Rapid ertapenem susceptibility testing and Klebsiella pneumoniae carbapenemase phenotype detection in Klebsiella pneumoniae isolates by use of automated microscopy of immobilized live bacterial cells

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Rapid Ertapenem Susceptibility Testing and Klebsiella pneumoniae Carbapenemase Phenotype Detection in Klebsiella pneumoniae Isolates by Use of Automated Microscopy of Immobilized Live Bacterial Cells

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We evaluated detection of ertapenem (ETP) resistance and *Klebsiella pneumoniae* carbapenemase (KPC) in 47 *Klebsiella pneumoniae* isolates using a novel automated microscopy system. Automated microscopy correctly classified 22/23 isolates as ETP resistant and 24/24 as ETP susceptible and correctly classified 21/21 isolates as KPC positive and 26/26 as KPC negative.
100%) and 100% specificity (95% CI, 83% to 100%) for detection of ETP resistance. There was one instance of susceptibility reported by automated microscopy for an isolate that tested as resistant by disk diffusion (KP-505; 13-mm ETP zone diameter, microscopy score of 0.93). Growth controls had an average and standard deviation of 55 ± 27 growing clones per field of view, a number which was therefore the number of viable clones in all channels. The average resistance score and standard deviation was 0.46 ± 0.41 (range, 0.00 to 1.25) for ETP-susceptible isolates and 1.89 ± 0.24 (range, 0.93 to 2.17) for ETP-resistant isolates. Images of ETP-resistant isolate wu33 and ETP-susceptible isolate wu9 at 0, 90, and 180 min after exposure to 10 μg/ml ETP are shown in Fig. 3. Upon exposure to 10 μg/ml ETP, ETP-resistant strains showed robust growth with minimal lysis, with most clones growing in a spheroidal clone morphology. In contrast, ETP-susceptible strains exhibited rapid growth arrest and lysis (Fig. 3).

The microscopy system classified 21/21 isolates as KPC positive and 26/26 strains as KPC negative compared to PCR results. Upon exposure to MEM alone, spheroidal clone morphology was observed for KPC-positive clones with minimal lysis, with most clones growing in a spheroidal clone morphology. In contrast, ETP-susceptible strains exhibited rapid growth arrest and lysis (Fig. 3).

The microscopy system classified 21/21 isolates as KPC positive and 26/26 strains as KPC negative compared to PCR results. Upon exposure to MEM alone, spheroidal clone morphology was observed for KPC-positive clones with minimal lysis (Fig. 4). Rapid growth arrest and lysis were observed for KPC-positive isolates in the presence of MEM plus NPBA as well as for all KPC-negative isolates upon exposure to MEM and MEM plus NPBA. The microscopy system achieved 100% sensitivity (95% CI, 81% to 100%) and 100% specificity (95% CI, 83% to 100%) for KPC characterization of the isolates. The mean inhibition score and standard deviation was 0.54 ± 0.19 (range, 0.19 to 0.91) for KPC-positive isolates and −0.75 ± 0.38 (range, −1.00 to −0.08) for KPC-negative isolates. Images of KPC-positive isolate wu15 at 0, 90, and 180 min are shown in Fig. 4. All 21 KPC-positive strains were classified as ETP resistant by microscopy analysis and by disk diffusion.

Of note, two strains evaluated in this study (KP-456 and KP-505) were negative for KPC by PCR but resistant to both ETP and MEM using disk diffusion. The microscopy analysis correctly classified both strains as KPC negative. Microscopy analysis correctly classified KP-456 as ETP resistant but incorrectly classified KP-505 as ETP susceptible (Fig. 2). Additional susceptibility testing of isolate KP-505 demonstrated that the isolate was susceptible to piperacillin-tazobactam, ticarcillin-clavulanic acid, cefotetan, amikacin, and tobramycin and resistant to gentamicin; this profile is different from those of the KPC-producing strains in this study. The KPC-producing strains were typically gentamicin susceptible and resistant to piperacillin-tazobactam, ticarcillin-clavulanic acid, amikacin, and tobramycin. Together, these data suggest that in isolate KP-505, carbapenem resistance may be attributed to an ESBL-producing strain with a porin or efflux mutation(s). Visual examination of the time-lapse images for isolate KPC-505 revealed that the microscopy error was due to technical limitations, including loss of focus in two of the six fields of view and very low clone counts in the remaining four fields of view, issues which weighted the scoring algorithm toward a score in the susceptible range. Improvements in instrument focus, setting of strict requirements for the number of clones analyzed prior to reporting, and algorithm optimization may help to reduce or eliminate these sources of error in future analyses. For isolate KP-456, additional susceptibility testing results revealed cefepime susceptibility and cefotetan resistance, suggesting that in this isolate, an acquired AmpC with a porin and/or efflux mutation(s) may be a plausible

