Early detection of extended-spectrum β-lactamase from blood culture positive for an *Enterobacteriaceae* using βLACTA test

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Abstract

Bacterial pellets from *Enterobacteriaceae* positive blood cultures prepared using ammonium chloride were tested for rapid detection of β-lactamase using the commercial βLACTA test and read after 30 minutes. During 7 months, 137 bacterial pellets were tested prospectively. βLACTA test exhibited a sensitivity of 75% and a specificity of 100% for the detection of third-generation cephalosporin resistance. False negative tests were mainly observed with hyperproduced chromosomal or plasmid-borne AmpC.

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Results of positive blood culture are important since they help to customize the antimicrobial therapy. MALDI-TOF MS has dramatically modified the impact of positive blood-culture results, especially for *Enterobacteriaceae* [1]. Indeed, correct identification at species level was obtained in less than 2 hours for 87% of cases [1,2]. However, early recognition of bacterial resistance mechanisms such as β-lactamases, directly from positive blood culture, remains challenging and important to early tailor the antimicrobial therapy.

Recently, a rapid commercial test to detect β-lactamases targeting third-generation cephalosporin (3GC) called the βLACTA test (Bio-Rad, Marnes-la-Coquette, France) was evaluated on colonies [3,4]. This test is based on the cleavage of a chromogenic cephalosporin, HMRZ-86. This cephalosporin contain a carboxypropyloxyimino group comparable to ceftazidime which protect this compound from hydrolysis by class A, C and D β-lactamase but is hydrolyzed in presence of β-lactamases such as ESBLs, carbapenemases (KPC, MBL) and acquired or derepressed AmpC [3–7].

The objective of our study was to apply the βLACTA test directly to ammonium chloride-prepared bacterial pellets from blood cultures positive for an *Enterobacteriaceae*. First, we tested this assay on blood cultures spiked with various *Enterobacteriaceae* strains exhibiting different antibiotic resistance mechanisms characterized molecularly [8,9]. Then, this method was tested prospectively on clinical blood cultures positive for an *Enterobacteriaceae* identified at species level by MALDI-TOF MS [2].

Spiked blood cultures were prepared as follow: the β-lactamase resistant and susceptible *Enterobacteriaceae* strains were subcultured twice on Columbia agar. Then, blood culture vials (BACTEC Lytic anaerobic/F) were inoculated with 5 ml of human blood containing ~3 cfu/ml of bacteria to obtain a detection time between 9 to 11 hours using the Bactec FX system (Becton Dickinson, Sparks, USA).

Bacterial pellets from spiked blood culture or clinical positive blood culture were prepared as reported [2,10]. Briefly, 5 ml from positive vial (BACTEC Lytic anaerobic/F or Plus aerobic/F or Peds/F) were mixed with 40 ml sterile water and centrifuged at 1000*g* for 10 min. The supernatant was removed and the pellet was suspended in 1 ml of ammonium chloride and centrifuged at 140*g* for 10 min. The supernatant was discarded and the pellet suspended in 200µl of water. The βLACTA test was performed as follow: 5µl of bacterial pellet was mixed with 25µl of βLACTA test reagents R1 and R2 in 96 wells plates (Corning, NY, USA) gently agitated and maintained at 20°C for 30 min before reading. Two reference strains of *Klebsiella pneumoniae* were used as positive and negative controls (ATCC BAA-1705, *bla*$^{KPC+}$; ATCC BAA-1706 *bla*$^{KPC-}$). The test was considered as positive or doubtful when the enzymatic reaction turned from yellow to red or orange.

For clinical blood culture positive for *Enterobacteriaceae*, bacterial pellets were used for direct antibiotic susceptibility testing using AST-N242 cards (VITEK2 with software version

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5.04, bioMérieux, Marcy-l'Etoile, France) as described [11]. EUCAST standards (version 2012) were used for categorical interpretation. Phenotypic tests, double disks synergy tests, cefepime ± clavulanate E-tests (Etest ESBL PM/PML, bioMérieux) and cefotetan ± cloxacillin E-test (AmpC Etest CN/CNI, bioMérieux) were used to investigate 3GC resistance and to confirm the presence of ESBL, AmpC or hyperproduced β-lactamase of *K.oxytoca* K1 [12–15].

The table presents the results on spiked blood cultures and on positive clinical blood cultures. All ESBL strains gave a positive reaction, except one that produced a doubtful color (*Escherichia coli* TEM-53). One of 2 *E. coli* with plasmid-borne AmpC gave a doubtful result, whereas 80% (12/15) of chromosomal wild type AmpC or derepressed AmpC were negative from species naturally producing AmpC β-lactamase. One *K. oxytoca* strain with K1 hyperproduction gave a doubtful reaction. Five tests with Oxa-48 (n=3), NDM (n=1) and KPC (n=1) remained negative.

