Evaluation of the BL-RED™ test for the rapid detection of third-generation cephalosporins resistance in *Enterobacterales* on early culture of positive blood cultures.

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Context

The spread of multidrug resistant *Enterobacterales* is a major public health problem. Bacteremia caused by ESBL-producing *Enterobacterales* are associated with increased rates of treatment failure, mortality and hospital costs. Delayed administration of effective antibiotic therapy is a major risk factor for increased mortality. Thus, rapid optimization of antibiotic therapy, according to the organism and its resistance profile, is a major objective. In recent years, rapid diagnostic tests have been developed to determine directly from blood cultures the identification of the microorganism involved and its resistance phenotype notably to beta-lactam antibiotics. These techniques help to promote the proper use of antibiotics by rapidly adapting an effective treatment and reducing the medico-economic impact through de-escalation of antibiotic therapy and shorter hospital stays.

Objective

The aim of this study was to evaluate the performances of the BL-RED™ electrochemical test (Beta-Lactamase Rapid Electrochemical Detection, CORIS BioConcept, Belgium) and another colorimetric technique routinely used in our laboratory β-LACTA™ (Bio-Rad, France) for the detection of third-generation cephalosporins (3GC) resistance in early subcultures of positive blood cultures, from a panel of strains of *Enterobacterales* from our laboratory with characterized resistance mechanism and positive blood cultures from patients prospectively included between April and July 2023.

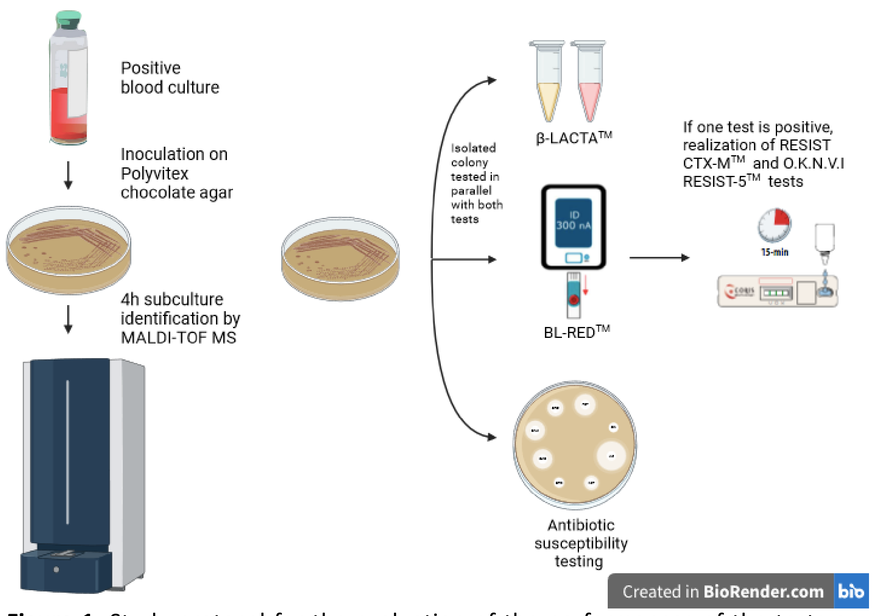

Figure 1. Study protocol for the evaluation of the performances of the tests on positive blood cultures.

Results

The distribution of the 123 strains isolated from prospectively included blood cultures and identified by MALDI-TOF mass spectrometry (Brucker, USA), as well as their resistance mechanism determined by performing an AST, is summarized in **Table 1**. All strains producing ESBL or plasmid mediated AmpC were screened for resistance genes by NGS sequencing.

All the discrepancies between the tests and our gold standard comparator method are shown in **Table 2**, with the strain species, the results of both tests and thephenotype determined by AST. 10 strains showed discordant results with 3GC resistance not detected by either test. One TEM-3 ESBL producing strain was detected by the β-LACTA™ test but not by the BL-RED™ test.

The global analytical performances of BL-RED™ test and β-LACTA™ tests for the detection of 3GC resistance are presented in **Table 3**.

