboratories. Results obtained with the Vitek system were compared to MICs determined by a standardized broth microdilution method. For purposes of comparison, susceptibility categories (susceptible, intermediate, or resistant) were assigned on the basis of the results of both methods. The result of the broth microdilution test was considered definitive. The total category error rate with the Vitek system and the recent clinical isolates (11,902 organism-antimicrobial comparisons) was 4.5%, i.e., 1.7% very major errors, 0.9% major errors, and 1.9% minor errors. The total category error rate calculated from tests performed with the challenge set (i.e., 8,800 organism-antimicrobial comparisons) was 5.9%, i.e., 2.2% very major errors, 1.1% major errors, and 2.6% minor errors. Very major error rates higher than the totals were noted with Enterobacter cloacae versus ampicillin-sulbactam, aztreonam, ticarcillin, and ticarcillin-clavulanate and with P. aeruginosa versus mezlocillin, ticarcillin, and ticarcillin-clavulanate. Major error rates higher than the averages were observed with Proteus mirabilis versus imipenem and with Klebsiella pneumoniae versus ofloxacin. Excellent overall interlaboratory reproducibility was observed with the Vitek system. The importance of inoculum size as a primary determinant in the accuracy of susceptibility test results with the Vitek system was clearly demonstrated in this study. Specifically, when an inoculum density fourfold higher than that recommended by the manufacturer was used, high rates of false resistance results were obtained with cell wall-active antimicrobial agents versus both the Enterobacteriaceae and P. aeruginosa.

The Vitek system (bioMerieux Vitek, Hazelwood, Mo.) is an automated method for performing same-day identification and antimicrobial susceptibility tests on nonfastidious bacteria. It was first introduced in 1979 and has received broad application in clinical microbiology laboratories worldwide. Numerous published reports have described the antimicrobial susceptibility testing capability of the Vitek system. Many of these studies have addressed susceptibility testing of Enterococcus species (2, 8, 16, 22–25, 27, 29) or staphylococci (3, 4, 7, 8, 14, 26, 30, 31). Several published investigations have described acceptable levels of accuracy when the Vitek system was used to determine the antimicrobial susceptibility patterns of members of the family Enterobacteriaceae and Pseudomonas aeruginosa (6, 9–11, 13, 19–21, 28, 32). A single exception might be detection of extended-spectrum β-lactamase-producing Klebsiella and Escherichia species with current test materials (12). Recently, two reports that describe high rates of false resistance with imipenem and aztreonam when selected Enterobacteriaceae and Pseudomonas spp. were tested with the currently available Vitek system have appeared (1, 15). Because of these two reports, a prospective, controlled multicenter study was conducted in an effort to assess the accuracy of the contemporary Vitek system as a means for determining the antimicrobial susceptibility of clinical isolates of facultative gram-negative bacilli.

MATERIALS AND METHODS

Organisms tested. Between 231 and 320 unique patient isolates of Enterobacteriaceae and P. aeruginosa were examined in each of four medical centers: Good Samaritan Hospital, Phoenix, Ariz.; Veterans Administration Medical Center, Tampa, Fla.; University of Iowa Hospitals and Clinics, Iowa City, Iowa; and Primary Children’s Medical Center, Salt Lake City, Utah (Table 1). In addition, a collection of 200 common stock strains was examined in each center (Table 1).

Study design. Antimicrobial susceptibility tests were performed in each of four laboratories using the GNS-F6 card and the bioMerieux Vitek instrument according to the manufacturer’s instructions. Inocula of clinical isolates were prepared directly from primary plates. The 200 isolates in the common challenge set were tested after two subcultures. The following 11 antimicrobial agents were used: ampicillin, ampicillin-sulbactam, aztreonam, ciprofloxacin, imipenem, mezlocillin, ofloxacin, piperacillin, ticarcillin, ticarcillin-clavulanate, and trimethoprim-sulfamethoxazole (TMP-SMX). After testing, all of the clinical isolates were shipped on tryptic soy agar slants to the University of Massachusetts Medical Center (UMMC), where stock cultures were prepared and frozen at –70°C. Stock cultures of the challenge set were also maintained at UMMC at 70°C.

