Supplementary Material

Hofko M et al., Detection of carbapenemases by real-time PCR and melt-curve analysis on the BD MAX™ System

Supplementary Material and Methods

Characterization of isolates by the German National Reference Laboratory

Laboratories in Germany were asked to send isolates fulfilling the following criteria to the German National Reference Laboratory (NRL) for multidrug-resistant bacteria (Department of Medical Microbiology, Ruhr-University Bochum): (a) Enterobacteriaceae with elevated MICs for ertapenem (MIC≥0.5 mg/l), meropenem or imipenem (MIC≥2 mg/l) or decreased inhibition zone diameters (≤22 mm for imipenem or meropenem or ≤25 mm for ertapenem) with the exception of *Proteus* spp., *Morganella morganii* and *Providencia* spp. with elevated MIC or decreased inhibition zone solely for imipenem and a meropenem MICs of ≤0.25 mg/l. (b) *Pseudomonas aeruginosa* with resistance to imipenem, meropenem and ceftazidime. (c) *Acinetobacter baumannii* with resistance to imipenem and meropenem. Several phenotypic tests were performed routinely: the modified Hodge Test (1), the combined disk test with EDTA (2) and the combined disk test with boronic acid (3, 4) (Enterobacteriaceae only). In addition, the following PCRs followed by sequencing were performed routinely: bla_{KPC} (5) (Enterobacteriaceae and *P. aeruginosa* only), bla_{GES} (6) (*P. aeruginosa* only), bla_{OXA-48} (7) (Enterobacteriaceae only), $bla_{OXA-23/-24/-58}$ (8) (*Acinetobacter* spp. only), bla_{VIM} (2), bla_{IMP} (2) and bla_{NDM} (9). If the phenotypic tests suggested the presence of a carbapenemase further PCRs for rarely occurring carbapenemases were performed. If negative, a microbiological bioassay was performed similar as described previously (10).

Collections of isolates

Two different collections from the National Reference laboratory were investigated in our study. (i), 129 isolates were selected. 89 had defined carbapenemase resistance genes, 40 were negative for carbapenemases. The positive strains comprised the following carbapenemase genes (details in Table S2): bla_{KPC} type (n=12), bla_{GES} type (n=4), bla_{NDM} type (n=12), bla_{VIM} type (n=25), bla_{IMP} type (n=12), bla_{OXA-48} type (n=15), bla_{OXA-23} type (n=6) and other bla_{OXA} type (n=6), with 3 of 89 strains carrying two carbapenemase genes. 40 strains

had decreased carbapenem susceptibility due to non-carbapenemase based mechanisms such as ESBL, AmpC and/or decreased membrane permeability. (ii), In a second collection a total of 152 isolates sent to the NRL for analysis of decreased carbapenem susceptibility in an entire half-month period (Jan, 21st to Feb, 6th 2013) were analyzed in a blinded study setup. Isolates were tested at the NRL as described above and in parallel analyzed with the here described assay. The blinding was lifted only after completion of the investigation, compared to the NRL data and discrepant results were analyzed on new isolates once. 65/152 (42.8%) of the isolates harbored carbapenemase genes.

Table S1

Primers for the detection of carbapenemase resistance genes

target	primer	sequence (5'-3')	amplicon size [bp]	reference
16S rRNA	16S-fw	TGG AGC ATG TGG TTT AAT TCG A	159	*In-house*
	16S-rv	TGC GGG ACT TAA CCC AAC A		
KPC	KPC-fw	ATG TCA CTG TAT CGC CGT CT	893	(11)
	KPC-rv	TTT TCA GAG CCT TAC TGC CC		
GES	GES-C	GTT TTG CAA TGT GCT CAA CG	371	(12)
	GES-D	TGC CAT AGC AAT AGG CGT AG		
IMP-1	IMP-1F	TGA GCA AGT TAT CTG TAT TC	740	(13)
	IMP-1R	TTA GTT GCT TGG TTT TGA TG		
IMP-2	IMP-2F	GGC AGT CGC CCT AAA ACA AA	737	(13)
	IMP-2R	TAG TTA CTT GGC TGT GAT GG		
VIM-1	VIM-1F	TTA TGG AGC AGC AAC CGA TGT	920	(13)
	VIM-1R	CAA AAG TCC CGC TCC AAC GA		
VIM-2	VIM-2F	AAA GTT ATG CCG CAC TCA CC	865	(13)
	VIM-2R	TGC AAC TTC ATG TTA TGC CG		
OXA-48(like)	OXA-48A	TTG GTG GCA TCG ATT ATC GG	744	(7)
	OXA-48B	GAG CAC TTC TTT TGT GAT GGC		
NDM	NDM-A	TCG ATC CCA ACG GTG ATA TT	287	*In-house*
	NDM-B	TGG ATC AAG CAG GAG ATC AA		
OXA-23(like)	OXA-23f	GAT CGG ATT GGA GAA CCA GA	501	(8)
	OXA-23r	ATT TCT GAC CGC ATT TCC AT		