Species	Wild-type	Low level penicillinase	High level penicillinase	Hyperproduced SHV	Inhibitor resistant penicillinase	OXA NS	Overproduced AmpC	Plasmid-mediated AmpC	ESBL	Total
Citrobacter koseri	1									1
Enterobacter cloacae complexe	5				1		3		2	11
Escherichia coli	26	2	18		9	5		2	8	70
Klebsiella oxytoca	3									3
Klebsiella pneumoniae	13		1	1		1		1	4	21
Klebsiella variicola	1									1
Klesbiella oxytoca	1									1
Morganella morganii	2									2
Proteus mirabilis	2	1								3
Salmonella spp.	1	1	1							3
Serratia sp.	7									7
Total	**62**	**4**	**20**	**1**	**10**	**6**	**3**	**3**	**14**	**123**

Table 1. Distribution of strains isolated from prospectively included blood cultures.

Discussion

In this study, we evaluated the performances in the detection of 3GC resistance of the BL-RED™ and β-LACTA™ tests. For both tests, it seems that there are interesting performances for the detection of 3GC resistance by production of ESBL, suggesting that these two tests may provide useful results to early therapeutic guidance, notably switching 3GC for carbapenems.

3GC resistance due to overproduced chromosomal AmpC or plasmid-mediated AmpC is poorly detected by both tests in our study.

Due to their principle based on the hydrolysis of 3GC analogs, both tests cannot detect 3GC resistance due to impermeability or efflux pumps, although these resistance mechanisms are less frequent than the production of beta-lactamases for *Enterobacterales*.

Our study has some limitations, notably its monocentric design. Moreover, we tested limited numbers of ESBL, chromosomal AmpC (group 3 *Enterobacterales*) or plasmid-mediated AmpC producing strains. The small number of 3GC resistant strains due to other enzymatic mechanisms, for instance hyperproduction of chromosomal enzymes OXY and SHV, NS and extended spectrum OXA or carbapenemases, is also a limitation.

One of the major advantages of the BL-RED™ test is that the reader gives a value, enabling traceability of results and avoiding uninterpretable results or operator-related errors which can occur with the β-LACTA™ test.

To rapidly optimize the antibiotic treatment for septic patients, we suggest a simple and affordable algorithm for positive blood cultures based on MALDI-TOF MS identification and BL-RED™ or β-LACTA™ test for the detection of 3GC resistant *Enterobacterales* (**Figure 2**).

Material and methods

The **BL-RED™ test** principle is based on the presence of a 3GC-analog substrate in the reagent, which is hydrolyzed in the presence of a 3GC-active beta-lactamase, releasing an electro-conductive product.

The **β-LACTA™ test** principle is based on the hydrolysis of a chromogenic cephalosporin analog substrate (HMRZ-86), resulting in a color change from yellow to red within 10 min for a positive test.

The **gold standard comparator method** realized in this study was an antibiotic susceptibility testing (AST) by the agar disc diffusion method according to EUCAST-SFM 2023 recommendations. 3GC resistance was determined with 10µg ceftazidime and 5µG cefotaxime SIRscan® discs (i2a, France). Strains categorized as resistant or susceptible at high exposure for ceftazidime and/or cefotaxime were considered resistant to 3GC.

Panel of *Enterobacterales* strains with characterized resistance mechanism

A panel of 56 *Enterobacterales* isolated at the department of bacteriology of Saint-Antoine Hospital from clinical samples was tested. Various beta-lactam resistance phenotypes were included: **3GC-susceptible strains (n = 27)** and **3GC-resistant strains (n = 29)**, with ESBL producing strains (n = 23), hyperproduced chromosomal or plasmid-mediated AmpC cephalosporinase producing strains (n = 3), hyperproduced OXY beta-lactamase producing strain (n = 1) and carbapenemase producing strains (n = 8). Presence of genes of ESBLs, plasmid-mediated AmpC cephalosporinases and carbapenemases was confirmed by specific PCR.

For each strain, 1mL of a 0.5 McFarland suspension was injected into sterile aerobic blood culture (BACT/ALERT® FA Plus, Biomérieux, France). Blood cultures were incubated for 16h at 35±2°C. Subcultures were grown on PolyViteX chocolate agar (Biomérieux, France), incubated for 4 hours at 35±2°C. Isolated colonies were identified by MALDI-TOF mass spectrometry (Brucker, USA), and AST was performed.

Positive blood cultures prospectively included

Positive patient blood cultures were prospectively included and tested between April and July 2023. Blood cultures flagged positive before 11 a.m. by the BACT/ALERT® VIRTUO® automated system (Biomérieux, France) were subcultured onto PolyViteX chocolate agar (Biomérieux, France), with a 4-hour incubation period at 35±2°C. Colonies present on the 4-hour subcultures identified by MALDI-TOF mass spectrometry (Brucker, USA) as *Enterobacterales* were tested.

