

## Objectives

The increased carbapenem-resistance in *Enterobacteriaceae* necessitates building of the capacity in microbiology laboratories to rapidly and reliably detect such strains to allow proper management of treatments and implementation of infection control measures.

A number of phenotypic carbapenemase detection kits have been developed that rely on the inhibition of different enzyme classes by different inhibitors. These kits may be used for the preliminary identification of carbapenemase-coding genes such as KPC, metallo-beta-lactamases (MBLs) (e.g. IMP, VIM, NDM-1) and OXA-48.

In this study we aimed to evaluate the performances of two commercial products developed for this purpose in a collection of carbapenemase-producing isolates.

## Methods

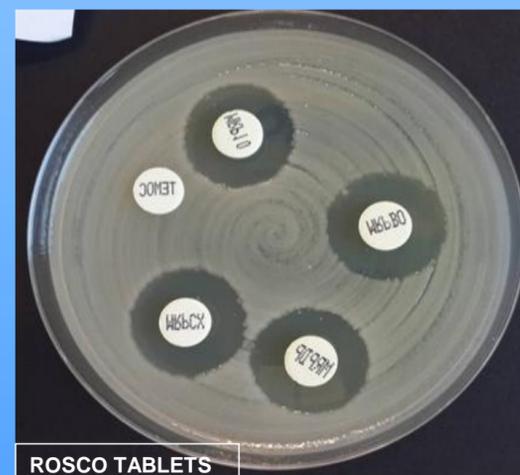
A collection of previously characterized *E. coli* (n=11) and *K. pneumoniae* (n=34) isolates which harbour one or two carbapenemase gene(s) was tested to assess the performances of the commercial kits. The collection consisted of OXA-48 (n=12), OXA-48+NDM-1 (n=11), NDM-1 (n=16), IMP (n=3), KPC (n=2), and OXA-48+VIM (n=1) positive isolates.



The isolates were tested in parallel with MASTDISCS D70C carbapenemase detection kit and temocillin disc (30 µg) (Mast Group Ltd., UK), and Rosco KPC/MBL and OXA-48 confirmation kit (Rosco Diagnostica, Denmark). In both methods the inhibition zone diameters of meropenem disc impregnated with a) MBL, b) KPC carbapenemase, and c) AmpC beta-lactamase inhibitor are compared to inhibition zone diameter of meropenem disc without inhibitors. Due to lack of a specific inhibitor for OXA-48, temocillin resistance is used in favour to presence of OXA-48 gene in the absence of any synergy with inhibitor containing discs. Discs (Mast) or tablets (Rosco) were placed on Mueller-Hinton agars (bioMérieux, France) that was streaked with 0.5 McFarland bacterial suspension and the plates were incubated overnight. The inhibition zone diameters around the discs/tablets were measured and the results were evaluated according to manufacturer's instructions.

## Results

For single carbapenemase gene harboring isolates, Mast kit detected 26/33 (78.8%) and Rosco kit detected 28/33 (84.8%) the underlying resistance mechanism (KPC, MBL and OXA-48) correctly. For isolates carrying two carbapenemase genes (OXA-48+MBL), however, Mast kit detected 9/12 (75.0%) and Rosco kit detected 12/12 (100%) of the isolates correctly as MBL positive (Table 1).



For isolates that carry OXA-48+MBL, resistance to temocillin resistance can be regarded as a clue for the presence of OXA-48, however 8 and 9 of 21 non-OXA-48-producing isolates showed temocillin-resistance with Mast and Rosco discs, respectively.

**Table 1.** Evaluation of the performances of Mast and Rosco phenotypic carbapenemase detection kits for *Escherichia coli* and *Klebsiella pneumoniae* isolates carrying carbapenemase genes

Carbapenemase gene	Organism	Mast		Rosco	
		Phenotype	n	Phenotype	n
IMP	<i>E. coli</i> (n=2)	MBL	1/2	MBL	2/2
		Negative	1/2		
	<i>K. pneumoniae</i> (n=1)	MBL	0/1	MBL	1/1
		Negative	1/1		
KPC	<i>K. pneumoniae</i> (n=2)	KPC	1/2	KPC	2/2
		Negative	1/2		
NDM-1	<i>E. coli</i> (n=4)	MBL	4/4	MBL	4/4
	<i>K. pneumoniae</i> (n=12)	MBL	10/12	MBL	11/12
		OXA-48	2/12	OXA-48	1/12
OXA-48	<i>E. coli</i> (n=5)	OXA-48	5/5	OXA-48 (n=3)	3/5
				MBL	1/5
				Negative	1/5
	<i>K. pneumoniae</i> (n=7)	OXA-48	5/7	OXA-48	5/7
		Negative	2/7	KPC	1/7
				Negative	1/7
OXA-48 + NDM-1	<i>K. pneumoniae</i> (n=11)	MBL	8/11	MBL	11/11
		OXA-48	3/11		
OXA-48 + VIM	<i>K. pneumoniae</i> (n=1)	OXA-48	1/1	MBL	1/1

MBL: metallo-beta-lactamase

## Conclusion

- Our results suggest that phenotypic carbapenemase detection kits tested in this study can be helpful to routine laboratories for preliminary identification of carbapenemase-producing isolates.
- The combined positive isolates diminish the differentiation power and thus the added value of these kits.
- As a practical approach, monitoring of the local epidemiology with molecular methods at the institution level and adopting a phenotypic method that fits the institution's needs might be recommended.