Evaluation of the Modified Carbapenem Inactivation Method for the detection of carbapenemase activity in Enterobacteriaceae in a routine laboratory

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Introduction

The resistance mechanisms behind carbapenem-resistant Enterobacteriaceae (CRE) are heterogenous and carbapenem resistance is conferred by either production of a carbapenemase (CP-CRE) or an extended-spectrum β-lactamase and/or AmpC in combination with cell membrane altrations (non-CP-CRE). The proportions of CP- and non-CP-CRE in clinical isolates show a considerable variability. Carbapenemases are transmitted via mobile genetic elements and resistance can spread to other bacteria. Studies suggest that CP-CRE are more virulent than non-CP-CRE and clinical outcomes in patients with CP-CRE are poorer [1]. Further, CP-CRE frequently carry multiple other resistance mechanisms.
The rapid worldwide dissemination of carbapenemases has led to an increase in the prevalence of CP-CRE. The fast and reliable detection of CP-CRE is of great importance for treatment and implementation of infection control measures to avoid spread of resistance and potential outbreaks. A novel phenotypic test, the Carbapenem Inactivation Method (CIM), has shown promising results in detecting carbapenemase activity [2]. Therefore, we contrasted the modified version of the CIM against the established Modified Hodge Test (MHT) for the purpose of integrating this method in our diagnostic workflow [3].

Methods

120 Enterobacteriaceae isolates from clinical specimens collected between 2014-2016 and indicative for carbapenem-resistance (Imipenem and Meropenem MIC ≥1 mg/l, Ertapenem ≥0,5 mg/l) were included in this study. The mean inhibitory concentration was determined by Vitek®2 (bioMérieux, France) and confirmed by gradient diffusion test (Etest, bioMérieux, France). The MHT was performed as described in CLSI-document M100-21 (Clinical & Laboratory Standards Institute, USA). Briefly, for the mCIM a meropenem disk was incubated for three hours in a suspension of a 10 µl loop of the bacterial isolate and 400 µl water. This meropenem disk was transferred on a Mueller-Hinton agar plate inoculated with a susceptible *Escherichia coli* strain. After a second incubation step (over night) carbapenemase activity could be detected by the absence of an inhibition zone (Figure 1). The results were verified by molecular detection of genes coding for carbapenemases.

Results

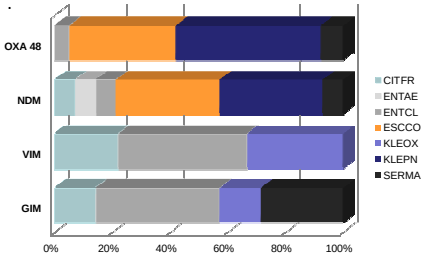

Figure 2:
Distribution of the carbapenemase enzymes in CP-CRE

From the 120 investigated strains 69 were carbapenemase producing isolates (including OXA- [n=38], NDM- [n=14], VIM- [n=9], GIM- [n=7], and KPC-type [n=1, not shown]). The most commonly recovered organisms were *Klebsiella pneumoniae* (36 %), *Escherichia coli* (28 %), and *Enterobacter cloacae complex* (14 %).

All PCR-positive strains showed carbapenemase activity by mCIM (sensitivity = 100 %), while MHT failed in the detection of one NDM-carbapenemase.
In both phenotypic methods we obtained false positive results (MHT [n=18] and CIM [n=6]). While we could not detect a specific pattern for the MHT, the false positive results derived from the CIM were restricted to the genus *Enterobacter* (*E.*) [*E. aerogenes* (n=1) and *E. cloacae* complex (n=5)], suggesting an influence by the presence of other mechanisms of resistance, such as AmpC in combination with membrane impermeability or active drug efflux.
Interestingly, the distribution of carbapenemase types derived from hospitals in Düsseldorf deviates in part from the EuSCAPE listing [4]. Herein we have a large proportion of NDM-type and only one CP-CRE with KPC. Of regional feature is the evidence of GIM-type-carbapenemase.

