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1 **A nitrocefin disc supplemented with ertapenem for rapid screening**
2 **of carbapenemase-producing Enterobacteriaceae**

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5 **Running title:** Screening of carbapenemases by nitrocefin

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20 ABSTRACT

21 Reliable, simple and rapid methods for laboratory detection of carbapenemases are important for
22 an appropriate antibiotic administration. A nitrocefin disc containing ertapenem for rapid
23 screening of carbapenemase production among Enterobacteriaceae is developed in the present
24 study. A total of 87 molecularly-confirmed Enterobacteriaceae including 31 carbapenemase
25 producers and 56 non-carbapenemase producers, were tested with nitrocefin discs supplemented
26 with and without ertapenem (20 µg/ disc). Nitrocefin discs with ertapenem successfully
27 discriminated all 31 carbapenemase and all non-carbapenemase producers within 30 minutes.
28 The sensitivity and specificity of the method were 100%. The minimum inhibitory
29 concentrations (MICs) of ertapenem against all carbapenemase-producing isolates ranged from 1
30 to ≥ 256 µg/mL. This simple test could help to minimize the treatment failure and control the
31 dissemination of infections caused by carbapenemase-associated resistant bacteria. It is a
32 promising approach that could be performed routinely in any laboratory.

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34 Keywords: Nitrocefin disc, ertapenem, carbapenemase, Enterobacteriaceae

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41 **1. Introduction**

42 Since carbapenems have been extensively used for the treatment of infections caused by AmpC
43 and extended-spectrum β -lactamase (ESBL)-producing isolates, an emergence of carbapenem-
44 resistant Enterobacteriaceae (CRE) has been identified (Jacoby, 2009; Perez et al., 2007).
45 Prevalence of CRE has been increasing dramatically, becoming one of the most serious clinical
46 issues with increased morbidity and mortality worldwide (Logan and Weinstein, 2017). The
47 mechanism of resistance to carbapenems, the last line β -lactam antibiotics for the treatment of
48 Gram-negative bacterial infections, is predominantly mediated by enzymes called
49 “carbapenemases” (Bush and Fisher, 2011). Resistance also involves a variety of other
50 mechanisms, including overproduction of AmpC β -lactamase or ESBL combined with decreased
51 porin channel for carbapenem entry, and increased efflux pump activity (Thomson, 2010).
52 Colistin, fosfomycin and tigecycline are among the most frequently used antibiotics for the
53 treatment of carbapenem resistance (Lee and Doi, 2014).

54 The carbapenemase gene (bla_{NmcA}) was first proven in the chromosome of *Enterobacter cloacae*
55 in France in 1993 (Naas and Nordmann, 1994). In 1995, a plasmid-encoded $bla_{IMP.1}$ was
56 described in Japan in *Serratia* spp. This metallo- β -lactamase (MBL) was able to hydrolyze
57 carbapenem antibiotics (Ito et al., 1995). A few years later, carbapenem-hydrolyzing bla_{KPC-1} and
58 bla_{KPC-2} gene were identified in a plasmid of *Klebsiella pneumoniae* (Smith Moland et al., 2003;
59 Yigit et al., 2001). Furthermore, OXA-48 carbapenemase was also first discovered and identified
60 in an isolate of carbapenem-resistant *K. pneumoniae* in Istanbul, Turkey (Poirel et al., 2004).
61 Even though numerous clinically significant carbapenemase-encoded genes have been identified
62 recently, they can be molecularly grouped into Ambler class A (KPC and GES), class B (MBL:

63 NDM, VIM, and IMP), and class D with carbapenemase activity (OXA-48-like) (Nordmann,
64 2014).

65 Currently, treatment options available for the treatment of infections caused by CRE are limited.
66 Development of diagnostic tools to detect the presence of carbapenemase in Enterobacteriaceae
67 is necessary to guide clinicians for appropriate antimicrobial therapy, as well as to minimize
68 treatment failure (Teethaisong et al., 2016). A number of innovative methods such as genotypic
69 (e.g. multiplex PCR and real-time PCR), phenotypic (e.g. disc-inhibitor synergy test), and
70 biochemical tests (e.g. Carba NP test), have been developed for the detection of carbapenemase-
71 producing isolates (Osei Sekyere et al., 2015; Tamma et al., 2017). However, they are relatively
72 time-consuming, or expensive and difficult to perform.

