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

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Background: Carbapenemase-producing Enterobacteriaceae (CPE) and non-fermenters (CPNF; Pseudomonadaceae and *Acinetobacter* sp.) have been increasingly reported worldwide. The most clinically-relevant carbapenemases belong either to Ambler class A (KPC-type), Ambler class B, i-e metallo-β-lactamases (MBLs such as IMP-, VIM- and NDM-types) or Ambler Class D (OXA-48-like in Enterobacteriaceae, *Acinetobacter* OXA group: OXA-23, OXA-40, OXA-58, OXA-143 and the intrinsic OXA-51-like enzymes, OXA-198 in *P. aeruginosa*).

Material/methods: The Amplidiag® Carba-R+VRE, a qualitative multiplex nucleic acid-based in-vitro diagnostic test intended for the detection of carbapenemase-producing bacteria and vancomycin resistant enterococci, has been tested on a collection of 100 characterized Gram-negative isolates with reduced susceptibility to carbapenems, and on 200 isolates collected at the National Reference Center for Antibiotic Resistance from 20st January to 10th February 2016. The markers detected by this assay are $bla_{\text{KPC-like}}$, $bla_{\text{NDM-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{IMP-like}}$, $bla_{\text{OXA-48-like}}$, *Acinetobacter* OXA genes including $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/-40}}$, $bla_{\text{OXA-58}}$, and $bla_{\text{OXA-51}}$ including upstream promoter IS*Aba*1, and *vanA* and *vanB*. DNA was extracted using QiaAmp DNA extraction kits or by boiling extract.

Results: The Amplidiag® CarbaR+VRE was able to detect all KPC, NDM, VIM, IMP, OXA-48 and variants including OXA-162, -181, -204, -232, -244. Similarly, all *A. baumannii* producing OXA-23, OXA-24/-40, OXA-58 as well as overexpression of the chromosomally-encoded OXA-51-like β-lactamase, due to the presence of IS*Aba1* upstream of the $bla_{\text{OXA-51}}$ gene were detected. The most prevalent carbapenemases encountered in *P.*

aeruginosa have also been detected. However, 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 by the Amplidiag® CarbaR+VRE assay. The PCR worked equally well on purified DNA and on boiling extracted DNA from a colony. The biological performances of the Amplidiag® CarbaR+VRE on the prospective study of 200 CREs isolates collected at the NRC were of 100% and 96% sensitivity and specificity, respectively.

Conclusions: Amplidiag® Carba-R's performances were high as it was able to detect the five major carbapenemases: NDM, VIM, IMP, KPC, and OXA-48, as well as OXA-type carbapenemases from *Acinetobacter* sp. It can provide a result directly on colonies growing on selective screening media within two hours. This test needs now to be evaluated on rectal swabs, since it is able to detect the most worrisome resistant traits in Gram negative bacteria.