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Evaluation of the Amplidiag Carba + MCR kit for the accurate detection of carbapenemase-producing and colistin resistant bacteria

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Introduction

As carbapenemase-producing Gram-negative bacilli (CP-GNB) co-producing MCR-1 are now emerging[1], thus compromising the use of last resort antibiotics (carbapenems and colistin), there is an urgent need for accurate and fast diagnostic tests. Amplidiag® CarbaR+VRE assay displayed a high sensitivity and specificity on colonies of CP-GNB, but failed to detect GES carbapenemase producing organisms (GES-CPO), which represent 11% of the carbapenemase producing *P. aeruginosa* (CP-*Pa*) in France[2]. Moreover, this assay was not validated on clinical samples such as rectal swabs.

Objectives

Evaluation of the Amplidiag® Carba-R+MCR assay (Mobidiag, Paris, France), a multiplex nucleic acid-based *in vitro* diagnostic test intended for the detection of CP-GNB, including GES carbapenemase-producing organisms (GES-CPO), and colistin resistance genes *mcr*-1/-2 from rectal swabs and from cultured colonies.

Methods

- ✓ Retrospective evaluation : colonies of 215 Gram negative rods
- ✓ Prospective study : 51 consecutive enterobacterial isolates collected at the French National Reference Center,
- ✓ DNA extracted from 100 rectal swabs (including 40 positives) sent to the hygiene unit for CP-GNB screening.
- ✓ Multiplex PCR was performed to detect carbapenemase and *mcr*-1/-2 genes using a fluorophore-labelled Taqman probe on a CFX96 thermocycler (Biorad, Marnes la Coquette, France).
- ✓ Results were automatically interpreted thanks to the Analyzer software (Mobidiag, Paris, France).

Results

Table 2. Global performances of the Amplidiag Carba+MCR® kit on DNA extracted from clinical rectal swabs (n= 100)

Culture on ChromID® Carba SMART medium *(n)*[a]			Amplidiag® Carba+MCR Ct values							
Direct inoculation from the rectal swab	After enrichment in ertapenem 0.5 µg/ml containing BHI	carbapenemase *(n)*[a]	KPC	NDM	VIM	IMP	OXA-48-like	AcOXA	MCR	GES
Negative *(60)*	Negative *(60)*	-	-	-	-	-	-	-	-	-
E. coli + *K. pneumoniae (2)*	Not performed	KPC-2	23-24	-	-	-	-	-	-	-
Negative	*K. pneumoniae*	KPC-3	25	-	-	-	-	-	-	-
Negative (GeneXpert® result :VIM)	Negative[b]	VIM-type	-	-	33	-	-	-	-	-
E. coli	Not performed	NDM-5	-	31	-	-	-	-	-	-
K. pneumoniae (1), A. baumannii (1)	Not performed	NDM-1	-	32	-	-	-	-	-	-
K. pneumoniae + *A. baumannii (3)*	Not performed	NDM- 1 + OXA-23	-	23-29	-	-	-	25-27	-	-
Negative	*A. baumannii*	NDM-1	-	33	-	-	-	- (38)[c]	-	-
Negative	*A. baumannii*	NDM-1	-	- (39)	-	-	-	-	-	-
Negative	*A. baumannii*	NDM-1	-	-	-	-	-	-	-	-
A. baumannii (2)	Not performed	OXA-23	-	-	-	-	-	15-22	-	-
C. freundii (1), E. cloacae + *K. varicola (2), E. cloacae (4)*	Not performed	OXA-48	-	-	-	-	18-25	-	-	-
E. aerogenes	Not performed	OXA-48	-	-	-	-	34	-	-	-
E. coli (6), E. coli + *C. freundii (1)*	Not performed	OXA-48	-	-	-	-	19-29	-	-	-
K. pneumoniae (3), E. coli + *K. pneumoniae (5)*	Not performed	OXA-48	-	-	-	-	19-30	-	-	-
E. cloacae[d]	Not performed	OXA-48 + OXA-23	-	-	-	-	23	27	-	-
K. oxytoca[e]	Not performed	OXA-48	-	-	-	-	- (37)	-	-	-

Sensitivity : 92.5% [CI95 = 78.5% - 98.0%]
Specificity : 100% [CI95 = 92.5% - 100%]
Positive predictive value : 100% [CI95 = 88.3% - 100%]
Negative predictive value : 95.2% [CI95 = 85.8% - 98.8%]

[a] Number of isolates tested. Dark grey boxes represent discrepant results.
[b] After enrichment: No growth was detected on ChromID® Carba SMART after an overnight culture of the rectal swab, but after overnight culture of 100 µl of the eSwab in Brain Heart Infusion with one ertapenem disk (10µg) before plating.
[c] The negativity cutoff value being fixed at Ct ≥ 35 by the manufacturer, this sample is counted as negative.
[d] Although no culture of *A. baumannii* was obtained with this sample, a in house PCR confirmed the presence of the bla_{OXA-23} gene.
[e] Only 2 cfu were obtained on ChromID Carba Smart.

Table 1. Global performances of the Amplidiag Carba+MCR® kit on *Enterobacteriaceae, P. aeruginosa,* and *Acinetobacter spp.* colonies grown on MH agar isolates with decreased susceptibility to carbapenems (n=187) or to colistin (n=28).