73 Nitrocefin, a chromogenic cephalosporin, is a substrate for β -lactamase enzymes. The reaction
74 can visually be observed by a change its original yellow color to red. Nitrocefin assay is one of
75 the most common methods to detect the presence of β -lactamases in both Gram-negative and
76 Gram-positive bacteria ((Kaase et al., 2008)). Carbapenem antibiotics prevent extended spectrum
77 β -lactamase (ESBL) and AmpC β -lactamase-producing bacteria from hydrolyzing nitrocefin
78 (Goessens et. al., 2013). The present study, therefore, developed a novel chromogenic method
79 using a nitrocefin disc impregnated with ertapenem for rapid screening of carbapenemase
80 production in Enterobacteriaceae isolates.

81

82 **2. Materials and methods**

83 2.1 Bacterial isolates

84 A total of 87 Enterobacteriaceae isolates with confirmed molecular types of β -lactamase
85 enzymes were used in the present study as summarized in Table 1. The bacterial strains used

86 have been described in our previous study (Teethaisong et al., 2016; Teethaisong et al., 2017),
87 with an addition of KPC-producing *K. pneumoniae* 4018. Thirty-one carbapenemase-producing
88 isolates (11 MBLs, 9 KPCs and 11 OXA-48), fifty-six non-carbapenemase-producing strains and
89 a β -lactamase-negative *E. coli* ATCC 25922 were used to investigate nitrocefin discs
90 supplemented with and without ertapenem for the rapid screening of carbapenemase production
91 in Enterobacteriaceae.

92 2.2 Minimum inhibitory concentration (MIC) determination

93 The MICs of ertapenem were assessed by a standard broth microdilution method according to
94 the Clinical and Laboratory Standards Institute (CLSI) guideline (Clinical Laboratory Standards
95 Institute, 2012). The organisms (18-h culture) were collected by centrifugation and washed twice
96 with sterile saline before adjusting to obtain a 0.5 Mcfarland standard. MIC determination was
97 performed by adding 20 μ L of adjusted and diluted organisms (5×10^6 CFU/mL) to the wells
98 containing 20 μ L of a doubling dilution series of ertapenem (final concentration ranging from 0.
99 25 - 256 μ g/mL) and 160 μ L of Mueller-Hinton culture medium. The total volume in each well
100 was 200 μ L. The 96-well microtiter plate was then incubated for 20 h at 37°C. Wells without
101 bacteria and ertapenem were used as controls. The experiment was carried out in triplicate. The
102 lowest concentration showing no visible growth was noted as MIC value. The susceptibility
103 profile of ertapenem was interpreted in accordance with CLSI susceptibility breakpoint:
104 Susceptible ≤ 0.5 , intermediate = 1 and resistant > 1 μ g/mL (Clinical Laboratory Standards
105 Institute, 2014).

106 2.3 Disc preparation and experimental procedure

107 For the preparation of discs, nitrocefin discs (MAST Diagnostic group, UK) were impregnated
108 with and without 10 μ L of ertapenem (Sigma-Aldrich) at a concentration of 2 mg/mL to obtain

109 ertapenem final amount of 20 µg/disc. Following discs had been air-dried in a cabinet, they were
110 stored at -20°C. The discs were allowed to equilibrate to room temperature before use. To
111 screen for the presence of carbapenemases in Enterobacteriaceae, two nitrocefin discs (one with
112 and one without ertapenem) were directly placed on 18-h culture colonies grown on LB agar.
113 Following incubation at 37°C for 30 min, the result was visually observed and interpreted. The
114 development of a red color in the area of both discs with and without ertapenem was denoted as a
115 positive result for carbapenemase, while no color changes in both discs or color change only in
116 nitrocefin without ertapenem disc indicated no carbapenemase production.

