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Evaluation of a simple phenotypic method for the detection of carbapenemase-producing Enterobacteriaceae

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ABSTRACT

We investigated the performance of a phenotypic test, the Carbapenemase Detection Set (MAST-CDS), for the identification of carbapenemase-producing Enterobacteriaceae. Our results indicated that MAST-CDS is rapid, easily performed, simple to interpret, and highly sensitive for the identification of carbapenemase producers, particularly imipenemase producers.

1. Introduction

Resistance to carbapenems in Gram-negative bacteria, including Enterobacteriaceae, is an increasingly serious problem globally, since the clinical utility of these antimicrobials is compromised (Hawkey and Jones, 2009). This resistance is facilitated by carbapenemases, such as Amber class A (*Klebsiella pneumoniae* carbapenemase [KPC] enzymes), class B (metallo β-lactamases [MBLs]; imipenemase [IMP], Verona integron-encoded MBL [VIM], and New Delhi MBL [NDM] enzymes), and class D (oxacillinase [OXA]-48-like enzymes) β-lactamases. The genes encoding these enzymes are mainly carried on transferable plasmids that also harbor resistance genes against various antibiotics, including quinolone and aminoglycosides (Koyano et al., 2013). Therefore, it is crucial for infection control procedures and epidemiological investigations that carbapenemase-producing Enterobacteriaceae be identified accurately.

To date, some phenotypic confirmation methods have been described for the identification of carbapenemase-producing Enterobacteriaceae, such as *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., and *Serratia* spp. (Birgy et al., 2012; Tsakris et al., 2010; van Dijk et al., 2014). Recently, the Carbapenemase Detection Set (MAST-CDS; Mast Group, Merseyside, UK), which is based on the hydrolysis of carbapenemases with inhibitors against MBL, KPC, and AmpC enzymatic activity, has become commercially available. Although its performance has been evaluated by Doyle et al. (2012), this test has been shown to be only 40% sensitive for IMP producers. Moreover, IMP producers have been reported more frequently in southern Europe and Asia and are the predominant MBLs in Japan (Ito et al., 1995; Nordmann et al., 2011); the additional data derived from studies of a large number of IMP producers have increased the usefulness of this test.

We here investigated the performance of the commercially available phenotypic test MAST-CDS for the identification and presumptive characterization of carbapenemase-producing Enterobacteriaceae, including a large number of IMP producers, and compared its performance with that of other phenotypic confirmation tests, viz., the modified Hodge test (MHT) for carbapenemases, the combination test using dipicolinic

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acid (CT-DPA) for MBLs, and the combination test using clavulanic acid (CT-CVA) for extended-spectrum β-lactamases (ESBLs).

2. Materials and methods

2.1. Bacterial strains

A total of 38 carbapenemase-producing Enterobacteriaceae, including 2 reference strains, viz., *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-2146, which had been already confirmed to harbor the genotypes bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and $bla_{OXA-48-like}$ by previously published methods (Dallenne et al., 2010; Poirel et al., 2004; Saito et al., 2014), was used in this study. The test strains included 2 KPC producers (2 *K. pneumoniae*), 29 IMP producers (6 *Citrobacter freundii*, 20 *Enterobacter cloacae*, 1 *Klebsiella oxytoca* and 2 *Serratia marcescens*), 4 NDM producers (2 *Escherichia coli* and 2 *K. pneumoniae*), and 3 OXA-48-like producers (1 *E. coli* and 2 *K. pneumoniae*); these are listed in Table 1. Furthermore, 36 carbapenemase-non-producing Enterobacteriaceae (9 ESBL producers, 20 ampicillinase C [AmpC] producers, and 7 β-lactamases non-producers), which had been confirmed phenotypically using the AmpC and ESBL Detection Set (Mast Group), were also included (Table 1).

2.2. Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) of imipenem, meropenem, ceftazidime, and cefotaxime was determined by broth microdilution methods, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011).

