Failure of the MicroScan WalkAway System To Detect Heteroresistance to Carbapenems in a Patient with Enterobacter aerogenes Bacteremia

N. C. Gordon and D. W. Wareham

Division of Infection, Barts & The London NHS Trust, London, United Kingdom, and Centre for Infectious Disease, Institute of Cell and Molecular Science, Barts & The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

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We report the failure of the automated MicroScan WalkAway system to detect carbapenem heteroresistance in Enterobacter aerogenes. Carbapenem resistance has become an increasing concern in recent years, and robust surveillance is required to prevent dissemination of resistant strains. Reliance on automated systems may delay the detection of emerging resistance.

Prompt administration of effective antimicrobial therapy is crucial to improving the outcome of severe infection (4) and has led to the increased use of potent broad-spectrum agents before the results of susceptibility tests are known. The global increase in cephalosporin resistance, due to dissemination of extended-spectrum β-lactamases, has led to a heightened use of carbapenems as the treatment of choice when gram-negative bacteria are the primary concern. Although carbapenems remain the most active agents, resistance in members of the Enterobacteriaceae family is increasing and is likely to be facilitated by wider use. A national resistance alert highlighting the importance of carbapenemase-producing strains of Klebsiella spp. and Enterobacter spp. was recently issued in the United Kingdom following the isolation of a carbapenemase-producing Klebsiella pneumoniae isolate in a patient with no significant travel or contact history (13).

Rapid methods for determining antimicrobial susceptibility play an important role in ensuring the adequate and appropriate use of antimicrobial agents. A number of automated platforms, such as Vitek II (bioMérieux, Marcy l’Etoile, France), Phoenix (Becton Dickinson, Maryland), and the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Deerfield, IL) have been developed and are used throughout the world. These aim to provide combined identification and susceptibility testing within 4 to 18 h, compared to conventional dilution or disc diffusion methods, which can take up to 48 h to provide definitive results. Here we highlight a problem with the MicroScan WalkAway 96plus system, which was unable to detect a carbapenem-heteroresistant phenotype in a clinical isolate of Enterobacter aerogenes.

Recently we used a selective chromogenic medium, CHROMagar KPC (CHROMagar, Paris, France) (11), to screen for colonization with carbapenem-resistant enterobacteriaceae in critically ill patients. A single isolate (EA1), identified as E. aerogenes using the API 20E kit (bioMérieux), was recovered from a perineal swab obtained from a patient who had received more than 21 days of carbapenem therapy. Susceptibility testing using the British Society for Antimicrobial Chemotherapy disc diffusion method (1) revealed resistance to all cephalosporins and carbapenems tested. As determined by Etest, the MICs of ertapenem, imipenem, and meropenem were all >32 µg/ml while the MIC of doripenem was 16 µg/ml. Susceptibility testing using an agar dilution method at the National Reference Facility (Health Protection Agency, London, United Kingdom) confirmed panresistance to cephalosporins and carbapenems.

Thirteen days after the isolation of EA1, a blood culture from the same individual, processed using the BacT/Alert system (bioMérieux), grew gram-negative rods. Further identification and susceptibility testing of this isolate were carried out using the Microscan WalkAway system using the Negative Combo 42 panel, which reported E. aerogenes (EA2) resistant to all cephalosporins, due to production of an extended-spectrum β-lactamase, yet susceptible to carbapenems.

In view of the previous isolation of the carbapenem-resistant isolate EA1, carbapenem susceptibility testing of isolate EA2 was repeated using disc diffusion and Etest methodology. When colonies of EA2 taken from the primary plate subcultured directly from the positive blood culture bottles were used, a resistant subpopulation of colonies with an MIC of >32 µg/ml could repeatedly be seen growing within the zones of inhibition surrounding ertapenem and imipenem Etest strips (Fig. 1). In order to ascertain whether the discrepancy between the Etest and MicroScan results was simply an inoculum phenomenon, MicroScan susceptibility testing of both EA1 and EA2 was repeated using a fourfold-higher inoculum (turbidity equivalent to a 2 McFarland standard). This confirmed carbapenem resistance in EA1 but still did not detect resistance in EA2 (Table 1). MicroScan results in all cases were obtained after the standard 18-h incubation.