117

118 **3. Results**

119 The present study demonstrates the use of a nitrocefin disc with ertapenem for rapid screening of
120 carbapenemase-producing isolates. The performance of the method was determined by a sum of
121 87 known molecular types of β-lactamase-producing Enterobacteriaceae. The distribution of
122 MICs of ertapenem among 31 carbapenemase producers and 56 non-carbapenemase producers
123 are presented in Fig. 1. In KPC producers, the MICs of ertapenem ranged from 16 to ≥ 256
124 µg/mL, while in MBL producers, MICs ranged from 8 to ≥ 256 µg/mL. These MIC values
125 indicated that all MBL and KPC producers were resistant to ertapenem. MICs of ertapenem
126 against OXA-48 producers ranged from 1 to 256 µg/mL. These values trended lower than those
127 MICs of KPC and MBL producers. The susceptibility profile of one OXA-48-E. coli was
128 intermediate. Furthermore, the majority of non-carbapenemase producers were susceptible to
129 ertapenem. Five AmpC-producing isolates were found in the intermediate range. The MIC of
130 ertapenem against a reference E. coli ATCC 25922 was ≤ 0.25 µg/mL which was classified as
131 susceptible.

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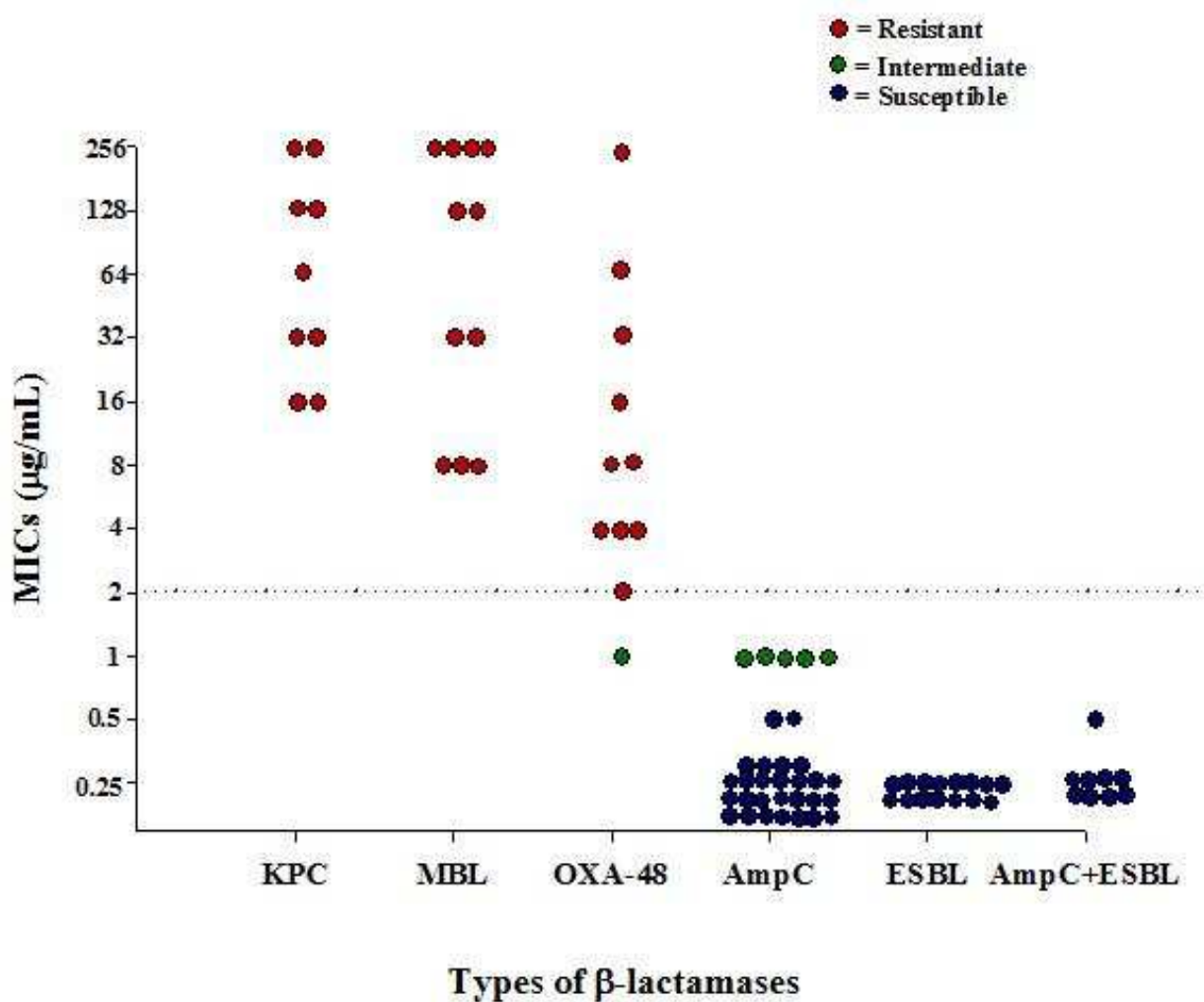
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147 **Fig. 1. The distribution of MIC values of ertapenem against distinct types of β -lactamase-**
 148 **producing Enterobacteriaceae.** Red dotted line indicates resistant breakpoint according to CLSI
 149 guidelines.

