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Comparative evaluation of selective agar media, MALDI-TOF MS, PCR and phenotypic methods for the detection of Carbapenemase-producing Enterobacteriaceae.

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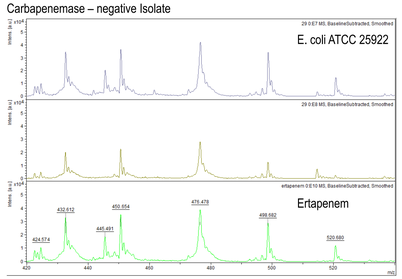
OBJECTIVES

Three selective agar media, Brilliance CRE (BCR) (Oxoid) Chromagar KPC (CKP) (MAST) and chromID CARBA agar (CIC) (bioMérieux), a MALDI-TOF MS Carbapenemase-detection method, the modified Hodge test (CDC; www.cdc.gov) and the VITEK 2 (bioMérieux) were evaluated for the detection of Carbapenem-resistant *Enterobacteriaceae*. Results were verified by Etest and a gel-based PCR with Carbapenemase specific primers.

Carbapenemase – negative Isolate



Carbapenemase – positive Isolate

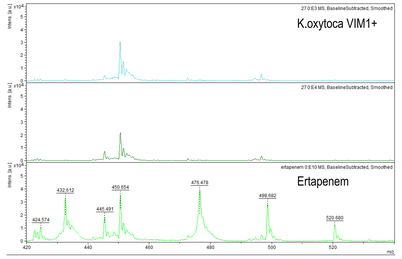


Figure 4: MALDI-TOF MS Carbapenemase-Detection

Figure 1: Brilliance CRE

METHODS

In total 31 strains with defined Carbapenemase resistance mechanisms were investigated. These included isolates producing OXA-48 (n=11), NDM-1 (n=7), KPC (n=7), VIM (n=3)- Carbapenemases and with porin-loss (n=3). Additionally 15 carbapenemase-negative *Enterobacteriaceae* with other resistance mechanisms (ESBL, ampC ...) were included as negative control. Species tested were *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Provicencia rettgeri, Enterobacter* species. The Agar plates were inoculated with a 1:10000 dilution of a 0.5 McFarland suspension and incubated for 24-48 hours at 35+-2°C (Figures 1-3). The modified Hodge test and the MALDI-TOF Carbapenemase detection method were performed according to literature (1, 2) (Figure 4). Etest and VITEK 2 were performed according to manufacturer´s instructions. For Carbapenemase-PCR, the Qiagen Hot Start Master Mix (Qiagen) was used.

Table1: Comparative results of different Carbapenemase detection methods

Study Nr	Spezies	Resistance-mechanism	Brilliance CRE	Chromagar KPC	chromID Carba	Etest IPM	Etest MEM	Etest ERT	Etest DOR	VITEK 2	Maldi	Hodge
LL5	E. coli	KPC	+	+	+	4	1	3	1	R	+	+
LL31	E. coli	KPC	+	+	+	12	32	32	4	I	0	0
LL7	K. pneumoniae	KPC	+	+	+	3	4	32	2	R	+	+
LL28	K. pneumoniae	KPC	+	+	+	32	6	8	3	I	+	+
LL29	K. pneumoniae	KPC	+	+	+	32	32	32	32	R	+	+
LL30	K. pneumoniae	KPC	+	+	+	32	8	16	4	R	+	+
LL36	K. pneumoniae	KPC	+	+	+	32	32	32	4	I	+	+
LL13	E. cloacae	NDM-1	+	0	0	0,75	0,125	3	0,125	S	0	0
LL43	E. coli	NDM-1	+	+	+	32	32	32	12	R	+	+
LL23	Enterobacter sp.	NDM-1	+	+	+	3	3	>32	2	R	+	0
LL11	K. pneumoniae	NDM-1	0	+	+	32	32	32	32	R	+	+
LL12	K. pneumoniae	NDM-1	+	+	+	32	8	32	32	R	+	+
LL44	K. pneumoniae	NDM-1	+	+	+	32	32	32	4	R	+	+
LL14	P. rettgeri	NDM-1	+	+	0	>32	>32	>32	>32	R	+	0
LL8	E. coli	OXA-48	+	+	0	4	3	12	0,5	R	+	+
LL38	E. coli	OXA-48	+	+	+	32	1	32	0,5	I	0	0
LL45	E. coli	OXA-48	0	0	0	4	1,5	32	0,75	R	+	+
LL47	E. coli	OXA-48	0	0	0	6	1	4	8	R	+	+
LL9	K. pneumoniae	OXA-48	+	+	+	32	32	32	0,25	R	+	+
LL10	K. pneumoniae	OXA-48	+	+	+	32	32	32	32	R	0	0
LL35	K. pneumoniae	OXA-48	+	0	+	32	4	32	3	S	+	+
LL40	K. pneumoniae	OXA-48	+	+	+	32	16	32	4	R	+	+
LL41	K. pneumoniae	OXA-48	0	0	+	8	3	2	1	R	+	+
LL48	K. pneumoniae	OXA-48	0	0	0	0,38	0,032	0,125	0,04	R	+	0
LL27	K.pneumoniae	OXA-48	+	+	0	2	2	0,125	0,75	S	+	+
LL22	E. coli	Porin-loss+ampC	+	+	+	2	0,25	0,047	0,032	I	0	0
LL39	K. pneumoniae	Porin-loss+ESBL	+	+	+	3	3	32	0,5	R	0	0
LL42	K. pneumoniae	Porin-loss+ESBL	+	+	+	1,5	4	32	0,75	R	0	0
LL1	E. coli	VIM	+	+	+	4	0,75	1	1	R	+	+
LL34	K. oxytoca	VIM	+	0	+	32	1,5	4	1,5	R	+	+
LL3	K. pneumoniae	VIM	+	+	+	32	32	32	32	R	+	+

