Revised manuscript

Article Type

Short communication (Note)

Title

Performance evaluation of BD Phoenix™, an automated microbiology system, for the screening of IMP-producing Enterobacteriaceae

Authors

Hiromi Yamakawa
Kosuke Kosai
Yasuhide Kawamoto
Norihiko Akamatsu
Junichi Matsuda
Norihito Kaku
Naoki Uno
Yositomo Morinaga
Hiroo Hasegawa
Katsunori Yanagihara

Affiliation

Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan
Department of Laboratory Medicine, Nagasaki University Graduate School of
Biomedical Sciences, Nagasaki, Japan

*Correspondence

Kosuke Kosai, MD, PhD
Department of Laboratory Medicine, Nagasaki University Hospital
1-7-1 Sakamoto, Nagasaki, Nagasaki 852-8501, Japan
Tel: +81-95-819-7574; Fax: +81-95-819-7422
E-mail: k-kosai@nagasaki-u.ac.jp

Running title: Accurate detection of IMP producer by BD Phoenix™
Abstract

BD Phoenix™ is an automated bacterial identification and susceptibility testing system. Here, its performance in screening IMP-producing *Enterobacteriaceae* was evaluated. The system identified 97.8% of IMP producers as being nonsusceptible to imipenem or meropenem, which was higher than that identified by the broth microdilution method (91.3%, imipenem; 41.3%, meropenem).

Keywords: Carbapenemase; IMP; Resistance; MIC; *Enterobacteriaceae*
Carbapenemase-producing *Enterobacteriaceae* (CPE) has emerged as a significant public health concern. It has been reported that performing appropriate empirical antibiotic therapy is difficult in patients with bacteremia caused by CPE, with very high mortality rates (Daikos et al., 2012; Doi and Paterson, 2015; Girometti et al., 2014). Additionally, plasmid-mediated carbapenemase producers are particularly problematic, because plasmids harboring resistant genes can be transferred among different bacterial genera or species (Lutgring and Limbago, 2016). Therefore, CPE detection is crucial for implementing appropriate therapy as well as for infection control.

Although resistance is determined by antimicrobial susceptibility testing (AST), some CPEs have low minimal inhibitory concentration (MIC) values for carbapenems, and are thus overlooked (Daikos and Markogiannakis, 2011; Giske et al., 2013). Several methods for detecting carbapenemase genes or activity, such as the Carba NP test and carbapenem inactivation method (CIM), have been developed and shown to be useful (Osei Sekyere et al., 2015; Tijet et al., 2016). However, because they cannot fully replace AST, they need to be performed in addition to AST, which requires additional time and cost. It is also impractical to perform these tests for all *Enterobacteriaceae* including those with lower MICs in daily practice; thus, more effective screening
methods using AST are desirable.

The AST results for identical bacteria sometimes differ among methods such as the broth microdilution (BMD) method and automated AST systems (Patel et al., 2013).

BD Phoenix™ is an automated identification and susceptibility testing system that provides rapid and accurate detection of antimicrobial resistance. The AST method in the BD Phoenix™ system is a broth-based microdilution method that not only measures turbidity, but also utilizes the redox indicator to enhance the detection of bacterial growth (Carroll et al., 2006; Snyder et al., 2008), enabling it to detect resistant bacteria with high sensitivity. In this study, we determined if this system could effectively identify CPEs as being nonsusceptible to carbapenems compared with the conventional BMD method.