150

151 The use of nitrocefin discs with and without ertapenem successfully differentiated
152 carbapenemase producers from other types of β -lactamase producers within 30 min as illustrated
153 in Fig. 2. The study demonstrated that the color of both discs with and without ertapenem
154 developed from yellow to red in all 31 carbapenemase producers. In non-carbapenemase
155 producers, the color change from yellow to red was observed only in the area of an ertapenem-
156 free disc. No color change in both discs was seen in a control strain *E. coli* 25922. The sensitivity
157 and specificity of the method were 100%. Interestingly, the diagnostic method developed in this
158 study can detect the presence of OXA-48 carbapenemase enzyme in an ertapenem-intermediate
159 *E. coli*. It can also discriminate the absence of carbapenemase in ertapenem-intermediate isolates
160 harboring hyperexpressed AmpC β -lactamase. Furthermore, some of ESBL and AmpC-
161 producing strains used in the current study were also previously found to be hyperproduction of
162 ESBL and AmpC β -lactamases (Teethaisong et al., 2016 and 2017).

163 4. Discussion

164 The first carbapenemase detection assay was based on antibiotic susceptibility profile from disc
165 diffusion method and MIC determination. The interpretation relies on susceptibility breakpoint.
166 Ertapenem is thought to be a good indicator for the detection of carbapenemase-associated
167 resistance in Enterobacteriaceae as it usually shows higher MIC values compared with those of
168 imipenem and meropenem (Nordmann et al., 2012; Vading et al., 2011). Due to reduced
169 sensitivity and specificity, detection of carbapenemase producers based on susceptibility profile
170 is not frequently conducted (Nordmann and Poirel, 2013). Since then, various methods have been
171 developed and described. Carba NP, a biochemical test, is one of the most popular methods that
172 has been extensively used by many laboratories to detect the presence of carbapenemases in
173 Gram-negative bacteria. This test can provide the result in less than 2 h (Dortet et al., 2012).

174 However, the preparation for manual Carba NP is relatively difficult. In addition to Carba NP, a
175 β -CARBATM test showed good performance in detection of carbapenemases-producing isolates
176 with sensitivity 100% of KPC, 100% of IMP, 96.4% of VIM, 85.3% of NDM, 80.5% of OXA-48-
177 like carbapenemases. This test exhibited poor specificity for non-KPC Ambler class A and
178 OXA-48-like carbapenemases (Bernabeu et al., 2017). A method developed in the present study
179 is a simple promising method that successfully screen carbapenemase production in
180 Enterobacteriaceae within 30 min. It could be an alternative method for identifying
181 carbapenemase-mediated resistance in Enterobacteriaceae and other Gram-negative bacilli.

