Evaluation of a Loop-Mediated Isothermal Amplification assay to detect Extended-Spectrum-β-Lactamase- and/or Carbapenemase-Producing *Enterobacteriaceae* and *Acinetobacter* directly from bronchoalveolar lavage fluid

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INTRODUCTION

Carbapenem resistance represents a developing **global public health threat** which translates into **increased mortality** in hospitalized patients **and hospital costs.**

Rapid detection of these resistant isolates **is essential** for adequate empirical treatment and infection control purposes.

The **aim** of this study was to **evaluate the efficacy of the eazyplex® SuperBug Complete A and the SuperBug CRE system** (Amplex Diagnostics GmbH, Gars-Bahnhof, Germany), based on loop-mediated isothermal amplification (LAMP), **to detect the presence of carbapenemases and/or CTX-M-type ESBLs** in *Acinetobacter* spp and *Enterobacteriaceae* directly **from bronchoalveolar lavage fluid** (BALF) samples.

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RESULTS & CONCLUSION

Table 1. *Enterobacteriaceae* strains tested with eazyplex® SuperBug CRE, sensitivity and detection value in minutes:seconds.

Strain	Resistance mechanism (*bla*)	Sensitivity (CFU/mL)	eazyplex® Superbug CRE (minutes:seconds)
K. pneumoniae	NDM	10^3	18:45
E. coli	NDM	10^2	10:30
	CTX-M-1	10^2	8:15
K. pneumoniae	NDM	10^3	NEGATIVE
	OXA-48	10^3	NEGATIVE
	CTX-M-1	10^3	12:00
K. pneumoniae	NDM	10^3	17:00
	CTX-M-1	10^2	16:30
E. coli	NDM	10^3	13:30
	CTX-M-1	10^2	22:15
K. pneumoniae	NDM	10^3	NEGATIVE
	OXA-48	10^3	NEGATIVE
	CTX-M-1	10^3	17:15
K. pneumoniae	OXA-48	10^3	NEGATIVE
K. pneumoniae	KPC	10^3	NEGATIVE
E. asburiae	KPC	10^2	25:30
	CTX-M-15	10^2	26:00
K. pneumoniae	KPC	10^3	21:00
E. coli	KPC	10^3	22:50
K. pneumoniae	OXA-48	10^3	18:00
E. coli	OXA-48	10^3	20:00
E. coli	VIM	10^2	19:45
K. pneumoniae	VIM	10^3	27:00
K. pneumoniae	IMP	10^3	NEGATIVE

Table 2. *Acinetobacter* spp strains tested with eazyplex® SuperBug Complete A , sensitivity and detection value in minutes:seconds.

Strain	Resistance mechanism (*bla*)	Sensitivity (CFU/mL)	eazyplex® Superbug Complete A (minutes:seconds)
A. baumannii	OXA-23	10^2	16:15
A. baumannii	OXA-23	10^3	11:45
A. baumannii	OXA-23	10^2	21:15
A. baumannii	OXA-40	10^2	13:15
A. baumannii	OXA-40	10^3	13:30
A. baumannii	OXA-40	10^2	17:00
A. baumannii	OXA-58	10^2	18:30
A. baumannii	OXA-58	10^3	16:15
A. baumannii	OXA-58	10^2	21:30
A. pitii	NDM	10^3	20:00
A. dijkshoorniae	NDM	10^3	18:30

The **eazyplex® SuperBug Complete A and SuperBug CRE** assays proved to be a **powerful tool for the detection of different carbapenemases as well as CTX-M-type ESBLs** in *Acinetobacter* spp and *Enterobacteriaceae* **directly from BALF** with a **rapid resolution** time and a **high sensitivity.**

MATERIALS & METHODS

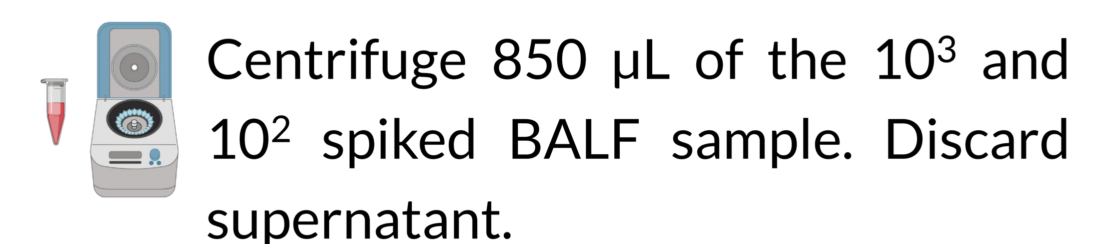
Negative BALF samples were selected and then spiked with an ESBL and/or carbapenemase-producing strain at a final concentration of 10^3 and 10^2 CFU/mL.

A total of 27 bacterial strains were evaluated:

- *Enterobacteriaceae:* OXA-48, NDM, VIM, CTX-M-1, CTX-M-15, KPC;
- *Acinetobacter* spp: OXA-23, OXA-40, OXA-58, NDM.

Isolate identification was performed with a MALDI-TOF/MS (Bruker Daltonics, Bremen, Germany) and detection of the genes encoding for ESBL and carbapenemases via conventional PCR.

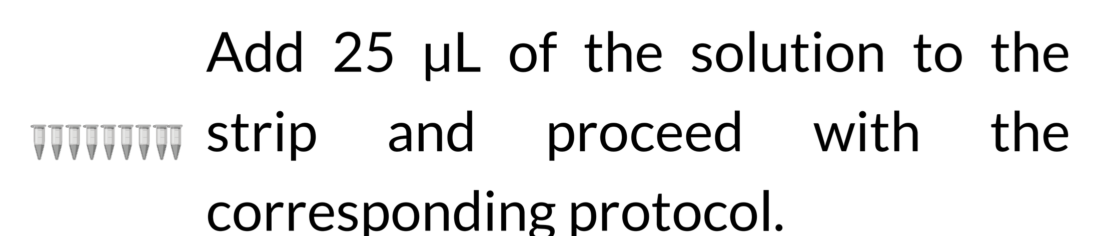
The protocol consisted in:



Centrifuge 850 μL of the 10^3 and 10^2 spiked BALF sample. Discard supernatant.

Resuspend in lysis buffer (RALF).

Incubate at 99° C for 2 minutes and centrifuge.



Add 25 μL of the solution to the strip and proceed with the corresponding protocol.

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