### TABLE 1 Characterization of bacterial strains

<table>
<thead>
<tr>
<th>Strain type</th>
<th>No. of isolates</th>
<th>No. of isolates with each result as determined by automated microscopy</th>
<th>Mean (range) zone diameter (mm) with a:</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC positive</td>
<td>21</td>
<td>21 positive, 0 negative</td>
<td>ETP 8 (6–11), MEM 10 (8–12)</td>
</tr>
<tr>
<td>KPC-2 positive</td>
<td>13</td>
<td>13 positive, 0 negative</td>
<td>ETP 12 (6–15), MEM 13 (12–14)</td>
</tr>
<tr>
<td>KPC-3 positive</td>
<td>8</td>
<td>8 positive, 0 negative</td>
<td>ETP 12 (6–15), MEM 13 (12–14)</td>
</tr>
<tr>
<td>KPC negative</td>
<td>26</td>
<td>0 positive, 26 negative</td>
<td>ETP 15 (13–16), MEM 18 (17–19)</td>
</tr>
<tr>
<td>Ertapenem resistant</td>
<td>2</td>
<td>0 positive, 2 negative</td>
<td>ETP 28 (26–35), MEM 27 (25–30)</td>
</tr>
<tr>
<td>Ertapenem susceptible</td>
<td>24</td>
<td>0 positive, 24 negative</td>
<td>ETP 28 (26–35), MEM 27 (25–30)</td>
</tr>
</tbody>
</table>

*The CLSI susceptibility breakpoint for ETP is ≥22 mm, and the CLSI susceptibility breakpoint for MEM is ≥23 mm.*

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**FIG 1** Bacterial cell immobilization by electrokinetic concentration. (Left) Sample suspension flows in and then stops. (Right) Electric field forces bacterial cells to the positively charged poly-L-lysine (PLL) capture coating. PLL binds cells upon contact and immobilizes them after the electric field turns off. ITO, indium tin oxide.
explanation for the observed carbapenem resistance in the absence of KPC.

The KPC type of each strain was determined (Table 1) as previously described (13). In addition, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) was used to assess the genetic relatedness of the KPC-producing strains. PCR products were resolved using DiversiLab DNA chips (bioMérieux, Durham, NC) on the Agilent 2100 system (Agilent Technologies, Santa Clara, CA). DiversiLab software was used to compare banding patterns and determine the similarity of the isolates (using a similarity index [SI]), with identical isolates having an SI of 85% or greater (14–17). Molecular typing of the specific KPC variants contained in the isolates identified 13 blaKPC-2- and 8 blaKPC-3-containing strains (Table 1). With ERIC-PCR, the isolates clustered into 4 major strain types: the first cluster with 5 isolates, the second with 3 isolates, the third with 12 isolates, and the fourth with 1 isolate.

State-of-the-art automated antimicrobial susceptibility testing systems vary in their ability to detect carbapenemase production (8, 18). Reliable detection of carbapenem resistance is important to guide selection of appropriate therapy. Automated microscopy, as evaluated in this study, detected ETP resistance and showed promising phenotypic results that were consistent with KPC expression, using data acquired in 3 h. Using ETP as an indicator, the automated microscopy method evaluated in this study was 96% sensitive and 100% specific for detection of carbapenem resis-
tance. This rapid result may be important for guiding antimicrobial therapy in critically ill patients and quickly initiating appropriate infection control measures.

ACKNOWLEDGMENTS
We thank James H. Jorgensen of the University of Texas Health Science Center for assisting with coordination of the KPC typing. We thank Steve Metzger, Alena Shamsheyeva, David Howson, and Christina Chantell of Accelerate Diagnostics, Inc. (Tucson, AZ), for useful discussions on study design, data analysis and interpretation, and assistance with manuscript preparation.

This study was funded through a grant from Accelerate Diagnostics, Inc., to the Washington University School of Medicine.

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