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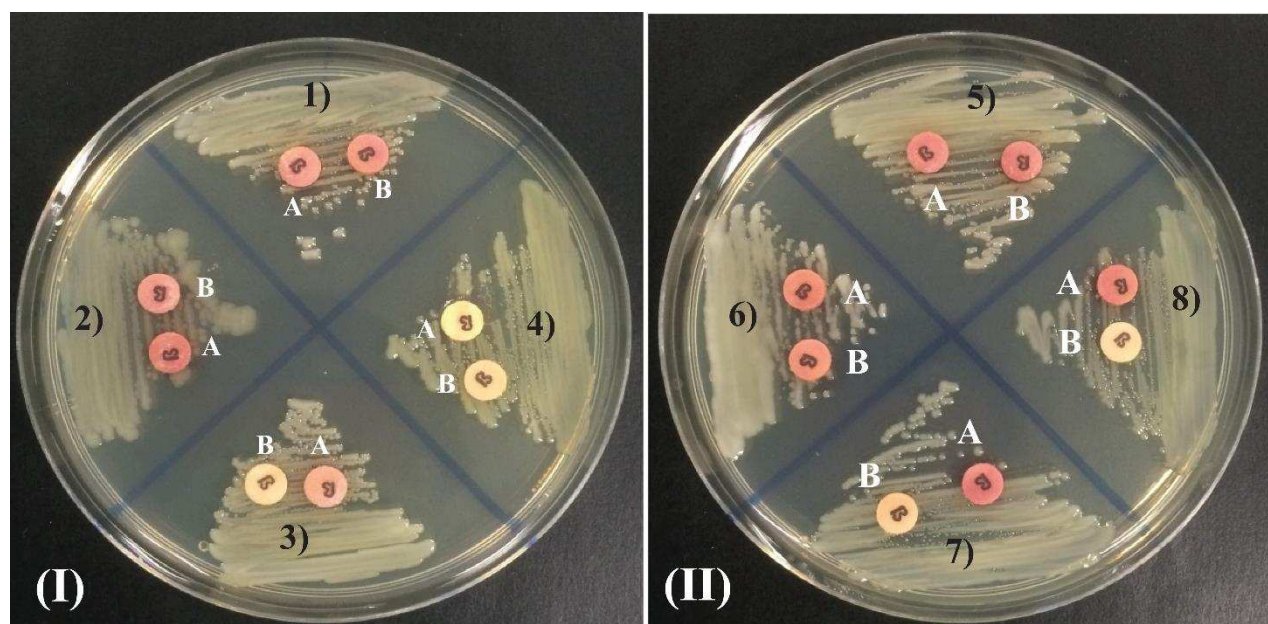
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194 **Fig. 2. Representative results from nitrocefin disc with and without ertapenem at 30 min of**
195 **incubation at 37°C.** A = nitrocefin disc alone; B = nitrocefin disc supplemented with ertapenem
196 (20 µg/disc). **(I)**, 1) = KPC-2-producing *K. pneumoniae* 4016; 2) = NDM-1-producing *E. coli*
197 4011; 3) = SHV-27 + TEM-53-producing *K. pneumoniae* 1012; 4) = a susceptible reference
198 strain *E. coli* ATCC 25922. **(II)**, 5) = IMP-1-producing *Klebsiella ozaenae* 403; 6) = OXA-48-
199 producing *K. pneumoniae* 4019; 7) = ACT-32-producing *E. cloacae* 2009; 8) = TEM-
200 214+SHV-12+ACT-32-*E. aerogenes* 3009.

201
202 The nitrocefin disc supplemented with ertapenem perfectly detected carbapenemase producers
203 with low ertapenem MIC values, particularly OXA-48-like-producing isolates. However, this
204 method needs to be further validated with carbapenem-susceptible isolates as well as with other
205 carbapenemase variants. It also requires larger sample sizes of carbapenemase-producing isolates
206 to confirm the robustness of this assay. The performance of the assay should be investigated with
207 other carbapenemase-producing Gram-negative bacteria such as *Pseudomonas* spp. and
208 *Acinetobacter* spp. Subjectivity in interpretation of the results is not only the common issue of
209 this method, but it is also a problem of all other chromogenic methods (Tijet et al., 2013).

210 In conclusion, the nitrocefin with ertapenem disc is very simple to perform and provides rapid
211 results to identify carbapenemase producers, with high sensitivity and specificity. This assay can
212 be carried out routinely in any microbiological laboratory. It could facilitate successful treatment
213 of carbapenemase-mediated resistance and indicate the isolates to be further evaluated by PCR or
214 sequencing for molecular epidemiological surveillance. This method is also potentially important
215 for follow-up investigations.