Species *(n)*[a]	β-lactam-, or colistin- resistance mechanism	Amplidiag® Carba+MCR Ct values[b] KPC, NDM, VIM, IMP, OXA-48-like, AcOXA	GES	MCR
ISOLATES WITH DECREASED SUSCEPTIBILITY TO CARBAPENEMS				
Non GES / Non-carbapenemase/ Non- targeted carbapenemase producers (45)				
E. coli (2), E. cloacae (4), E. asburiae (1) C. freundii (1), M. morganii (1), H. alvei (2), S. marcescens (2), K. oxytoca (1), K. pneumoniae (3), P. aeruginosa (17), A. baumannii (11)	↗↗↗ Case[c], ↗↗↗ Pase, CTX-M-15, TEM, SHV, OXA-163, OXA-405, PER-1, VEB-1, OXA-32, ↗↗↗ Case, OprD deficiency, Efflux, IMI, NmcA, GIM, AIM, SPM, DIM, OXA-198, None, PER-1, VEB-1, SCO-1, RTG-4, OXA-10, OXA-21, OXA-69, SIM, OXA-143, OXA-253	-	-[d]	-
Non GES Other carbapenemase producers (113)				
Enterobacteriaceae (53), P. aeruginosa (30) A. baumannii (30)	KPC, NDM, VIM, IMP, OXA-48-like, IS*Aba1*-OXA-51, OXA23, OXA-40, OXA-58	10-20	-	-
GES –Non-CPO (13)				
K. pneumoniae (1)	**GES-1**	-	-	-
A. baumannii (2)	**GES-11, GES-12** + OXA-51+IS*Aba1*	14	-	-
P. aeruginosa (2)	**GES-1, GES-9**	-	-	-
C. amalonaticus (1), E. cloacae (1), K. oxytoca (1)	**GES-7**	-	23-27	-
A. baumannii (5)	**GES-11/GES-12**, + OXA-23, + IS*Aba1*-OXA-51	11-16	26-32	-
GES -CPO (16)				
P. aeruginosa (1)	**GES-2**	-	-	-
E. cloacae (5), K. pneumoniae (2), C. braakii (1), C. youngae (1)	**GES-5, GES-6**	-	12-17	-
P. aeruginosa (2)	**GES-5**	-	14	-
A. baumannii (4)	**GES-14**	-	14-17	-
ISOLATES WITH DECREASED SUSCEPTIBILITY TO COLISTIN				
Non-/ Non-targeted-MCR producers (15)				
E. coli (4), K. pneumoniae (1), Salmonella spp.*(4)*	Δ*pmrB*[e], Δ*mgrB*, ND	-	-	-
E. coli (4), Salmonella spp.*(2)*	MCR-3, MCR-3.2, MCR-4, MCR-5	-	-	-
Targeted MCR producers (13)				
E. coli (4), Salmonella spp.*(3), K. pneumoniae (3)*	MCR-1, MCR-2	-	-	12-19
E. coli (3)	MCR-1 + OXA-48, + NDM-1	14-16	-	12-14

Sensitivity for carbapenemases: 99.2 % [CI95 = 95.1% – 100%], Sensitivity for GES-CPO (15/16) : 93.7%, for *mcr*-1/-2 : 100% [CI95 = 71.6% – 100%]
Specificity for carbapenemases: 86.2% [CI95 = 74.1% - 93.4%], Specificity among GES-Non-CPO (5/13): 38.5 %, for *mcr*-1/-2 : 100%[CI95 = 74.6% – 100%]

[a] Number of isolates tested. Dark grey boxes represent discrepant results.
[b] Ct values were rounded up
[c] ↗↗↗ Case, Abbreviation for overexpressed cephalosporinase.
[d] no amplification.
[e] Δ, mutation or deletion ; ND, Not Determined.

- ✓ The sensitivity for all carbapenemases in cultured colonies was 99.2 %, only one GES-CPO (GES-2-producing *Pa*) was not detected.
- ✓ Overall the specificity of detection of GES-carbapenemases was not optimal since GES-non-carbapenemase variants were also detected, but with higher Ct values (Ct 23 to 27 for the ESBL GES-7, unlike GES-CPOs (GES-5 and GES-6) that displayed lower Ct values of 12 to 17 (Table 1).
- ✓ Plasmid-encoded colistin resistance genes *mcr-1* and *mcr-2* were perfectly detected (100% sensitivity, Table 1). As claimed by the manufacturer, MCR variants other than MCR-1 and -2 (here MCR-3, -3.2, -4 and -5 were not detected (100% specificity, Table 1).
- ✓ With DNA extracted from rectal swabs, the sensitivity was 92.5 % (Table 2), sometimes higher than that of the selective culture media
- ✓ Specificity for all carbapenemases was 100%.

Conclusions

- The big 5 carbapenemase families including variants and the main acquired carbapenem-hydrolyzing oxacillinases from *A. baumannii* (OXA-23, OXA-24/-40, OXA-58, and the over-expressed OXA-51-like β-lactamase) were well detected (Table 1).
- Among the GES non-CPO, the specificity was lower and the cut off value of the Ct should be adapted.
- mcr-1/-2 were well detected.

The global performances of the Amplidiag® Carba-R+MCR were high (92-100% sensitivity and 86-100% specificity). Moreover, it can provide a result not only from colonies growing on MH or on selective screening media but also from DNA extracted from clinical rectal swabs in less than three hours (1 h of DNA extraction and less then 2-h for PCR).

References : 1. Beyrouthy et al. AAC 2017; 2. Oueslati et al. J Clin Microbiol 2018.