Table2: Summary of results of chromogenic media, MALDI-TOF, VITEK 2 and the Hodge-test for the detection Carbapenemase-producing Enterobacteriaceae

Resistance Mechanism/Species	Brilliance CRE		Chromagar KPC		chromID Carba		MALDI-TOF		VITEK2		Hodge-Test	
	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg
NDM-1 (total)	6	1	6	1	5	2	6	1	6	1	4	3
K. pneumoniae	2	1	3		3		3		3		3	
E. coli	1		1		1		1		1		1	
Enterobacter sp.	2		1	1	1	1	1	1	1	1		2
Providenica rettgeri	1		1			1	1		1			1
VIM (total)	3		2	1	3		3		3		3	
K. pneumoniae	1		1		1		1		1		1	
K. oxytoca	1			1	1		1		1		1	
E. coli	1		1		1		1		1		1	
KPC (total)	7		7		7		6	1	7		6	1
K.pneumoniae	5		5		5		5		5		5	
E. coli	2		2		2		1	1	2		1	1
OXA-48 (total)	7	4	6	5	6	5	9	2	9	2	8	3
K. pneumoniae	5	2	4	3	5	2	6	1	5	2	5	2
E. coli	2	2	2	2	1	3	3	1	4		3	1
Carbapenemases (total)	23	5	21	7	21	7	24	4	25	3	19	7
Porin-loss (total)	3		3		3		n.d.		3		n.d.	
E. coli	1		1		1				1			
K. pneumoniae	2		2		2				2			

Figure 2: Chrom ID CARBA agar

RESULTS

Of 31 *Enterobacteriaceae* strains with defined Carbapenem-resistance mechanism, all KPC and strains with porin-loss showed growth on the chromogenic media. One VIM- and one NDM1-positive *Klebsiella* strain showed no growth on CKP and on BCR, respectively. *Providencia rettgeri, Enterobacter* sp. and the OXA-48-positive *Enterobacteriaceae* strains showed variable growth on the chromogenic media. All VIM, NDM1, and KPC-producing *Klebsiella* and *E. coli* strains were correctly detected with the MALDI-TOF-Carbapenemase method, the Hodge-test and the VITEK 2. *Providencia rettgeri, Enterobacter* sp. and the OXA-48-positive *Enterobacteriaceae* showed variable results. The strains without Carbapenem-resistance mechanism showed no growth on the agar media and a negative result with the MALDI-TOF method, the Hodge test and the VITEK 2 (Tables 1,2).

CONCLUSIONS

Due to the variability of Carbapenemase-producing *Enterobactericeae* detection methods rely on a combination of different approaches. All tests should be performed in combination with PCR in case of negative or discrepant results.

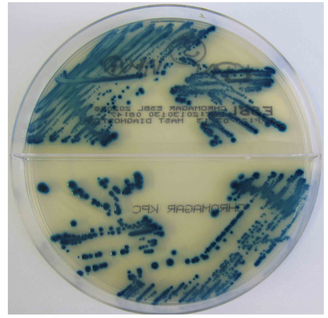


Figure 3: Chromagar KPC

References

1. Burckhardt I, Zimmermann S. 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J. Clin. Microbiol. 49:3321-3324.
2. Sparbier K, et al. 2012. Matrix-assisted laser desorption ionization–time of flight mass spectrometry-based functional assay for rapid detection of resistance against ß-lactam antibiotics. J. Clin. Microbiol. 50:927-937.