



# Evaluation of Amplidiag CarbaR+MCR on clinical isolates

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## Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) represent a major health concern. The rapid implementation of infection control measures necessitates efficient and reliable detection tools of carbapenemase production. Recently, Amplidiag® CarbaR+MCR (Mobidiag Ltd, Espoo, Finland), a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test, was launched on the market for the detection of carbapenemase and *mcr-1* and *mcr-2* producing strains. This assay was evaluated prospectively.

## Material and methods:

The Amplidiag® CarbaR+MCR is a multiplex real-time PCR qualitative test which targets a large diversity of carbapenemase encoding genes including *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>OXA-48-like</sub>, and *bla*<sub>GES</sub>; the *Acinetobacter* OXA-carbapenemase genes including *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-58-like</sub>, the chromosomal *bla*<sub>OXA-51-like</sub> with upstream promoter *ISAbal* and the plasmidic gene *mcr-1* and *mcr-2* which confer resistance to colistin.

All isolates sent to the Belgian National Reference Center for Antimicrobial Resistance in Gram- bacteria between the 1<sup>st</sup> of January to 1<sup>st</sup> of June 2018 (n=110) were tested with the Amplidiag® CarbaR+MCR using ISO15189 certified PCR and sequencing as comparator.

In addition, 24 collection isolates harbouring rarer resistance genes were analysed.

The assay was evaluated on boiling extracted DNA (1 colony in 100 µL, 5 min spinning and 5 µL supernatant used for amplification)

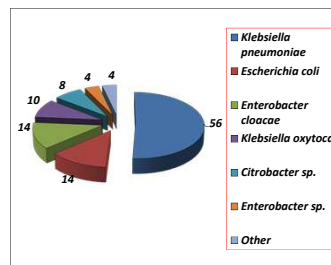


Figure 1: Species included in the prospective study

Other species : *Klebsiella variicola*, *Providencia rettgeri*, *Raoultella ornithinolytica*, *Serratia marcescens*

## Results

- 100 % concordance with the expected results for the detection of carbapenemases and of MCR coding genes included in our collection
- GES-5, GES-6 and GES-14 are well detected as carbapenemase while GES-12 (non carbapenemase) is not detected as expected
- MCR-1 and MCR-2 are well detected but not MCR-3 and MCR-5 as announced by the manufacturer
- OXA-carbapenemases from *Acinetobacter* spp. are efficiently detected including overexpressed OXA-51 (with upstream *ISAbal*)

N°	Species	Sequencing data	Amplidiag CarbaR+MCR results
1	<i>Pseudomonas aeruginosa</i>	KPC-2	KPC
2	<i>P. aeruginosa</i>	VIM-1	VIM
3	<i>P. aeruginosa</i>	VIM-2	VIM
4	<i>P. aeruginosa</i>	VIM-4	VIM
5	<i>P. aeruginosa</i>	NDM-1	NDM
6	<i>P. aeruginosa</i>	IMP-7	IMP
7	<i>P. aeruginosa</i>	IMP-13	IMP
8	<i>P. aeruginosa</i>	NDM-1 + GES-5	NDM, GES-CPO
9	<i>P. aeruginosa</i>	VIM-5 + NDM-1	NDM, VIM
10	<i>Acinetobacter baumannii</i>	GES-14	GES-CPO
11	<i>Acinetobacter pittii</i>	VIM-4	VIM
12	<i>A. baumannii</i>	NDM-1	NDM
13	<i>A. baumannii</i>	OXA-23	AcOXA
14	<i>A. baumannii</i>	OXA-24	AcOXA
15	<i>A. baumannii</i>	OXA-58	AcOXA
16	<i>A. baumannii</i>	OXA-72	AcOXA
17	<i>A. baumannii</i>	<i>ISAbal</i> -OXA51 + GES-12	AcOXA
18	<i>A. baumannii</i>	NDM-1 + OXA-23	AcOXA, NDM
19	<i>A. pittii</i>	VIM-4 + OXA-23	AcOXA, VIM
20	<i>Citrobacter braakii</i>	GES-6	GES-CPO
21	<i>Escherichia coli</i>	<i>mcr-1</i>	MCR
22	<i>E. coli</i>	<i>mcr-2</i>	MCR
23	<i>E. coli</i>	<i>mcr-3</i>	Negative
24	<i>Salmonella</i> sp	<i>mcr-5</i>	Negative

Table 1: Summary on 24 reference isolates. AcOXA: OXA-Carbapenemase of *Acinetobacter* including *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-58-like</sub>, and the chromosomal *bla*<sub>OXA-51-like</sub> with upstream *ISAbal*

Carbapenemases	Enterobacteriaceae (n=110), Reference results	Amplidiag® CarbaR+MCR results
ClassA	KPC -3 (n=6)	KPC
ClassB	NDM-1 (n=2), NDM-5 (n=1); VIM-1 (n=10) and VIM-4 (n=2); IMP-8 (n=1)	NDM, VIM and IMP respectively
ClassD	OXA-48 (n=39), OXA-244 (n=1)	OXA-48/181
Multiple carbapenemases	IMP-8 + OXA-48 (n=1)	OXA-48/181, IMP
Non carbapenemase	Overexpressed AmpC-ESBL - impermeability (n=47)	Negative

Table 2: Summary of the results obtained with the 110 prospective clinical isolates All Amplidiag® results are concordant with the reference results

## Conclusions

- The Amplidiag® CarbaR+MCR detected all targeted genes included in the collection and in the routine clinical isolates
- The test allows the detection of most carbapenemases encountered in Enterobacteriaceae AND in non-fermenters including GES carbapenemase which is currently unique on the market
- On colonies, with boiling extract the results are obtained in batch in less than 1h30
- Further evaluation on screening samples is required to confirm the performance of the assay directly from fecal swabs as claimed by the manufacturer