At UMMC, both clinical isolates and strains from the challenge set were subsequently reconstituted from frozen stocks and subcultured twice on sheep blood agar plates incubated at 35°C for 20 to 24 h, and MICs were determined with the same 11 antimicrobial agents tested with the GNS-F6 card (see above).
RESULTS

The results of testing of clinical isolates in the four participating laboratories are depicted in Table 2. Several observations could be made from these data. In general, there was excellent concordance among the four laboratories with respect to error rates for individual antimicrobial agents. Furthermore, in no case either for data from individual laboratories or for the data analyzed collectively was the aggregate error rate (i.e., very major plus major plus minor errors) for a specific agent found to exceed 10%. Three agents were found to yield total aggregate error rates of <3.0%: ciprofloxacin (1.4%), ofloxacin (2.6%), and TMP-SMX (2.2%). Total aggregate error rates of 3 to 7% were observed with seven drugs: ampicillin (3.2%), aztreonam (4.6%), imipenem (3.4%), mezlocillin (6.2%), piperacillin (6.8%), ticarcillin (4.7%), and ticarcillin-clavulanate (6.5%). The final agent, ampicillin-sulbactam, yielded a total aggregate error rate of 8.4%. The overall aggregate error rate calculated from the total of 1,082 clinical isolates tested versus 11 antimicrobial agents with the GNS-F6 card was 4.5% (1.7% very major, 0.9% major, and 1.9% minor errors).

The distributions of errors in each of the three error categories were roughly equivalent for most antimicrobial agents (Table 2). Essentially the same observations could be made from the results of testing of a set of 200 common challenge strains in the four laboratories (data not shown).

As seen in Table 1, five species were tested in numbers large enough to permit analysis individually: E. coli (n = 163 total clinical isolates in all four laboratories), Proteus mirabilis (n = 127), Klebsiella pneumoniae (n = 154), Enterobacter cloacae (n = 144), and P. aeruginosa (n = 158). Large numbers of these species were also included in the challenge set. In eight cases, individual organism-antimicrobial combinations yielded discrepancy rates much higher than the total category error rates calculated from the entire organism collection. For E. cloacae and the indicated agents, the rates were as follows: ampicillin-sulbactam, 5.6% very major errors; aztreonam, 4.2% very major errors; ticarcillin, 7.6% very major errors; and ticarcillin-clavulanate, 7.6% very major errors. For P. aeruginosa, the rates were as follows: mezlocillin, 5.1% very major errors;
TABLE 2. Results of comparisons between Vitek GNS-F6 results and broth microdilution MIC determinations with clinical isolates of Enterobacteriaceae and P. aeruginosa tested in four laboratories versus 11 antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Laboratory A (n = 231)</th>
<th>Laboratory B (n = 320)</th>
<th>Laboratory C (n = 299)</th>
<th>Laboratory D (n = 232)</th>
<th>Total (n = 1,082)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VM M mi</td>
<td>VM M mi</td>
<td>VM M mi</td>
<td>VM M mi</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0 (0.0)</td>
<td>8 (3.5)</td>
<td>3 (1.3)</td>
<td>4 (1.3)</td>
<td>1 (0.0)</td>
</tr>
<tr>
<td>Ampicillin-</td>
<td>11 (4.8)</td>
<td>1 (0.4)</td>
<td>5 (2.2)</td>
<td>11 (3.4)</td>
<td>1 (0.0)</td>
</tr>
<tr>
<td>sulbactam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>5 (2.1)</td>
<td>11 (4.8)</td>
<td>5 (2.1)</td>
<td>5 (1.6)</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (0.9)</td>
<td>6 (1.9)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0.0)</td>
<td>9 (3.9)</td>
<td>3 (1.3)</td>
<td>0 (0.0)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Mezlocillin</td>
<td>2 (0.9)</td>
<td>7 (3.0)</td>
<td>7 (3.0)</td>
<td>2 (0.7)</td>
<td>1 (0.0)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0 (0.0)</td>
<td>2 (0.9)</td>
<td>1 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.0)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>5 (2.2)</td>
<td>5 (2.2)</td>
<td>7 (3.0)</td>
<td>8 (2.5)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>6 (2.6)</td>
<td>3 (1.3)</td>
<td>2 (0.9)</td>
<td>11 (3.4)</td>
<td>5 (1.7)</td>
</tr>
<tr>
<td>Ticarcillin-</td>
<td>6 (2.6)</td>
<td>0 (0.0)</td>
<td>4 (1.7)</td>
<td>16 (5.0)</td>
<td>8 (2.7)</td>
</tr>
<tr>
<td>clavulanate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>1 (0.4)</td>
<td>2 (0.9)</td>
<td>0 (0.0)</td>
<td>4 (1.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>36 (1.4)</td>
<td>48 (1.9)</td>
<td>38 (1.5)</td>
<td>66 (1.9)</td>
<td>56 (1.6)</td>
</tr>
</tbody>
</table>

* VM, very major; M, major; mi, minor.