We evaluated 62 Enterobacteriaceae (33 K. pneumoniae and 29 Enterobacter cloaceae complex) that were clinically isolated at Nagasaki University Hospital. The MICs were simultaneously measured using the BD Phoenix™ Automated Microbiology System (BD Diagnostics) according to the manufacturer’s instructions, and using MIC plates customized by Eiken Chemical Co., Ltd. for the BMD method according to the Clinical and Laboratory Standard Institute (CLSI) protocol. Susceptibility was determined according to CLSI definitions, namely, MICs ≤1 and ≥2 μg/mL for
imipenem and meropenem were considered susceptible and nonsusceptible, respectively (CLSI, 2014). The presence of IMP-type metallo-β-lactamase (MBL) and *K. pneumoniae* carbapenemase (KPC) genes were evaluated by PCR in all 62 strains. Briefly, DNA was extracted using the boiling method with minor modifications (Motoshima et al., 2010). The PCR primers used to amplify the IMP and KPC genes were as follows: IMP forward, 5’-GGAATAGAGTGCTTAAYTCTC-3’; IMP reverse, 5’-GGTTTAAYAAAACAACCACC-3’; KPC forward, 5’-CGTCTAGTTCTGCTGTCTTG-3’; and KPC reverse, 5’-CTTGTCATCCTTGTTAGGCG-3’ (Poirel et al., 2011). PCR amplification was performed under the following conditions: 10 min at 94°C, 40 cycles of 30 s at 94°C, 40 s at 52°C, 1 min at 72°C, and 5 min at 72°C for the final extension. We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the two methods for identifying CPEs as being nonsusceptible to carbapenems. The 95% confidence intervals for sensitivity, specificity, PPV, and NPV were calculated using R statistical software (https://cran.ism.ac.jp/) (Kosai et al., 2017). Of the 62 strains tested, 46 (25 *K. pneumoniae* and 21 *E. cloacae* complex) tested positive in the IMP genetic screen and were deemed IMP producers. Sixteen strains (8 *K. pneumoniae* and 8 *E. cloacae* complex) did not possess the IMP gene and
were considered non-IMP producers. No KPC gene was detected in the strains tested in this study. The results were consistent with previous reports showing that IMP MBLs are widespread in Japan (Fukigai, et al., 2007; Livermore, et al., 2000; Tojo, et al., 2014). Table 1 shows the susceptibility patterns of the strains examined. Both methods successfully identified 10 IMP-producing *K. pneumoniae* and 9 IMP-producing *E. cloacae* as being nonsusceptible to both imipenem and meropenem. The BMD method identified 15 IMP-producing *K. pneumoniae* as being susceptible to meropenem, whereas the BD Phoenix™ system determined that only 1 was susceptible to imipenem. Similarly, although the BMD method identified 12 and 4 IMP-producing *E. cloacae* complex as being susceptible to meropenem and imipenem, respectively, the BD Phoenix™ system only identified 1 IMP-producing *E. cloacae* complex as being susceptible to meropenem. The BD Phoenix™ system appropriately identified all non-IMP producers as being susceptible to both carbapenems, whereas the BMD method identified one non-IMP-producing *E. cloacae* complex as being nonsusceptible to imipenem.

The sensitivity, specificity, PPV, and NPV of the two methods for identifying IMP producers as nonsusceptible are presented in Table 2. Those of the BD Phoenix™ system were 97.8%, 100.0%, 100.0%, and 94.1%, respectively, when using either
imipenem or meropenem for screening. When both drugs were used for screening, the
results were excellent (100.0%). In addition to IMP producers, it has been reported that
the BD Phoenix™ system is able to successfully detect CPEs that produce other types
of carbapenemases such as KPC, Verona integron-encoded MBL (VIM), New Delhi
MBL (NDM), and OXA-48 as nonsusceptible strains (Doern, et al., 2011; Woodford, et
al., 2010).

Conversely, the BMD method showed extremely low sensitivity (41.3%) and
NPV (37.2%) when using meropenem. The sensitivity, specificity, PPV, and NPV of the
BMD method using imipenem were 91.3%, 93.8%, 97.7%, and 78.9%, respectively,
which was similar to the results obtained when both drugs were used for screening in
the BMD method. Although the BMD method effectively identified IMP producers as
being nonsusceptible to imipenem it overlooked 58.7% of IMP producers when
meropenem was used for screening. Therefore, if the BMD method is routinely used in
our hospital, imipenem should be adopted above meropenem for screening
IMP-producing Enterobacteriaceae. However, because hydrolytic efficiencies and drug
susceptibility patterns vary depending upon the combination of drug and carbapenemase
type (Doern et al., 2011; Tzouvelekis et al., 2012), drugs used for screening should be
determined based on regional epidemiology. Thus, it is important that surveillance be
continued including drug susceptibility patterns and carbapenemase types of CPE in each region. Because this study focused on the detection of IMP-producers, the ability of the BD Phoenix™ system to detect bacteria with other resistant mechanisms, such as decreased outer membrane permeability, was not analyzed.

