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 plasmidborne 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-B6. This cephalosporin contain a carboxypropyloxyimino group comparable to cefazidime 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 anerobic/F or Plus aerobic/F or Peds/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 1000g for 10 min. The supernatant was removed and the pellet was suspended in 1 ml of ammonium chloride and centrifuged at 140g 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, blakPC⁰; ATCC BAA-1706 blakPCK⁺). 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...
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 ± clavulenate E-tests (Etest ESBL PM/PML, bio-Mérieux) 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-lactamases 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        | 1        |
|                                  | Plasmid-borne AmpC    | 1         |          |          |
|                                  | OXA-48                | 1         |          |          |
| Klebsiella pneumoniae (12)       | ESBL (2 NDM, 2 KPC, 1 VIM) | 12    | 9        |          |
| Enterobacter spp. (11)           | AmpC hyperproduction (1 Oxa-48) | 1   |          |          |
| Serratia marcescens (2)          | AmpC hyperproduction & KPC | 1   |          |          |
| Morganella morganii (2)          | AmpC hyperproduction & NDM | 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        | 3        |          |
|                                  | AmpC                  | 23        |          |          |
|                                  | ESBL                  | 5         | 1        |          |
| Klebsiella pneumoniae (28)       | 3GC susceptible       | 23        |          |          |
|                                  | ESBL                  | 4         |          |          |
| Enterobacter spp. (8)            | Wild AmpC (inducible AmpC) | 7   |          |          |
|                                  | AmpC hyperproduction  | 3         |          |          |
| 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   |          |          |

*TEM-53
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)
The quality of our ammonium chloride-based pellet used here apparently overcame this interference. However, larger numbers of clinical isolates should be tested in the future to confirm the reliability 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.

References

[1] Clerc O, Prod’hom G, Vogne C, Bizzini A, Calandra T, Greub G. Impact of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALdi-TOF) on the clinical management of patients with gram-negative bacteremia: a prospective observational study. Clin Infect Dis 2012.

[2] Prod’hom G, Bizzini A, Durussel C, Bille J, Greub G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol 2010;48(4):1481–3.

[3] Renvoie A, Decr D, Amars-Guerel R, Huang TD, Jost C, Podgajen I, et al. Evaluation of the betaLacta test, a rapid test detecting resistance to third-generation cephalosporins in clinical strains of Enterobacteriaceae. J Clin Microbiol 2013;51(12):4012–7.

[4] Morosini MI, Garcia-Castillo M, Tato M, Gijon D, Valverde A, Ruiz-Garbajosa P, et al. Rapid detection of beta-lactamase-hydrolyzing extended-spectrum cephalosporins in Enterobacteriaceae by use of the new chromogenic betaLact test. J Clin Microbiol 2014;52(5):1741–4.

[5] Hanaki H, Kubo R, Nakano T, Kurihara M, Sunagawa K. Characterization of HMRZ-86: a novel chromogenic cephalosporin for the detection of extended-spectrum beta-lactamases. J Antimicrob Chemother 2004;53(5):888–9.

[6] Hanaki H, Yamazaki H, Harada H, Kubo R, Kobayashi T, Atsuda K, et al. The synthesis of 7-substituted-3-dinitrostyryl cephalosporins and their ability for detecting extended spectrum beta-lactamases (ESBLs). J Antimicrob Chemother 2005;58(1):69–73.

[7] Hanaki H, Koide Y, Yamazaki H, Kubo R, Nakano T, Atsuda K, et al. Substrate specificity of HMRZ-86 for beta-lactamases, including extended-spectrum beta-lactamases (ESBLs). J Infect Chemother Off J Jpn Soc Chemother 2007;13(6):390–5.

[8] Larüge MF, Zinzius C, Wenger A, Bille J, Poirel L, Nordmann P. Extended-spectrum beta-lactamases of the CTX-M type now in Switzerland. Antimicrob Agents Chemother 2007;51(8):2855–60.

