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Title

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

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

35 **Abstract**

36 BD Phoenix™ is an automated bacterial identification and susceptibility testing system.

37 Here, its performance in screening IMP-producing *Enterobacteriaceae* was evaluated.

38 The system identified 97.8% of IMP producers as being nonsusceptible to imipenem or

39 meropenem, which was higher than that identified by the broth microdilution method

40 (91.3%, imipenem; 41.3%, meropenem).

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42 **Keywords**: Carbapenemase; IMP; Resistance; MIC; *Enterobacteriaceae*

43 **Text**

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

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

61 methods using AST are desirable.

62 The AST results for identical bacteria sometimes differ among methods such as
63 the broth microdilution (BMD) method and automated AST systems (Patel et al., 2013).
64 BD Phoenix™ is an automated identification and susceptibility testing system that
65 provides rapid and accurate detection of antimicrobial resistance. The AST method in the
66 BD Phoenix™ system is a broth-based microdilution method that not only measures
67 turbidity, but also utilizes the redox indicator to enhance the detection of bacterial
68 growth (Carroll et al., 2006; Snyder et al., 2008), enabling it to detect resistant bacteria
69 with high sensitivity. In this study, we determined if this system could effectively
70 identify CPEs as being nonsusceptible to carbapenems compared with the conventional
71 BMD method.

72 We evaluated 62 *Enterobacteriaceae* (33 *K. pneumoniae* and 29 *Enterobacter*
73 *cloacae* complex) that were clinically isolated at Nagasaki University Hospital. The
74 MICs were simultaneously measured using the BD Phoenix™ Automated Microbiology
75 System (BD Diagnostics) according to the manufacturer's instructions, and using MIC
76 plates customized by Eiken Chemical Co., Ltd. for the BMD method according to the
77 Clinical and Laboratory Standard Institute (CLSI) protocol. Susceptibility was
78 determined according to CLSI definitions, namely, MICs ≤1 and ≥2 μg/mL for

79 imipenem and meropenem were considered susceptible and nonsusceptible, respectively
80 (CLSI, 2014). The presence of IMP-type metallo-β-lactamase (MBL) and *K.*
81 *pneumoniae* carbapenemase (KPC) genes were evaluated by PCR in all 62 strains.
82 Briefly, DNA was extracted using the boiling method with minor modifications
83 (Motoshima et al., 2010). The PCR primers used to amplify the IMP and KPC genes
84 were as follows: IMP forward, 5'-GGAATAGAGTGGCTTAAYTCTC-3'; IMP reverse,
85 5'-GGTTTAAYAAAACAACCACC-3'; KPC forward,
86 5'-CGTCTAGTTCTGCTGTCTTG-3'; and KPC reverse,
87 5'-CTTGTCATCCTTGTTAGGCG-3' (Poirel et al., 2011). PCR amplification was
88 performed under the following conditions: 10 min at 94°C, 40 cycles of 30 s at 94°C, 40
89 s at 52°C, 1 min at 72°C, and 5 min at 72°C for the final extension. We calculated the
90 sensitivity, specificity, positive predictive value (PPV), and negative predictive value
91 (NPV) of the two methods for identifying CPEs as being nonsusceptible to carbapenems.
92 The 95% confidence intervals for sensitivity, specificity, PPV, and NPV were calculated
93 using R statistical software (https://cran.ism.ac.jp/) (Kosai et al., 2017).
94 Of the 62 strains tested, 46 (25 *K. pneumoniae* and 21 *E. cloacae* complex)
95 tested positive in the IMP genetic screen and were deemed IMP producers. Sixteen
96 strains (8 *K. pneumoniae* and 8 *E. cloacae* complex) did not possess the IMP gene and

97 were considered non-IMP producers. No KPC gene was detected in the strains tested in
98 this study. The results were consistent with previous reports showing that IMP MBLs
99 are widespread in Japan (Fukigai, et al., 2007; Livermore, et al., 2000; Tojo, et al.,
100 2014). Table 1 shows the susceptibility patterns of the strains examined. Both methods
101 successfully identified 10 IMP-producing *K. pneumoniae* and 9 IMP-producing *E.*
102 *cloacae* as being nonsusceptible to both imipenem and meropenem. The BMD method
103 identified 15 IMP-producing *K. pneumoniae* as being susceptible to meropenem,
104 whereas the BD Phoenix™ system determined that only 1 was susceptible to imipenem.
105 Similarly, although the BMD method identified 12 and 4 IMP-producing *E. cloacae*
106 complex as being susceptible to meropenem and imipenem, respectively, the BD
107 Phoenix™ system only identified 1 IMP-producing *E. cloacae* complex as being
108 susceptible to meropenem. The BD Phoenix™ system appropriately identified all
109 non-IMP producers as being susceptible to both carbapenems, whereas the BMD
110 method identified one non-IMP-producing *E. cloacae* complex as being nonsusceptible
111 to imipenem.