The results of this investigation indicate that the currently available bioMerieux Vitek system, when used in conjunction with the GNS-F6 card according to the manufacturer’s recommendations, provides accurate susceptibility test results with numerous Enterobacteriaceae and P. aeruginosa versus ampicillin, ampicillin-sulbactam, aztreonam, ciprofloxacin, imipenem, mezlocillin, ofloxacin, piperacillin, ticarcillin, ticarcillin-clavulanate, and TMP-SMX. An overall category error rate of <10% has been established as a standard of performance for susceptibility tests (17). Included in this target percentage are very major error rates of ≤1.5% and major error rates of ≤3.0% (17). When analyzed by antimicrobial agent without respect to the organism tested, the total aggregate very major rate was found to exceed the 1.5% standard in five cases, i.e., ampicillin-sulbactam (4.1%), mezlocillin (1.8%), piperacillin (1.8%), ticarcillin (2.6%), and ticarcillin-clavulanate (4.1%). In no case did the total major error rate exceed 3.0% or was the total aggregate error rate greater than 10%. Indeed, in most instances, the error rates obtained with the study agents with the Vitek system and the GNS-F6 card were well below these limits.

Certain specific organism-antimicrobial combinations appeared to yield disproportionately high very major or major error rates. In the first category were E. cloacae versus ampicillin-sulbactam, aztreonam, ticarcillin, and ticarcillin-clavulanate and P. aeruginosa versus mezlocillin, ticarcillin, and ticarcillin-clavulanate. In the second category were P. mirabilis versus imipenem and K. pneumoniae versus ofloxacin. These observations are generally consistent with several previously published evaluations of the Vitek system (6, 9–11, 19–21, 28, 30, 43).
The higher very major error rate observed with *P. aeruginosa* and mezlocillin, ticarcillin, and ticarcillin-clavulanate may have been influenced by the fact that no intermediate MIC interpretive category has been established for these agents versus *P. aeruginosa* (18). As a result, even a 1-dilution MIC discrepancy could yield a very major error. The problem of false resistant ofloxacin results with *K. pneumoniae* with the GNS-F6 card has been previously described in the literature (5). The problem was solved by a manufacturer reformulation of the ofloxacin used in the test card (5). Updated cards containing the reformulated ofloxacin (i.e., GNS-F8) are now commercially available.

This study was intentionally conducted in clinical microbiology laboratories of four distinctly different hospital settings, an acute-care community hospital, a Veterans Administration medical center, a tertiary-care referral hospital in an academic medical center, and a pediatric hospital. It was reasoned that this diversity would provide the most rigorous challenge of the Vitek system. Surprisingly little interlaboratory variability was observed. This suggests that the utility of the Vitek susceptibility test system is not influenced by patient demographics or geographic location.

Finally, the results of this study underscore the importance of inoculum size as a determinant in the accuracy of results obtained with the Vitek system when *Enterobacteriaceae* and *P. aeruginosa* are tested. Not surprisingly, the antimicrobial agents most effected by a high inoculum were cell wall-active compounds, i.e., aztreonam, mezlocillin, piperacillin, imipenem, ampicillin-sulbactam, ticarcillin-clavulanate, and ticarcillin. Inoculum-related errors with these agents manifest as either false resistance, a major error, or minor errors in this direction of false resistance. An inoculum effect with β-lactam antimicrobial agents has been well documented. It is possible that two recent reports of excessive numbers of major errors when the Vitek system and the GNS-F6 card were used to test aztreonam and imipenem versus *Enterobacteriaceae* and *P. aeruginosa* were the result of an inoculum effect (1, 15). In the current study, when care was exercised to use an inoculum equivalent to that recommended by the manufacturer, accurate results were obtained with these agents.

In conclusion, the results of this multicenter clinical laboratory evaluation of the Vitek system and the GNS-F6 card indicate that this combination as currently formatted represents an accurate and acceptable means for performing susceptibility tests with the *Enterobacteriaceae* and *P. aeruginosa* versus several antimicrobial agents.

ACKNOWLEDGMENTS

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