Table S2

Assay performance for detection of different carbapenemases on a reference collection

carbapene-mase type	β-lactamase NRL result	No.	Species	melt temp. (Tm) in mastermix (1) or (2)	BD MAX result	comparison to NRL[1]	
						con-cordant	dis-cordant
KPC-type	KPC-2	5	klpn(3), esco(1), klox(1)	92.8-93.5 (1)	KPC	5	
	KPC-3	5	klpn(3), esco(1), klox(1)	92.2-93.6 (1)	KPC	5	
GES-type	GES-1	1	psae	89.3 (1)	GES	1	
	GES-5	1	psae	89.5 (1)	GES	1	
	GES-11	1	acba	89.8 (1)	GES	1	
	GES-20	1	psae	89.5 (1)	GES	1	
NDM-type	NDM-1/-6	9	klpn(3), esco(3), encl(1), prmi(1), acba(1)	92.6-94.1 (2)	NDM	9	
	NDM-2	1	acba	92.6 (2)	NDM	1	
	NDM-3	1	esco	92.3 (2)	NDM	1	
VIM-type	VIM-1	11	klpn(2), klox(2), sema(1), esco(1), cifr(1), encl(3), psae(1)	90.5-92.2 (2)	VIM-1	11	
	VIM-2	8	klpn(1), psae(6), klox(1)	90.5-92.2 (1)	VIM-2	8	
	VIM-4	1	encl	90.4 (2)	VIM-1	1	
	VIM-5	1	psae	90.5 (2)	VIM-1	1	
	VIM-19	1	klpn	90.1 (2)	VIM-1	1	
	VIM-28	1	psae	90.8 (2)	VIM-1	1	
IMP-type	IMP-1	1	psae	84.7 (1)	IMP-1		
	IMP-2/-19	1	psae	84.9 (1)	IMP-2	1	
	IMP-4/-28	1	encl				1
	IMP-7	2	psae(2)				2
	IMP-8/-24	3	psae(3)				3
	IMP-13	1	sema				1
	IMP-14	1	encl				1
	IMP-15	1	psae	84.4 (1)	IMP-2	1	
	IMP-16	1	psae	85.3 (1)	IMP-2	1	
OXA-type	OXA-48	14	esco(2), klpn(7), enae(1), cifr(1), sema(1), klox(1), encl(1)	86.2-88.8 (2)	OXA-48	14	
	OXA-162	3	cifr(1), esco(1), klpn(1)	86.2-86.3 (2)	OXA-48	3	
	OXA-181	2	esco(1),klpn(1)	86.7; 87.6 (2)	OXA-48	2	
	OXA-204	1	klpn	86.5 (2)	OXA-48	1	
	OXA-23	6	acba(6)	84.2-85.0 (2)	OXA-23	6	
multiple	KPC-2, VIM-1	1	klpn	92.9 (1); 90.4 (2)	KPC, VIM-1	1	
	KPC-2, VIM-26	1	klpn	93.1 (1); 90.2 (2)	KPC, VIM-1	1	
	NDM-1/-6, OXA-48	1	klpn	92.4 (2); 86.1 (2)	NDM, OXA-48	1	
Total (positive strains)		**89**				**81** (91.0%)	**8** (9.0%)
No carba-penemase	negative	40			negative	40	0

acba, *Acinetobacter baumanii*; cifr, *Citrobacter freundii* enae, *Enterobacter aerogenes*; encl, *Enterobacter cloacae*; esco, *Escherichia coli*; klox, *Klebsiella oxytoca*; klpn, *Klebsiella pneumoniae*; prmi, *Proteus mirabilis*; psae, *Pseudomonas aeruginosa*; sema, *Serratia marcescens*

[1] 9 strains had to be tested twice due to mix-up or contamination.

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