The ability to detect resistant subpopulations of bacteria in susceptibility tests has been well described for a number of organisms and is usually termed “heteroresistance.” It has been implicated in glycopeptide resistance in Staphylococcus.
Carbapenem resistance in *Pseudomonas aeruginosa* (10), and resistance to both carbapenems and polymyxin E in *Acinetobacter baumannii* (8). Although the concept of heteroresistance has not to our knowledge been applied to *Enterobacter* spp., the ability of this organism to develop resistance to *β*-lactams during therapy is well established. Derepression of the chromosomal AmpC *β*-lactamase due to mutations in the *ampD* regulator can lead to cephalosporin resistance (5), while accompanying mutations in porin genes promote reduced susceptibility to carbapenems (2). The mechanisms of carbapenem resistance in the isolates described here have not yet been fully characterized. A modified Hodge test did not indicate carbapenemase production, and PCR screening using published primers did not detect any known class A (*bla*<sub>KPC</sub>), *bla*<sub>TEM</sub>, *bla*<sub>GES</sub>, and *bla*<sub>SFC</sub>), B (<em>bla</em>IMP, *bla*<sub>VIM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>SPM</sub>, *bla*<sub>F1</sub>, *bla*<sub>NDM</sub>, and *bla*<sub>KHM</sub>), or D ( *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-48-like</sub>, *bla*<sub>OXA-51-like</sub>, and *bla*<sub>OXA-58-like</sub>) carbapenemase genes, suggesting that impermeability and AmpC overexpression may be important, as has been described for other isolates recently recovered in the United Kingdom (3).

In previous assessments, MicroScan was found to perform better than other automated systems in the detection of carbapenem resistance in *K. pneumonia*, although it still failed to detect imipenem resistance in 53% (8/15) of the isolates tested (12). This may be partially attributable to differences in the inoculum, although the authors also reported variations in susceptibility dependent upon the reference method used in the comparison. This suggests variable expression of resistance determinants which could go undetected by some automated methods. Problems with the detection of imipenem resistance in *Pseudomonas aeruginosa* (6) and in *A. baumannii* using MicroScan have also been reported (7). In the *A. baumannii* evaluation, there was no difference in detection when a heavier inoculum was used.

Resistance to carbapenems is likely to become an increasing problem as their use becomes more widespread. Reliance on automated sensitivity testing, while it is more rapid than disc diffusion methods, may result in a delay in detecting or inability to detect the development of resistance. Where there is a high suspicion of emerging resistance, additional methods may therefore need to be employed.

We thank the staff of the Antimicrobial Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, London, United Kingdom, for carrying out reference susceptibility testing of *Enterobacter aerogenes* EA1.

### TABLE 1. Reported susceptibilities and MICs determined by agar dilution or MicroScan for EA1 and EA2

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml) of drug for isolate with indicated method&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EA1</th>
<th>MicroScan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agar dilution</td>
<td>MicroScan</td>
<td>EA2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2 (S)</td>
<td>&lt;16 (S)</td>
<td>&lt;16 (S)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;64 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;64 (R)</td>
<td>&gt;32 (R)</td>
<td>&gt;32 (R)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>256 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.125 (S)</td>
<td>&lt;1 (S)</td>
<td>&lt;1 (S)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;16 (R)</td>
<td>&gt;4 (R)</td>
<td>&gt;2 (S)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>32 (R)</td>
<td>&lt;4 (R)</td>
<td>&lt;4 (S)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>16 (R)</td>
<td>Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Piperaclidin-tazobactam</td>
<td>&gt;64 (R)</td>
<td>&gt;64 (R)</td>
<td>&gt;64 (R)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Resistance (R) or susceptibility (S) of the isolate is indicated in parentheses.

**REFERENCES**