For clinical blood cultures, 137 bacterial pellets were tested during 7 months. All 10 ESBL gave positive or doubtful results (Table 1). Two *K. oxytoca* β-lactamases with 3GC resistance and one *Hafnia alvei* were positive. All *Enterobacter* spp. and *Serratia marcescens* were negative.

Compared to phenotypic resistance to 3GC, the βLACTA test had a sensitivity of 75% (95%CI: 47.9–92.7%) and a specificity of 100% (95%CI: 97–100%) using data from clinical strains obtained from blood culture and considering doubtful and positive results as positive. Overall, the positive and negative predictive value were 100% (95%CI: 73.5–100%) and 96.8% (95%CI: 92–99.1%), respectively. Compared to the manufacturer's recommendations, we propose two adaptations for use with blood culture bacterial pellets: i) reading the test at 30 min, ii) interpretation of any colorimetric change to orange as doubtful, since this change may reflect a poor hydrolysis by β-lactamase from bacterial pellets or sometimes also seen with AmpC β-lactamase-producing *Enterobacteriaceae* with 3GC resistance or *K. oxytoca* with hyperproduced β-lactamase K1.

Our results confirm two recent studies that evaluated the βLACTA test on colonies of *Enterobacteriaceae* [3,4]. Both studies have observed an excellent sensitivity of 97.5%–100% to detect ESBL. In these studies, positive βLACTA tests were observed in 22% to 50% of derepressed AmpC and in 0% to 38% of plasmid-borne AmpC. Noteworthy, in a previous study using the chromogenic cephalosporin HMRZ-86 on blood cultures, only 42% of vials could be successfully tested, since lysed blood apparently interfered with the test's interpretation [16].

TABLE 1. βLACTA test results for spiked blood cultures and clinical blood cultures. Results are presented according to bacterial species and resistance mechanisms

Microorganisms (no. of isolates)	Phenotype or Genotype	No. with βLACTA result of:		
		Positive	Doubtful	Negative
Spiked blood cultures				
Escherichia coli (14)	ESBL	10	1[a]	
	Plasmid-borne AmpC		1	1
	OXA48			1
Klebsiella pneumoniae (12)	ESBL (2 NDM, 2 KPC, 1 VIM)	12		
Enterobacter spp. (11)	AmpC hyperproduction (1 Oxa-48)		1	9
	ESBL	1		
Serratia marcescens (2)	AmpC hyperproduction & KPC			1
	Wild AmpC (inducible AmpC) & Oxa-48			1
Morganella morganii (2)	AmpC hyperproduction & NDM			1
	ESBL	1		
Hafnia alvei (1)	Wild AmpC (inducible AmpC)	1		
Providencia stuartii (1)	Wild AmpC (inducible AmpC) & VIM	1		
Proteus vulgaris (1)	3GC susceptible			1
Klebsiella oxytoca (1)	K1 hyperproduction		1	
Clinical blood cultures				
Escherichia coli (85)	3GC susceptible			76
	AmpC			3
	ESBL	5	1[b]	
Klebsiella pneumoniae (28)	3GC susceptible			23
	ESBL	4		
	AmpC			1
Enterobacter spp. (8)	Wild AmpC (inducible AmpC)			7
	AmpC hyperproduction			1
Klebsiella oxytoca (5)	3GC susceptible			3
	K1 hyperproduction	2		
Serratia marcescens (4)	Wild AmpC (inducible AmpC)			4
Proteus spp. (4)	3GC susceptible			4
Citrobacter koseri (1)	3GC susceptible			1
Salmonella Enteritidis (1)	3GC susceptible			1
Hafnia alvei (1)	Wild AmpC (inducible AmpC)	1		

[a]TEM-53
[b]mixed positive blood culture for *E. coli* ESBL and *K. pneumoniae* 3GC susceptible (this mixed culture was detected only following subculture, being not detected neither by Gram staining of blood culture suspension nor by MALDI-TOF performed on the bacterial pellet, which identified only one species with a score >2)

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The quality of our ammonium chloride-based pellet used here apparently overcame this interference. However, larger number of clinical isolates should be tested in the future to confirm the fiability of this test.

An alternative rapid test for the detection of ESBL from positive blood cultures is based on the colorimetric detection of hydrolysis of cefotaxime in presence of a pH indicator [17]. The results of this test applied to spiked blood cultures or blood cultures bacterial pellets showed an excellent sensitivity and specificity [17,18]. The authors mentioned that few ESBL strains susceptible to cefotaxime were not detected. MALDI-TOF assays allowing detection of extended β-lactamase or carbapenemase directly from blood-culture bacterial pellets in 90 minutes to 4 hours were also described [19,20].

In conclusion, the application of the βLACTA test on ammonium chloride-prepared bacterial pellets from blood culture was found reliable to detect ESBL with a 100% positive predictive value and may help clinicians managing patients with Gram negative bacteremia.

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Conflicts of interest

The authors have no conflict of interest.

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