Acknowledgements
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References

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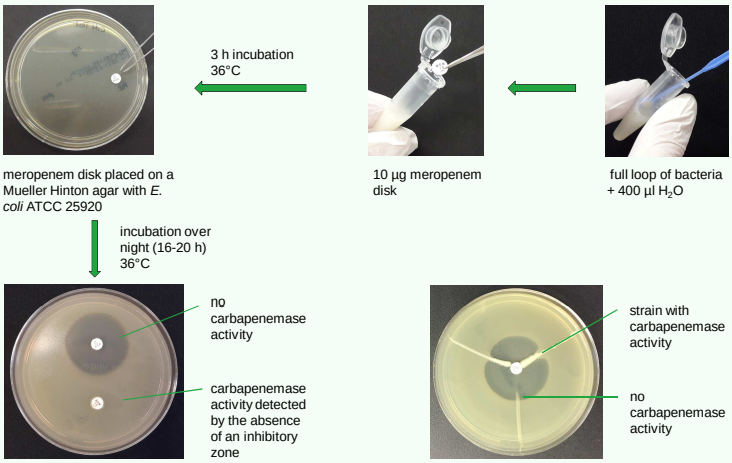

Figure 1:
a) Modified Carbapenem Inactivation Method (mCIM) b) MHT

Results

Species	Carbapenemase (PCR)	n	mCIM	Hodge	Imipenem	MIC (mg/l) Meropenem	Ertapenem
Citrobacter braakii	neg	2	neg	neg (1), **pos** (1)	2-4	0,25	0,25-0,5
Citrobacter freundii	**VIM-1**	2	pos	pos	≥ 16	4	4
	GIM-1	1	pos	pos	4	4	4
	NDM*	1	pos	pos	≥ 8	≥ 16	≥ 8
	neg	2	neg	neg (1), **pos** (1)	2 - 16	0,25- 16	1- 8
Enterobacter aerogenes	**NDM***	1	pos	pos	≥ 16	≥ 16	≥ 8
	neg	1	**pos**	**pos**	2	0,25	4
	neg	4	neg	neg (3), **pos** (1)	2 - 4	0,25 - 4	0,25 - 8
Enterobacter cloacae Complex	**GIM-1**	3	pos	pos	8 - ≥ 16	≥ 16	≥ 8
	NDM*	1	pos	pos	≥ 16	≥ 16	≥ 8
	OXA-48	2	pos	pos	4 - ≥ 16	4 - 8	8 - 8
	VIM-1	4	pos	pos	≥ 16	≥ 16	4 - 8
	neg	6	**pos**	neg (1), **pos** (5)	0,25 - 4	0,5 - 2	2 - 4
	neg	11	neg	neg (5), **pos** (6)	1 - ≥ 16	1 - ≥ 16	0,25 - ≥ 8
Escherichia coli	**NDM***	1	pos	pos	≥ 16	≥ 16	≥ 8
	NDM-5 + OXA-181	4	pos	pos	8 - ≥ 16	≥ 16	≥ 8
	OXA-48	9	pos	pos	1 - 4	0,5 - 4	2 - 8
	OXA-244	5	pos	pos	0,25 - 2	0,25 - 2	4 - ≥ 8
	neg	7	neg	neg	0,25 -4	0,25 - 2	0,25 - ≥ 8
Hafnia alvei	neg	1	neg	**pos**	1	4	≥ 8
Klebsiella oxytoca	**GIM-1**	1	pos	pos	0,5 - 1	1 - 2	4
	VIM-1	2	pos	pos	≥ 16	1	0,5
	VIM-4	1	pos	pos	≥ 16	4	2
	neg	1	neg	**pos**	0,25	2	4
Klebsiella pneumoniae	**NDM***	5	pos	pos	≥ 16	≥ 16	≥ 8
	OXA-48	18	pos	pos	2 - ≥ 16	1 - ≥ 16	4 - ≥ 8
	OXA-232	1	pos	pos	≥ 16	≥ 16	≥ 8
	KPC-2	1	pos	pos	≥ 16	≥ 16	≥ 8
	neg	14	neg	neg (11), **pos** (3)	0,25 - 8	0,25 - ≥ 16	0,5 - ≥ 8
Serratia marcescens	**GIM-1**	2	pos	pos	8 – ≥ 16	≥ 16	4 – ≥ 8
	NDM*	1	pos	**neg**	≥ 16	≥ 16	≥ 8
	OXA-48	3	pos	pos	4 - ≥ 16	1 - 2	4 - ≥ 8
	neg	2	neg	**pos**	1	0,25 - 0,5	4 - ≥ 8

* NDM-1 or -5

Table 1:
Characteristics of the Enterobacteriaceae isolates included in this study

Conclusion

Detection of carbapenemases is important for epidemiological and infection control purposes and planning of therapeutic strategies.

mCIM:
- easy to perform and easy to read
- cost-effective
- highly robust
- diagnostic performance: 100% negative predictive value
  91% positive predictive value

- ➢ Reliable screening method that can be integrated in an algorithm intended to detect carbapenemases in Enterobacteriaceae in a routine laboratory.
- ➢ As a phenotypic method mCIM may also detect carbapenemase activity even in strains with so far unknown carbapenemase encoding genes.
- ➢ High proportion of NDM carbapenemase-type may also be attributable to flourishing medical tourism from highly endemic countries.