BMR-01

Evaluation of Amplidiag CarbaR-VRE kit for the detection of carbapenemase-producing bacteria

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Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) and carbapenemase-producing non-fermenters (CPNF; Pseudomonadaceae and *Acinetobacter* sp.) have been increasingly reported worldwide. Therefore, reliable detection of carbapenemase production is essential for the prompt implementation of infection control measures able to prevent clonal expansion or transfer of carbapenemase genes.

We evaluated the performance of Amplidiag® CarbaR+VRE (Mobidiag Ltd, Espoo, Finland), a qualitative multiplexed nucleic acid-based in vitro diagnostic test.

Matériels et méthodes:

The Amplidiag® CarbaR+VRE is a qualitative multiplexed nucleic acid-based diagnostic test intended for the detection of carbapenemase: targeted genes are *bla*KPC, *bla*NDM, *bla*VIM,*bla*IMP, *bla*OXA-48-like, *Acinetobacter* OXA genes including *bla*OXA-23, *bla*OXA-24/-40, *bla*OXA-58, and *bla*OXA-51 with upstream promoter IS*Aba*1.

- The Amplidiag® CarbaR+VRE has been tested on a collection of **100 well-characterized isolates** with a reduced susceptibility to carbapenems, and **200 isolates** collected at the **National Reference Center for Antibiotic Resistance** between the 20st January to 10th February 2016.
- OXA-23 and OXA-58 producing P. mirabilis were also tested.
- PCR was evaluated on extracted DNA (as recommended by the manufacturer), on boiling extracted DNA and directly on colonies

Conclusions

The Amplidiag® CarbaR+VRE was able to detect all targeted genes including variants.

As claimed by the manufacturer, other carbapenemases such as GES-like carbapenemases (GES-2, GES-5 in *P. aeruginosa*, GES-14 in *A. baumannii*), GIM-1, AIM-1, SPM-1, DIM-1 or OXA-198 in *P. aeruginosa*, or OXA-143-like in *A. baumannii* were not detected.

The Amplidiag® CarbaR+VRE assay **is well adapted to the French epidemiology** with a **good sensitivity and specificity**.

The **main advantage** of this test is that it contains **a large panel of targeted resistance determinants**.

Interestingly this assay could detect also A. *baumannii* carbapenemases such as OXA-23 and OXA-58 producing Enterobacteriaceae, as recently described in a OXA-58 or OXA-23-producing *P. mirabilis* isolates.

Results

100% detected Not detected

- **Results of the 100 well-characterized isolates**
- **Table 1: Summary on reference isolates**

	Enterobacteriaceae	Pseudomonadaceae	*Acinetobacter sp.*
Class_A Carbapenemases	**KPC Type:** n=9 (KPC-2,KPC-3)	**KPC-2:** n=2 **GES Type** n=2 (GES-2, GES-5)	**GES-12:** n=1
Class_D Carbapenemases	**OXA-48 Type:** n=15 (OXA-48, 162, 181, 204, 232, 244)	**OXA-198:** n=1	**OXA Ac :** n=13 (OXA-51, 23, 24/40, 72, 58,)
Class_B Carbapenemases	**NDM, VIM and IMP Type:** n=12 (NDM-1,4,5,6 and 7; VIM-1,2,4 and 19; IMP-1,8 and 11)	**VIM and IMP Type:** n=11 (VIM-1,2 and 4; IMP-1,2 and 13) **Others :** n=4 (GIM, AIM, SPM, DIM)	**NDM, VIM and IMP Type:** n=3 (NDM-1; VIM-4; IMP1)
Multiple carbapenemases	n=5		n=1 (NDM-1 + OXA-23)
Non carbapenemase	**Overexpressed AmpC/ ESBL/ impermeability Extended spectrum oxacillinase (OXA-163, OXA-405)**; n=9	**Efflux/ OprD deficiency AmpC + other ß-lactamases (GES-9, OXA-32)** n=11	n=2 (OXA-21, PER-1)

- **Results of the 200 isolates** collected at the **National Reference Center for Antibiotic Resistance => 100% specificity and sensitivity**
- **Table 2: Biological performances**

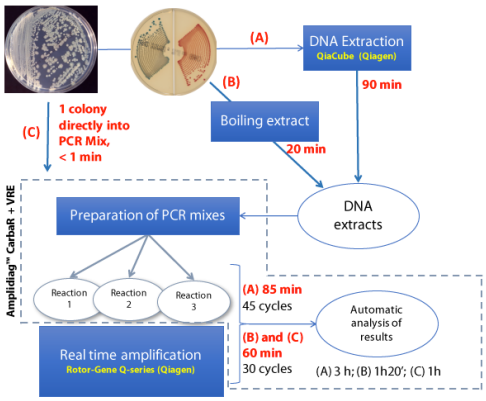
Test parameters	Prospective (All isolates) (=200)	Enterobacteriaceae (n=50)	Pseudomonas spp. (n=30)	A. baumannii (n=20)	Extrapolation to the French CPE epidemiology (2012-2015)* (n=9518)
Sensitivity	100% [95%CI = 95.4% - 100%]	97.6 % [95%CI = 85.6% - 99.9%]	65.0 % [95%CI = 40.9% - 83.7%]	83.3 % [95%CI = 57.7% - 95.6%]	**99.55 %** [95%CI = 99.24% - 99.74%]
Specificity	100% [95%CI = 95.3% - 100%]	88.9% [95%CI = 50.7% - 99.4%]	100% [95%CI = 65.6% - 100%]	100% [95%CI = 19.8% - 100%]	**99.98 %** [CI95 = 99.89 % - 99.99 %]
PPV	100% [95%CI = 95.4% - 100%]	N/A	N/A	N/A	**99.97 %** [CI95 = 99.80 % - 99.99 %]
NPV	100% [95%CI = 95.3% - 100%]	N/A	N/A	N/A	**99.76 %** [CI95 = 99.59 % - 99.86 %]

*Extrapolation of the results obtained for the *Enterobactericeae* to the French CPE epidemiological situation. During that period 118 KPC, 13 IMI, 1 FRI-1, 366 NDM, 115 VIM, 10 IMP, 2491 OXA-48, 120 OXA-181, 35 OXA-204, 8 OXA-232,10 OXA-244 and 33 multiple carbapenemase-producers (including 30 NDM + OXA-48-like, 1 NDM + VIM and 2 VIM + OXA-48-like) have been identified. Using our test results for *Enterobactericeae*, we would expect that all isolates except those producing IMI, FRI-1 and NDM + VIM (Total = 15) would be correctly identified. In addition the unique OXA-405-producing *S. marcescens* should give a false positive results.

Table 2. Results of the Amplidiag® *Proteus mirabilis.*

- **Results PCR on colonies => on MH plates: 100% specificity and sensitivity on MH**
  => On chromogenic media: required boiling extract for Enterobactericeae
- **Detects efficient OXA-23 and OXA-58-producing *P. mirabilis* => Not detected by other commercially avaiable tests**

Figure 1



- Figure 1: Experimental procedures. (A) corresponds to the experimental setup as recommended by the manufacturer. As DNA extraction is open, the Qiacube Automated DNA extractor was used. (B) correspond to Boiling DNA extraction and (C) one colony was pricked three times in each 20 µl PCR mix.