Performance of the eazyplex® SuperBug CRE in rapid detection of carbapenemases and CTX-M extended-spectrum β-lactamases in Enterobacteriaceae and *Pseudomonas* spp.

EV0803

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BACKGROUND

The emergence and spread of multidrug-resistant Gram-negative bacteria are posing a serious threat for healthcare systems globally. Currently, the most significant resistance mechanisms are extended-spectrum β-lactamases (ESBLs) and carbapenemases. Rapid detection of Gram-negative isolates that produces carbapenemases or ESBLs is important for the implementation of appropriate treatment and infection control strategies. The objective of this study was to evaluate the performance of the molecular assay, eazyplex SuperBug CRE, for the detection of the most common carbapenemase- and ESBL-genes in Enterobacteriaceae and *Pseudomonas* spp.

MATERIALS AND METHODS

The eazyplex® SuperBug CRE assay (Amplex Diagnostics) is based on loop-mediated isothermal amplification and real-time detection of target genes in a closed portable system (Genie II). The target enzymes and specificity for the assay includes KPC-2 to -15, NDM-1 to -7, OXA-48 (OXA-48, -162, -204, -244), VIM-1 to -37, OXA-181 (OXA-181, -232), CTX-M group 1, and CTX-M group 9. A collection of 75 clinical Enterobacteriaceae (*n*=53) and *Pseudomonas* spp. (*n*=22) isolates previously confirmed by molecular methods (PCR and/or Check-Point microarray) to harbour carbapenemase- and/or CTX-M genes were used in the evaluation (Table 1). The assay was performed according to the manufacturer's instructions (Figure 1).

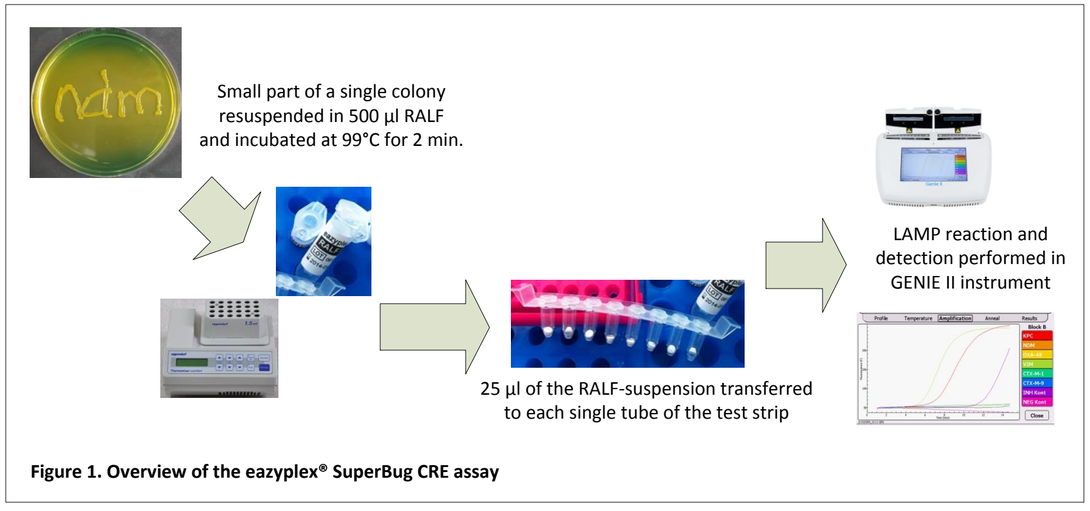

Figure 1. Overview of the eazyplex® SuperBug CRE assay

Table 1. Overview of bacterial strain collection and β-lactamases

Species	β-lactamase genes[a]						
	bla_{KPC}	bla_{NDM}	bla_{VIM}	$bla_{OXA-48-like}$	bla_{NDM}+$bla_{OXA-181}$	$bla_{CTX-M\ gr.\ 1}$	$bla_{CTX-M\ gr.\ 9}$
K. pneumoniae (*n*=33)	17	6	5	4	1	8	0
E. coli (*n*=15)	0	5	2	5	0	9	6
Other Enterobacteriaceae (*n*=5)[b]	2	3	0	0	0	1	0
Pseudomonas spp. (*n*=22)	0	1	21	0	0	0	0

[a] Several isolates harbored multiple β-lactamases. [b] 5 isolates comprising *Enterobacter* spp. (*n*=2), *Citrobacter koseri* (*n*=1), *Proteus mirabilis* (*n*=1) and *Providencia stuartii* (*n*=1).

RESULTS

The assay identified all target genes correctly with the exception of one bla_{VIM-7} positive *P. aeruginosa* isolate and one $bla_{OXA-181}$ positive *E. coli*. Thus, a sensitivity of 100% for all target genes was obtained except for bla_{VIM} and $bla_{OXA-181}$ (99% sensitivity, Table 2). Manual inspection of the data for the false-negative $bla_{OXA-181}$ positive *E. coli* revealed a clear amplification curve and high amplification rate (Figure 2). However, this was interpreted as negative by the software. No false-positive results were observed resulting in a specificity of 100% for all targets.

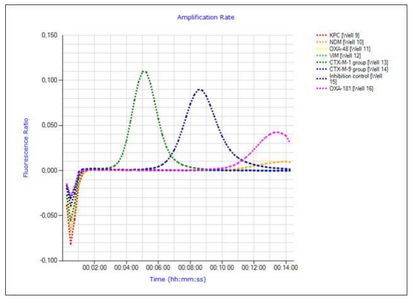

Figure 2. Amplification plot of $bla_{OXA-181}$ false-negative *E. coli* isolate

Table 2. Performance of the eazyplex® SuperBug CRE kit

Target gene	No.	False-negative	False-positive	Sensitivity (%)	Specificity (%)
bla_{KPC}	19	0	0	100	100
bla_{NDM}	16	0	0	100	100
bla_{VIM}	28	1	0	99	100
$bla_{OXA-48-like}$[a]	10	1	0	99	100
$bla_{CTX-M\ gr.\ 1}$	18	0	0	100	100
$bla_{CTX-M\ gr.\ 9}$	6	0	0	100	100

[a] Including $bla_{OXA-181}$

CONCLUSION

In conclusion the eazyplex® SuperBug CRE assay showed good sensitivity and specificity for all targets. The guidelines in the assay protocol should specify that bla_{VIM-7} is not detected and further developement of the software is necessary. The assay offers the posibility of rapid (20-30 min) detection of the most common carbapenemases and ESBLs from single bacterial colonies.

TRANSPARENCY DECLARATION

Montebello Diagnostics/Amplex provided kits, machinery and training for the evaluation.

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