For each blood culture, a colony isolated from 4-hour subcultures was tested in parallel by BL-RED™ test and by β-LACTA™ test. When one of the BL-RED™ test or β-LACTA™ test was positive, a colony was also tested by the RESIST CTX-M™ and O.K.N.V.I RESIST-5™ immunochromatographic tests.

The study protocol is represented in **Figure 1**.

Species	BL-RED™	β-LACTA™	Phenotype
Klebsiella oxytoca	Negative	Negative	HyperOXY
Enterobacter cloacae complexe	Negative	Negative	Overproduced chromosomal AmpC
Enterobacter cloacae complexe	Negative	Negative	Overproduced chromosomal AmpC
Enterobacter cloacae	Negative	Negative	Overproduced chromosomal AmpC
Enterobacter cloacae	Negative	Negative	Overproduced chromosomal AmpC
Citrobacter freundii	Negative	Negative	Overproduced chromosomal AmpC
Klebsiella pneumoniae	Negative	Negative	Oxacillinase narrow spectrum (OXA NS)
Klebsiella pneumoniae	Negative	Negative	Plasmid-mediated AmpC
Escherichia coli	Negative	Negative	Plasmid-mediated AmpC
Escherichia coli	Negative	Negative	Plasmid-mediated AmpC
Escherichia coli	Negative	Positive	TEM-3 ESBL

Table 2. Discrepancies between gold-standard and BL-RED™ or β-LACTA™ tests.

Method	Result	3GC		Sensitivity	Specificity	PPV*	NPV*	Accuracy (IC 95%)
		Resistant (n=49)	Susceptible (n=127)					
BL-RED™	Positive	38	0	78%	100%	100%	92%	94% (0.8909, 0.9684)
	Negative	11	127					
β-LACTA™	Positive	39	0	80%	100%	100%	93%	94% (0.898, 0.9724)
	Negative	10	127					

Table 3. Global analytical performances of BL-RED™ and β-LACTA™ tests for the detection of 3GC resistance.

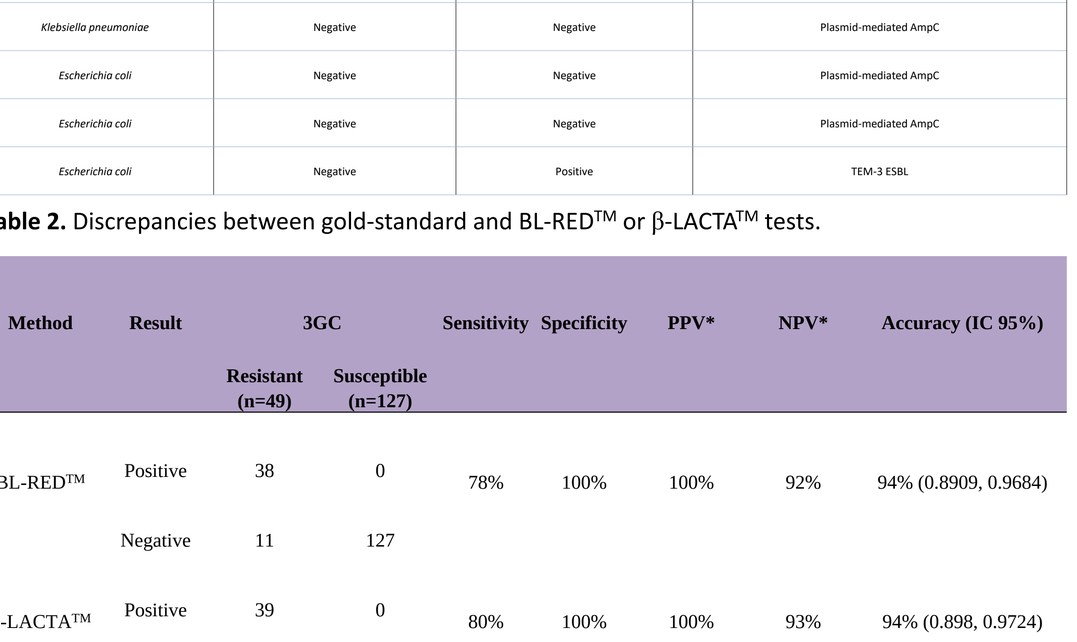

Figure 2. Algorithm proposed for the management of Enterobacterales positive blood cultures.

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