The results of this study demonstrated that the BD Phoenix™ system could detect IMP-producing *Enterobacteriaceae* with high accuracy, thereby making it suitable for daily screening of IMP producers. To effectively screen CPE, continuous surveillance is needed of regional CPE epidemiology with regard to drug susceptibility patterns, carbapenemase types, and their relationship.

Reagents, instrumentation, and funding were provided by Nippon Becton Dickinson Company, Ltd. This study was partially supported by the Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan (H28-Shinkou-Ippan-003), and a grant for research and development of diagnostic methods and therapies for antimicrobial-resistant bacteria from the Japan Agency for Medical Research and Development (AMED).
References


Lutgring, J.D., Limbago, B.M., 2016. The problem of carbapenemase-producing-carbapenem-resistant-<i>Enterobacte</i><i>r</i><i>ri</i><i>a</i><i>c</i><i>e</i><i>a</i> detection. J Clin Microbiol. 54, 529-534.


of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 70, 119-123.


Table 1. Comparison of drug susceptibility of IMP-producing *Enterobacteriaceae* and nonproducers between the BD Phoenix™ and BMD methods.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Carbapenemase gene</th>
<th>Susceptibility</th>
<th>BD Phoenix™</th>
<th>BMD method</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipenem</td>
<td>Meropenem</td>
<td>Imipenem</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>IMP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>E. cloacae</em> complex</td>
<td>IMP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMD, broth microdilution; ND, not detected; NS, not susceptible; S, susceptible
Table 2. Performance of the BD Phoenix™ and BMD methods for identifying IMP-producing Enterobacteriaceae as nonsusceptible strains for carbapenems.

<table>
<thead>
<tr>
<th>Method</th>
<th>Drug used for screening</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BD Phoenix™</strong></td>
<td>Imipenem</td>
<td>97.8 (45/46), (88.5–99.9)</td>
<td>100.0 (16/16), (79.4–100.0)</td>
<td>100.0 (45/45), (92.1–100.0)</td>
<td>94.1 (16/17), (71.3–99.9)</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>97.8 (45/46), (88.5–99.9)</td>
<td>100.0 (16/16), (79.4–100.0)</td>
<td>100.0 (45/45), (92.1–100.0)</td>
<td>94.1 (16/17), (71.3–99.9)</td>
</tr>
<tr>
<td></td>
<td>Both&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0 (46/46), (92.3–100.0)</td>
<td>100.0 (16/16), (79.4–100.0)</td>
<td>100.0 (46/46), (92.3–100.0)</td>
<td>100.0 (16/16), (79.4–100.0)</td>
</tr>
<tr>
<td><strong>BMD method</strong></td>
<td>Imipenem</td>
<td>91.3 (42/46), (79.2–97.6)</td>
<td>93.8 (15/16), (69.8–99.8)</td>
<td>97.7 (42/43), (87.7–99.9)</td>
<td>78.9 (15/19), (54.4–93.9)</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>41.3 (19/46), (27.0–56.8)</td>
<td>100.0 (16/16), (79.4–100.0)</td>
<td>100.0 (19/19), (82.4–100.0)</td>
<td>37.2 (16/43), (23.0–53.3)</td>
</tr>
<tr>
<td></td>
<td>Both&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.3 (42/46), (79.2–97.6)</td>
<td>93.8 (15/16), (69.8–99.8)</td>
<td>97.7 (42/43), (87.7–99.9)</td>
<td>78.9 (15/19), (54.4–93.9)</td>
</tr>
</tbody>
</table>

Data expressed as percentages, (95% confidence interval)

BMD, broth microdilution; PPV, positive predictive value; NPV, negative predictive value

<sup>a</sup>If strains were not susceptible to imipenem or meropenem, they were considered nonsusceptible.
Highlights

– IMP metallo-β-lactamase is a major carbapenemase found in *Enterobacteriaceae*.

– The broth microdilution method may overlook IMP-producing *Enterobacteriaceae*.

– The BD Phoenix™ accurately screened IMP-producing *Enterobacteriaceae*. 