Extended-spectrum β-lactamase (ESBL) and carbapenemases detection directly from rectal swabs with the rapid isothermal amplification-based eazyplex® SuperBug CRE assay

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Objectives: We evaluated the performance of the eazyplex® SuperBug CRE system, a loop-mediated isothermal amplification (LAMP)-based system, for the detection of the most common carbapenemases in addition to CTX-M-type ESBLs directly on rectal swabs collected at Geneva University Hospitals.

Methods: Out of 90 rectal swabs tested, 44 grew ESBL producing *Enterobacteriaceae*, 5 grew carbapenemase producing *Enterobacteriaceae* (2 KPC, 2 OXA-48, and 1 NDM-1), and 2 grew ESBL and carbapenemase producing *Enterobacteriaceae* (2 OXA-48). Phenotypic confirmation of ESBL production on culture isolates was performed with the double-disk synergy test and ESBL + AmpC Screen Kit (ROSCO DIAGNOSTICA). For carbapenemase production, the confirmation was assessed using home-brew real-time PCR assays. The corresponding rectal swabs (eSwab™, Copan) were tested by eazyplex® SuperBug complete B Assay, according to the manufacturer's instructions.

Results: The eazyplex® SuperBug CRE system correctly detected ESBL-encoding genes in 40/46 ESBL-positive isolates, and in the 36/46 corresponding rectal swabs. The remaining 6 culture isolates and corresponding rectal swabs tested ESBL-negative in eazyplex® were all identified as TEM-116 producers, following Sanger sequencing results. This target is absent from this assay. Absolute concordance (100%) was observed in all isolates with phenotypes compatible with the presence of a carbapenemase, with or without an ESBL, based on susceptibility patterns and phenotypic inhibitory profiles. No false-positive results were observed with the eazyplex® SuperBug complete assays. Determinations performed with the eazyplex® SuperBug CRE system took 20 to 30 min. In case of multiple positive signals or very late signals, the testing should be repeated with longer incubation time.

		Real Time PCR	eazyplex® SuperBug CRE assay		** PCR and sequencing ESBL genes
		Bacterial isolate	Rectal swab	*Bacterial isolate	Bacterial isolate
No. of samples	Species (phenotype) isolated from rectal swab	Target detected (no. of isolates)	Target detected (no. of samples)	Target detected (no. of isolates)	Target detected (no. of isolates)
21	*Escherichia coli* (ESBL)	ND	CTX-M-1 (10), CTX-M-1 and CTX-M-9 (1), CTX-M-9 (8)		TEM-116 (2)
1	*Escherichia coli* (ESBL)	ND	Negative	CTX-M-1 (1)	
1	*Klebsiella pneumoniae* (ESBL)	ND	CTX-M-1 and NDM		
10	*Klebsiella pneumoniae* (ESBL)	ND	CTX-M-1 (10)		
2	*Klebsiella pneumoniae* (ESBL)	ND	Negative	CTX-M-1 (2)	
1	*Escherichia coli and Klebsiella pneumoniae* (ESBL)	ND	CTX-M-1 and CTX-M-9		
2	*Escherichia coli and Klebsiella pneumoniae* (ESBL)	ND	CTX-M-1 (2)		
1	*Citrobacter* sp. (ESBL)	ND	Negative	Negative	TEM-116 (1)
3	*Enterobacter cloacae complex* (ESBL)	ND	CTX-M-1 (1)		TEM-116 (2)
1	*Morganella morganii* (ESBL)	ND	Negative	CTX-M-1 (1)	
1	*Proteus penneri* (ESBL)	ND	Negative	Negative	TEM-116 (1)
1	*Klebsiella pneumoniae* (carbapenemase-producing)	KPC (1)	KPC		
1	*Klebsiella pneumoniae* (carbapenemase-producing)	KPC (1)	KPC + NDM + CTX-M-1		
1	*Escherichia coli* (carbapenemase-producing)	NDM (1)	NDM + CTX-M-1		
2	*Escherichia coli* (carbapenemase-producing)	OXA-48 (2)	OXA-48 (2)		
1	*Escherichia coli* (ESBL and carbapenemase-producing)	OXA-48 (1)	OXA-48, OXA-181, CTX-M-1, and CTX-M-9		
1	*Escherichia coli and Klebsiella pneumoniae* (ESBL and carbapenemase-producing)	OXA-48 (1)	CTX-M-1, and CTX-M-9		

ND = no done

* eazyplex® SuperBug CRE was done on isolates when the detection directly from rectal swab was negative

** Discordant results were resolved with PCR and sequencing of ESBL genes

Conclusions: This study demonstrated that the eazyplex® SuperBug CRE isothermal amplification assay represents a promising platform with optimal sensitivity for the routine detection of many of the most prevalent carbapenemases as well as CTX-M-type ESBLs in *Enterobacteriaceae* directly from rectal swabs with a rapid resolution time. The implementation of this system in routine clinical laboratories should provide clinicians with early valuable information for the accurate management of patients carrying ESBL and/or carbapenemase producing *Enterobacteriaceae.*

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