216

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223 **References**

224 Bernabeu, S, Dortet, L, & Naas, T (2017) Evaluation of the beta-CARBA test, a colorimetric test for the
225 rapid detection of carbapenemase activity in Gram-negative bacilli. *J Antimicrob Chemother*, 72: 1646-
226 1658. 10.1093/jac/dkx061 Bush, K, & Fisher, JF (2011) Epidemiological expansion, structural
227 studies, and clinical challenges of new beta-lactamases from Gram-negative bacteria. *Annu Rev*
228 *Microbiol*, 65: 455-478. 10.1146/annurev-micro-090110-102911

229

230 Clinical Laboratory Standards Institute (2012) Method for dilution antimicrobial susceptibility
231 tests for bacteria that grow aerobically : CLSI document M7-A7; Ninth Edition. CLSI,
232 Wayne,PA

233 Clinical Laboratory Standards Institute (2014) Performance standards for antimicrobial
234 susceptibility testing; Twenty-fourth informational supplement update : CLSI document
235 M100-S24. CLSI, Wayne,PA

236 Dortet, L, Poirel, L, & Nordmann, P (2012) Rapid detection of carbapenemase-producing
237 *Pseudomonas* spp. *J Clin Microbiol*, 50: 3773-3776. 10.1128/jcm.01597-12

- 238 Ito, H, Arakawa, Y, Ohsuka, S, Wacharotayankun, R, Kato, N, & Ohta, M (1995) Plasmid-
239 mediated dissemination of the metallo-beta-lactamase gene bla_{IMP} among clinically
240 isolated strains of *Serratia marcescens*. *Antimicrob Agents Chemother*, 39: 824-829.
- 241 Jacoby, GA (2009) AmpC beta-lactamases. *Clin Microbiol Rev*, 22: 161-182, Table of Contents.
242 10.1128/cmr.00036-08
- 243 Kaase, M, Lenga, S, Friedrich, S, Szabados, F, Sakinc, T, Kleine, B, & Gatermann, SG (2008)
244 Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*.
245 *Clin Microbiol Infec*, 14: 614-616. <https://doi.org/10.1111/j.1469-0691.2008.01997.x>
- 246 Lee, C-S, & Doi, Y (2014) Therapy of infections due to carbapenem-resistant Gram-
247 negative pathogens. *Infect Chemother*, 46: 149-164. 10.3947/ic.2014.46.3.149
- 248 Logan, LK, & Weinstein, RA (2017) The Epidemiology of carbapenem-resistant
249 Enterobacteriaceae: The impact and evolution of a global menace. *J Infect Dis*, 215: S28-
250 s36. 10.1093/infdis/jiw282
- 251 Naas, T, & Nordmann, P (1994) Analysis of a carbapenem-hydrolyzing class A beta-lactamase
252 from *Enterobacter cloacae* and of its LysR-type regulatory protein. *Proc Natl Acad Sci U*
253 *S A*, 91: 7693-7697.
- 254 Nordmann, P (2014) Carbapenemase-producing Enterobacteriaceae: overview of a major public
255 health challenge. *Med Mal Infect*, 44: 51-56. 10.1016/j.medmal.2013.11.007
- 256 Nordmann, P, Gniadkowski, M, Giske, CG, Poirel, L, Woodford, N, & Miriagou, V (2012)
257 Identification and screening of carbapenemase-producing Enterobacteriaceae. *Clin*
258 *Microbiol Infect*, 18: 432-438. <http://dx.doi.org/10.1111/j.1469-0691.2012.03815.x>
- 259 Nordmann, P, & Poirel, L (2013) Strategies for identification of carbapenemase-producing
260 Enterobacteriaceae. *J Antimicrob Chemother*, 68: 487-489. 10.1093/jac/dks426

