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Note

Comparison of the in-house made Carba-NP and Blue-Carba tests: Considerations for better detection of carbapenemase-producing Enterobacteriaceae

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ABSTRACT

The in-house Carba-NP and Blue-Carba tests were compared using 30 carbapenemase- and 33 non-producing Enterobacteriaceae. Tests were read by three operators. 100% sensitivity was reported for both tests, but Carba-NP was slightly more specific than Blue-Carba (98.9% vs. 91.7%). We describe potential sources of error during tests' preparation and reading.

The continuous worldwide expansion of carbapenemase-producing Enterobacteriaceae (CPE) is a serious concern as infections caused by these pathogens have an increased mortality, morbidity, and associated health-care costs (Tängdén and Giske, 2015). Treatment options for CPE infections are often limited, since these organisms usually co-carry resistant determinants to other classes of antibiotics (Tängdén and Giske, 2015). Moreover, the heterogeneity of carbapenemase classes and types leads to a multiplicity of diverse carbapenem hydrolytic efficiencies and resistance phenotypes (Hrabák et al., 2014; Tängdén and Giske, 2015). Since carbapenem resistance mediated by carbapenemase production is continuously rising in Enterobacteriaceae, rapid, inexpensive, and reliable methods are urgently needed to identify CPE (Dortet et al., 2014a, 2014b).

Carba-NP and Blue-Carba are recent quick biochemical methods that detect carbapenemase activity when the enzyme breaks imipenem's β-lactam ring, leading to a pH decrease and consequent color shift of the pH-indicator in solution (Nordmann et al., 2012; Pires et al., 2013). Both methods proved to be fast (detection observed ≤2 h), highly sensitive, specific and very cheap. Further studies have evaluated both tests, emphasizing their reproducibility, high sensitivity and specificity (Pasteran et al., 2015a, 2015b, Vasoo et al., 2013). However, others have questioned the utility of these methodologies (Tijet et al., 2013). Moreover, studies comparing the performance of the two tests are still scarce and those evaluating the impact of operators' experience in reading and interpreting results are lacking.

Since commercial tests have just been launched into the market (Novais et al., 2015; Poirel and Nordmann, 2015), we aim to compare the in-house made Carba-NP and Blue-Carba tests using a characterized collection of carbapenemase-producing and non-producing Enterobacteriaceae in order to further identify potential sources of error.

Sixty-one previously characterized Enterobacteriaceae from different sources and countries (CPE, n = 30, including 9 NDM, 10 OXA-48, 5 KPC, 3 NDM plus OXA-48, 2 VIM, and 1 IMP producers; non-CPE, n = 33) recovered from cation adjusted Mueller–Hinton agar (Becton–Dickinson) were tested using Carba-NP and Blue-Carba, as previously described (Nordmann et al., 2012; Pires et al., 2013). Both assays were executed in parallel two times each in non-consecutive days. Tests were performed and read by two different operators with previous experience in both assays (OP1 and OP2); a third operator (OP3) with no

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Table 1
Results obtained for the Carba NP and Blue-Carba tests performed using a collection of well-characterized strains (30 CPE and 33 non-CPE).

Acquired β-lactamases	Species (no. of strains with the same assay results)	Carba NP test						Blue-Carba test						MIC (μg/ml)			Reference or ATCC strain
		Assay 1			Assay 2			Assay 1			Assay 2						
		OP1	OP2	OP3	OP1	OP2	OP3	OP1	OP2	OP3	OP1	OP2	OP3	IMP	ERT	MEM	
Carbapenemase producers[a]																	
Class A (n = 5)																	
KPC-2	*K. pneumoniae* (n = 3)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	≥8	≥64	≥64	This study
	K. pneumoniae (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	1	16	4	This Study
	K. pneumoniae (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	1	8	2	ATCC BAA-1705
Class B (n = 11)																	
IMP-1	*K. pneumoniae* (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	16	64	32	This Study
NDM-1	*K. pneumoniae* (n = 5)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	≥1	≥4	≥2	This Study, Principe et al. (2015)
	K. pneumoniae (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	++	≥64	≥64	≥64	This Study
	E. coli (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	16	≥64	≥64	This study
	E. coli (n = 1)	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++	8	≥64	≥64	ATCC BAA-2452
	E. cloacae (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	16	64	64	This Study
VIM-1	*K. pneumoniae* (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	8	0.5	1	This Study
VIM-2	*K. pneumoniae* (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	64	≥64	≥64	This Study
Class D (n = 10)																	
OXA-48	*K. pneumoniae* (n = 1)	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	4	32	16	This Study
	K. pneumoniae (n = 1)	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	++	4	64	4	This Study
	K. pneumoniae (n = 1)	+	++	+	++	+	++	++	++	+++	++	+++	++	4	≥8	≥16	This study
	K. pneumoniae (= 1)	+++	+++	++	+++	+++	++	+++	+++	+++	+++	++	+++	4	≥8	2	This Study
	K. pneumoniae (= 1)	++	++	+	++	++	+	+++	+++	+++	++	++	++	0.5	0.5	≤0.5	This Study
	K. pneumoniae (= 1)	++	++	++	+	++	+	++	+++	++	+++	+++	++	8	≥8	2	Giani et al. (2014)
	K. pneumoniae (= 1)	++	++	++	++	+++	++	++	++	++	++	++	++	4	≥8	2	Giani et al. (2014)
	E. coli (n = 1)	+++	+++	++	+++	+++	++	+++	+++	+++	++	++	++	0.5	4	4	This Study
	E.coli (n = 1)	+	+	+	++	++	++	++	++	++	+++	+++	+++	1	4	1	This Study
	Salmonella Kentucky (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	≤0.25	1	≤0.5	Seiffert et al. (2014)
Class B + class D (n = 3)																	
NDM-1 + OXA-48	*C. freundii* (n = 1)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	4	≥8	2	This Study
	K. pneumoniae (n = 2)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	≥4	≥4	≥16	This Study