2.3. Phenotypic β-lactamase testing

MAST-CDS was performed according to manufacturer's instructions. Briefly, disk A (meropenem 10 μg), disk B (meropenem 10 μg and dipicolinic acid 1000 μg as a MBL inhibitor), disk C (meropenem 10 μg and aminophenylboronic acid 600 μg as a KPC inhibitor), and disk D (meropenem 10 μg and cloxacillin 750 μg as an AmpC inhibitor) were placed on Mueller-Hinton agar plates on which the test strains had been inoculated. Plates were incubated at 37 °C for 24 h, after which the zone difference, ≥5 mm for disk B and ≥4 mm for disk C, as compared to disk A, was interpreted as indicating MBL- and KPC-producing bacteria, respectively. Moreover, a zone difference of both ≥4 mm for disk C and ≥5 mm for disk D, as compared to disk A, was interpreted as indicating AmpC producers with porin loss. MAST-CDS is not designed to identify OXA-48-like producers.

MHT was performed for the confirmation of carbapenemase producers as recommended in the CLSI guidelines (CLSI, 2011). CT-DPA was performed with broth microdilution methods, using imipenem and dipicolinic acid (400 μg/mL), an MBL inhibitor; a ≥8-fold decrease in the MIC caused by the presence of the inhibitor was taken as indicating MBL-positive bacteria. For confirmation of ESBL producers, CT-CVA was performed as recommended in the CLSI guidelines (CLSI, 2011). The AmpC and ESBL Detection Set (Mast Group) was also implemented according to manufacturer's instructions to identify ESBL and AmpC producers phenotypically.

3. Results

Among the carbapenemase producers investigated in this study, the MIC ranges of imipenem and meropenem of IMP producers were extremely broad (from 2 to >8 mg/L; Table 1). Moreover, although OXA-48-like producers showed low-level resistance to imipenem and meropenem (both 2 mg/L), KPC and NDM producers demonstrated high-level resistance to meropenem (MIC >8 mg/L) in particular. Of the 36 carbapenemase non-producers, 56% (5/9) of ESBL producers, 15% (3/20) of AmpC producers, and 86% (6/7) of carbapenemase, ESBL, and AmpC non-producers were not susceptible to imipenem (MICs of 2–4 mg/L), while all these strains were susceptible to meropenem (Table 1).

Table 1
Distribution of carbapenem MICs for carbapenemase-producing and non-producing Enterobacteriaceae.

β-lactamase type (no.)[a]	Species	Carbapenemase	MIC (mg/L) and no. of isolates									
			Imipenem					Meropenem				
			≤1	2	4	8	>8	≤1	2	4	8	>8
KPC (2)	*Klebsiella pneumoniae*	KPC-2					2					2
IMP (29)	*Citrobacter freundii*	IMP-1		1	2	2	1			2	1	3
	Enterobacter cloacae	IMP-1		8	8	2	2		4	7	5	4
	Klebsiella oxytoca	IMP-1		1						1		
	Serratia marcescens	IMP-1					1					1
	Serratia marcescens	IMP-11			1						1	
NDM (4)	*Escherichia coli*	NDM-1				1	1					2
	Klebsiella pneumoniae	NDM-1				1	1					2
OXA-48 (3)	*Escherichia coli*	OXA-48-like		1					1			
	Klebsiella pneumoniae	OXA-48-like		2					2			
ESBL (9)	*Escherichia coli*	ND[b]	3					3				
	Klebsiella pneumoniae	ND	1					1				
	Proteus mirabilis	ND		4	1			5				
AmpC (20)	*Citrobacter freundii*	ND	5					5				
	Escherichia coli	ND	10					10				
	Klebsiella pneumoniae	ND	1					1				
	Morganella morganii	ND		2				2				
	Providencia stuartii	ND		1				1				
	Serratia marcescens	ND	1					1				
Carbapenemase, ESBL and AmpC negative (7)	*Morganella morganii*	ND		1				1				
	Proteus mirabilis	ND		3	1			4				
	Proteus penneri	ND	1					1				
	Proteus vulgaris	ND		1				1				

[a] KPC, *Klebsiella pneumoniae* carbapenemase; IMP, imipenemase; NDM, New Delhi metallo β-lactamase; OXA, oxacillinase; ESBL, extended-spectrum β-lactamases; AmpC, ampicillinase C.
[b] ND, not detected.