- 261 Osei Sekyere, J, Govinden, U, & Essack, SY (2015) Review of established and innovative
262 detection methods for carbapenemase-producing Gram-negative bacteria. *J Appl*
263 *Microbiol*, 119: 1219-1233. 10.1111/jam.12918
- 264 Perez, F, Endimiani, A, Hujer, KM, & Bonomo, RA (2007) The continuing challenge of ESBLs.
265 *Curr Opin Pharmacol*, 7: 459-469. 10.1016/j.coph.2007.08.003
- 266 Poirel, L, Heritier, C, Tolun, V, & Nordmann, P (2004) Emergence of oxacillinase-mediated
267 resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, 48: 15-
268 22.
- 269 Smith Moland, E, Hanson, ND, Herrera, VL, Black, JA, Lockhart, TJ, Hossain, A, Johnson, JA,
270 Goering, RV, & Thomson, KS (2003) Plasmid-mediated, carbapenem-hydrolysing beta-
271 lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. *J Antimicrob Chemother*, 51: 711-
272 714.
- 273 Tamma, PD, Opene, BN, Gluck, A, Chambers, KK, Carroll, KC, & Simner, PJ (2017)
274 Comparison of 11 phenotypic assays for accurate detection of carbapenemase-producing
275 *Enterobacteriaceae*. *J Clin Microbiol*, 55: 1046-1055. 10.1128/jcm.02338-16
- 276 Teethaisong, Y, Eumkeb, G, Nakouti, I, Evans, K, & Hobbs, G (2016) A combined disc method
277 with resazurin agar plate assay for early phenotypic screening of KPC, MBL and OXA-
278 48 carbapenemases among *Enterobacteriaceae*. *J Appl Microbiol*, 121: 408-414.
279 10.1111/jam.13196
- 280 Teethaisong, Y, Evans, K, Nakouti, I, Tiomyom, K, Ketudat-Cairns, JR, Hobbs, G, & Eumkeb,
281 G (2017) The performance of a resazurin chromogenic agar plate with a combined disc
282 method for rapid screening of extended-spectrum-beta-lactamases, AmpC beta-

283 lactamases and co-beta-lactamases in Enterobacteriaceae. *Microbiol Immunol*, 61: 297-
 284 304. 10.1111/1348-0421.12499

285 Thomson, KS (2010) Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. *J*
 286 *Clin Microbiol*, 48: 1019-1025. 10.1128/jcm.00219-10

287 Tijet, N, Boyd, D, Patel, SN, Mulvey, MR, & Melano, RG (2013) Evaluation of the Carba NP
 288 Test for Rapid Detection of Carbapenemase-Producing Enterobacteriaceae and
 289 *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 57: 4578-4580.
 290 10.1128/AAC.00878-13

291 Vading, M, Samuelsen, O, Haldorsen, B, Sundsfjord, AS, & Giske, CG (2011) Comparison of
 292 disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella*
 293 *pneumoniae* with the EUCAST and CLSI breakpoint systems. *Clin Microbiol Infect*, 17:
 294 668-674. 10.1111/j.1469-0691.2010.03299.x

295 Yigit, H, Queenan, AM, Anderson, GJ, Domenech-Sanchez, A, Biddle, JW, Steward, CD,
 296 Alberti, S, Bush, K, & Tenover, FC (2001) Novel carbapenem-hydrolyzing beta-
 297 lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*.
 298 *Antimicrob Agents Chemother*, 45: 1151-1161. 10.1128/aac.45.4.1151-1161.2001