112 The sensitivity, specificity, PPV, and NPV of the two methods for identifying
113 IMP producers as nonsusceptible are presented in Table 2. Those of the BD Phoenix™
114 system were 97.8%, 100.0%, 100.0%, and 94.1%, respectively, when using either

115 imipenem or meropenem for screening. When both drugs were used for screening, the
116 results were excellent (100.0%). In addition to IMP producers, it has been reported that
117 the BD Phoenix^{TM} system is able to successfully detect CPEs that produce other types
118 of carbapenemases such as KPC, Verona integron-encoded MBL (VIM), New Delhi
119 MBL (NDM), and OXA-48 as nonsusceptible strains (Doern, et al., 2011; Woodford, et
120 al., 2010).

121 Conversely, the BMD method showed extremely low sensitivity (41.3%) and
122 NPV (37.2%) when using meropenem. The sensitivity, specificity, PPV, and NPV of the
123 BMD method using imipenem were 91.3%, 93.8%, 97.7%, and 78.9%, respectively,
124 which was similar to the results obtained when both drugs were used for screening in
125 the BMD method. Although the BMD method effectively identified IMP producers as
126 being nonsusceptible to imipenem it overlooked 58.7% of IMP producers when
127 meropenem was used for screening. Therefore, if the BMD method is routinely used in
128 our hospital, imipenem should be adopted above meropenem for screening
129 IMP-producing *Enterobacteriaceae*. However, because hydrolytic efficiencies and drug
130 susceptibility patterns vary depending upon the combination of drug and carbapenemase
131 type (Doern et al., 2011; Tzouvelekis et al., 2012), drugs used for screening should be
132 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.

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for carbapenem resistance in *Enterobacteriaceae*. J Clin Microbiol. 48, 2999-3002.

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

Strains	Carbapenemase gene	Susceptibility				N
		BD Phoenix™		BMD method		
		Imipenem	Meropenem	Imipenem	Meropenem	
K. pneumoniae	IMP	NS	NS	NS	NS	10
		NS	NS	NS	S	14
		S	NS	NS	S	1
	ND	S	S	S	S	8
E. cloacae complex	IMP	NS	NS	NS	NS	9
		NS	NS	NS	S	7
		NS	S	NS	S	1
		NS	NS	S	S	4
	ND	S	S	NS	S	1
		S	S	S	S	7
Total						62

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.

Method	Drug used for screening	Sensitivity	Specificity	PPV	NPV
BD Phoenix™	Imipenem	97.8 (45/46), (88.5–99.9)	100.0 (16/16), (79.4–100.0)	100.0 (45/45), (92.1–100.0)	94.1 (16/17), (71.3–99.9)
	Meropenem	97.8 (45/46), (88.5–99.9)	100.0 (16/16), (79.4–100.0)	100.0 (45/45), (92.1–100.0)	94.1 (16/17), (71.3–99.9)
	Both[a]	100.0 (46/46), (92.3–100.0)	100.0 (16/16), (79.4–100.0)	100.0 (46/46), (92.3–100.0)	100.0 (16/16), (79.4–100.0)
BMD method	Imipenem	91.3 (42/46), (79.2–97.6)	93.8 (15/16), (69.8–99.8)	97.7 (42/43), (87.7–99.9)	78.9 (15/19), (54.4–93.9)
	Meropenem	41.3 (19/46), (27.0–56.8)	100.0 (16/16), (79.4–100.0)	100.0 (19/19), (82.4–100.0)	37.2 (16/43), (23.0–53.3)
	Both[a]	91.3 (42/46), (79.2–97.6)	93.8 (15/16), (69.8–99.8)	97.7 (42/43), (87.7–99.9)	78.9 (15/19), (54.4–93.9)

Data expressed as percentages, (95% confidence interval)

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

[a]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^{TM} accurately screened IMP-producing *Enterobacteriaceae*.