[9] Vogne C, Prod’Hom G, Jaton K, Decosterd L, Greub G. A simple, robust and rapid approach to detect carbapenemases in Gram negative isolates by MALDI-TOF mass spectrometry: validation with triple quadrupole tandem mass spectrometry, microarray and PCR. Clin Microbiol Infect 2014.

[10] Croxatto A, Prod’Hom G, Durussel C, Greub G. Preparation of a blood culture pellet for rapid bacterial identification and antibiotic susceptibility testing. J Vis Exp: JoVE 2014;(92):e51985.

[11] Prod’hom G, Durussel C, Greub G. A simple blood-culture bacterial pellet preparation for faster accurate direct bacterial identification and antibiotic susceptibility testing with the VITEK 2 system. J Med Microbiol 2013;62(Pt 5):773–7.

[12] Edquist P, Ringman P, Liljequist BO, Wissel KT, Giske G. Phenotypic detection of plasmid-acquired AmpC in Escherichia coli—evaluation of screening criteria and performance of two commercial methods for the phenotypic confirmation of AmpC production. Eur J Clin Microbiol Infect Dis 2013;32(9):1205–10.

[13] Drieu L, Brossier F, Sougkoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 2008;14(Suppl. 1):90–103.

[14] Sturenburg E, Sobottka I, Noor D, Laufs R, Mack D. Evaluation of a new cefepime-clavulanate ESBL Etst to detect extended-spectrum beta-lactamases in an Enterobacteriaceae strain collection. J Antimicrob Chemother 2004;54(1):134–8.

[15] Potz NA, Colman M, Warner M, Reynolds K, Livermore DM. False-positive extended-spectrum beta-lactamase tests for Klebsiella oxytox acia strains hyper-producing K1 beta-lactamase. J Antimicrob Chemother 2004;53(3):545–7.

[16] Jain S, Andrews J, Fraise A, Brenwald N. Rapid detection of extended-spectrum beta-lactamase-producing Gram-negative bacilli in blood cultures. J Antimicrob Chemother 2007;60(3):652–4.

[17] Nordmann P, Dortet L, Poirel L. Rapid detection of extended-spectrum beta-lactamase-producing Gram-negative bacilli in blood cultures. J Antimicrob Chemother 2007;60(3):652–4.

[18] Drieu L, Brossier F, Sougkoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 2008;14(Suppl. 1):90–103.

[19] Sturenburg E, Sobottka I, Noor D, Laufs R, Mack D. Evaluation of a new cefepime-clavulanate ESBL Etst to detect extended-spectrum beta-lactamases in an Enterobacteriaceae strain collection. J Antimicrob Chemother 2004;54(1):134–8.

[20] Potz NA, Colman M, Warner M, Reynolds K, Livermore DM. False-positive extended-spectrum beta-lactamase tests for Klebsiella oxytocia strains hyper-producing K1 beta-lactamase. J Antimicrob Chemother 2004;53(3):545–7.

[21] Jain S, Andrews J, Fraise A, Brenwald N. Rapid detection of extended-spectrum beta-lactamase-producing Gram-negative bacilli in blood cultures. J Antimicrob Chemother 2007;60(3):652–4.

[22] Drieu L, Brossier F, Sougkoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 2008;14(Suppl. 1):90–103.

[23] Sturenburg E, Sobottka I, Noor D, Laufs R, Mack D. Evaluation of a new cefepime-clavulanate ESBL Etst to detect extended-spectrum beta-lactamases in an Enterobacteriaceae strain collection. J Antimicrob Chemother 2004;54(1):134–8.

[24] Potz NA, Colman M, Warner M, Reynolds K, Livermore DM. False-positive extended-spectrum beta-lactamase tests for Klebsiella oxytocia strains hyper-producing K1 beta-lactamase. J Antimicrob Chemother 2004;53(3):545–7.

[25] Jain S, Andrews J, Fraise A, Brenwald N. Rapid detection of extended-spectrum beta-lactamase-producing Gram-negative bacilli in blood cultures. J Antimicrob Chemother 2007;60(3):652–4.

[26] Drieu L, Brossier F, Sougkoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 2008;14(Suppl. 1):90–103.