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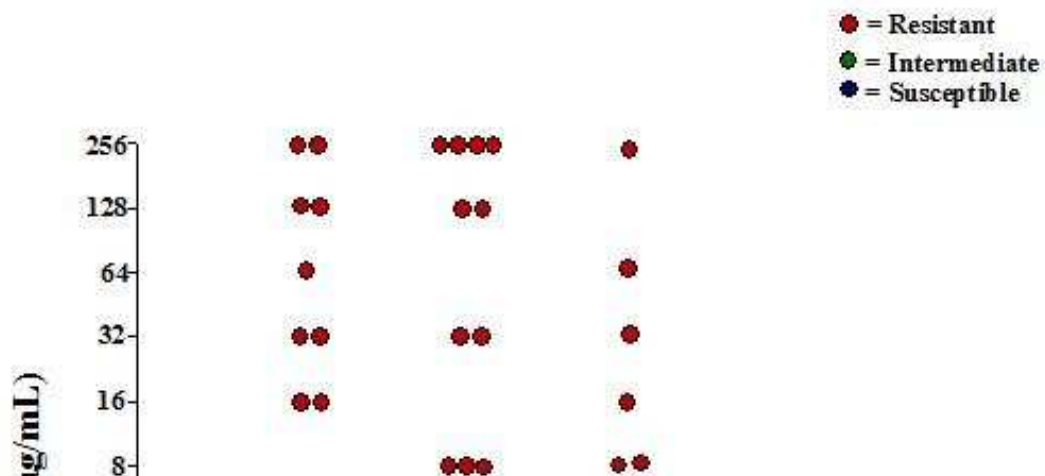
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312 **Fig. 1. The distribution of MIC values of ertapenem against distinct types of β -lactamase-**
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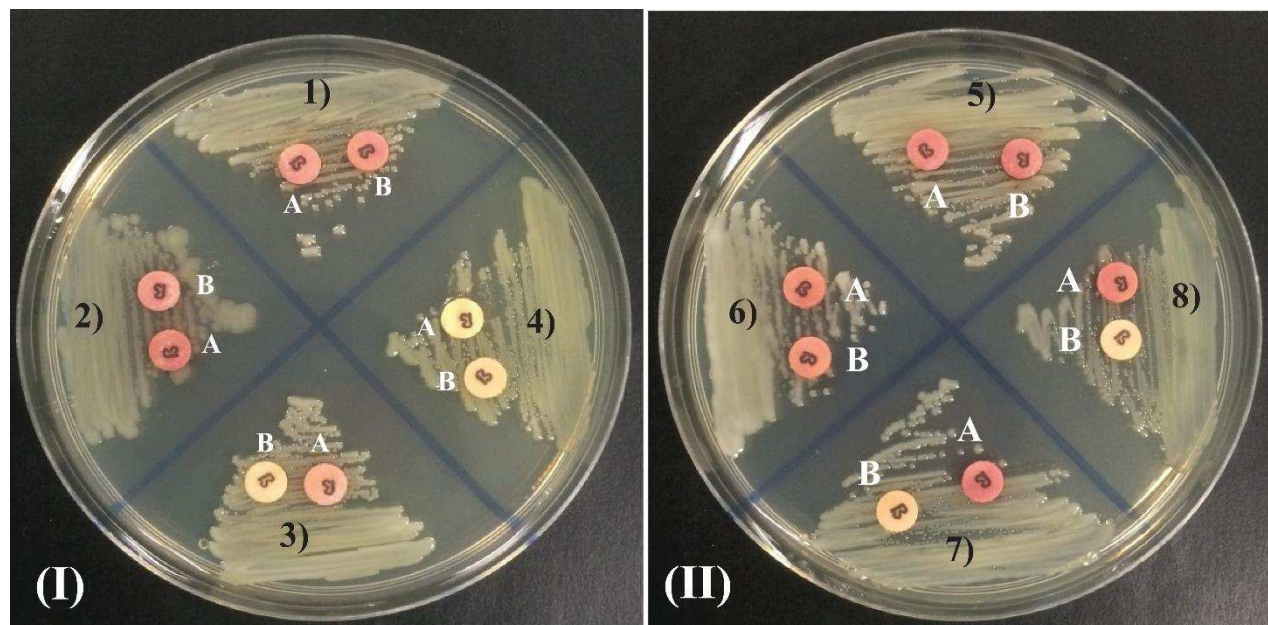
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333 214+SHV-12+ACT-32-*E. aerogenes* 3009.

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337 **Table 1**338 **Summary of carbapenemase-producing and non-carbapenemase-producing Enterobacteriaceae used in this study.**

Species	Carbapenemase producers (n = 31)					Non-carbapenemase producers (n = 56)		
	Ambler class A	Ambler class B MBL		Ambler class D	Ambler class A	Ambler class C	ESBL+AmpC	
	KPC	NDM	VIM	IMP	OXA-48 like	ESBL	AmpC	
<i>E. coli</i>	2	1	-	-	4	7	10	1
<i>K pneumoniae</i>	6	3	4*	-	5	8	3	-
<i>E. aerogenes</i>	-	-	-	-	-	-	7	5
<i>E. cloacae</i>	-	2	-	-	2	-	8	1
<i>M. morgani</i>	-	-	-	-	-	-	2	-
<i>C. freundii</i>	-	-	-	-	-	-	2	2
<i>K oxytoca</i>	1	-	-	-	-	-	-	-
<i>K ozaenae</i>	-	-	-	1	-	-	-	-
Total	9		11		11	15	32	9

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340 * One VIM-1 + SHV-12 and one VIM-1 + SHV-102 co-expressions

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342