New Microbiologica, 44, 3, 161-163, 2021, ISSN 1121-7138

FULL PAPER

Evaluation of the new BL-RED (β-Lactamase Rapid Electrochemical Detection) test in positive blood culture broths

Laurine Millière, Claire Duployez, Caroline Loïez, Frédéric Wallet

Service de Bactériologie-Hygiène, Centre de Biologie Pathologie, CHRU de Lille, 59037

SUMMARY

The rapid detection of extended-spectrum β-lactamase Enterobacterales (ESBL-E) in a positive blood culture is important in order to initiate an appropriate antibiotic therapy and thus decrease mortality. We evaluated the new BL-RED (β-Lactamase Rapid Electrochemical Detection) test in 100 positive blood culture broths to detect (in ten minutes) the presence or absence of ESBL-E. The BL-RED test appears to be an easy, rapid and reliable test to detect the presence of ESBL directly in Gram negative bacilli-positive blood culture broths, with good performances (sensibility =97.3%, specificity =90.5%, predictive positive value =85.7% and predictive negative value =98.3%). This test could be useful for therapeutic decisions and adjustments of sepsis empirical antibiotic therapy, particularly in wards where the ecology is unfavorable, such as in intensive care units.

Received April 12, 2021 *Accepted* September 27, 2021

INTRODUCTION

In recent decades, antimicrobial resistance has become a major threat to public health worldwide. Among *Enterobacterales*, the production of β-lactamases is the most prevalent type of β-lactam resistance. Among these β-lactamases, extended-spectrum β-lactamase (ESBL) and overproduced cephalosporinase enzymes are the major representative mechanisms hydrolyzing the majority of β-lactams, including third-generation cephalosporins (3GC) except carbapenems (Drieux *et al*., 2008; Bush *et al*., 2020). In case of sepsis, good and rapid microbiological documentation is of paramount importance for the success of therapeutic strategy, as well as for a quick start of adequate treatment. Therefore, the rapid detection of ESBL *Enterobacterales* in a positive blood culture is important for determining an appropriate antibiotic therapy with a decrease in mortality, thereby avoiding emerging antibiotic resistance and longer hospital stay (Kollef *et al*., 2000; Harbarth *et al*., 2003). Even if it is now possible to perform the antimicrobial susceptibility test directly on positive blood broth, 8-10 hours are needed to obtain the result for antimicrobial β-lactam susceptibilities requires using the Vitek 2 platform (Hogan *et al*., 2019; Höring *et al*., 2019). Recently, a new test called BL-RED (β-Lactamase Rapid Electrochemical Detection) test developed by Coris BioConcept (Gembloux, Belgium) is able to detect ESBL activity in ten minutes, directly on positive blood culture broth with a rod morphology evocating *Enterobacterales* on Gram staining. The aim of this study was to evaluate this new test on 100 positive blood cultures showing Gram-negative bacilli (GNB) staining evocating *Enterobacterales*.

Key words:
Blood culture, Sepsis, Third generation cephalosporins resistance, ESBL detection, Rapid test.

Corresponding author:
F. Wallet, M.D.
E-mail: frederic.wallet@chru-lille.fr

MATERIALS AND METHODS

One hundred positive blood culture broths with GNB staining evocating *Enterobacterales* were tested. These samples were taken from patients with a context of antimicrobial treatment or hospitalization in the three preceding months. The gold standard to evaluate this new test was the performed and expertized antimicrobial susceptibility test using the Vitek 2 AST-N233 and AST-XN05 cards (BioMérieux, Marcy-l'Étoile, France) associated with the MASTDISCS ID ESBL detection disc diffusion tests (Mast Diagnostics, Amiens, France) to confirm the presence of ESBL or overproduction of cephalosporinase (Drieux *et al*., 2008; Nourisson *et al*., 2015). In case of carbapenem resistance,

the O.K.N.V.I. Resist Coris test (CorisBioconcept, Gembloux, Belgium) allowed us to type the carbapenemase. The BL-RED test is based on a voltametric phenotypic test detecting ESBL activity with disposable carbon screen printed sensors using a 3GC-like β-lactamase chromogenic substrate. The principle of the electrochemical test is based on the hydrolysis of its β-lactam cycle, with the substratum converted into a catalytic product whose specific oxidation peak is detected as analytical response. The intensity of the peak current is proportional to the amount of ESBL, thus indicating the number of ESBL-producing bacteria in the sample. The tests were performed as recommended by the manufacturer, using 1.5 mL of blood culture broth; results were interpreted after 10 minutes.

RESULTS

Among the 100 GNB observed after Gram staining performed on blood culture broth, identification by MALDI-TOF spectrometry was as follows: 32 *Escherichia coli*, 31 *Klebsiella pneumoniae*, 2 *K. oxytoca*, 1 *K. variicola*, 17 *Enterobacter cloacae*, 6 *K. aerogenes*, 3 *Serratia marcescens*, 1 *Citrobacter freundii*, 1 *Morganella morganii*, 5 *Pseudomonas aeruginosa* and 1 *Acinetobacter junii*. The results of the new test were reported in *Table 1* in comparison with our gold standard, allowing classification of the presence of the resistance support. These results allowed us to determine a sensibility =97.3%, a specificity =90.5%, a predictive positive value (PPV) =85.7%, and a predictive negative value (PNV) =98.3%.

DISCUSSION

Compared to the princeps test developed in 2018, this second-generation test shows identical performance (sensibility =97.3/100%; specificity =90.5/80%) for the direct detection of resistance to 3GC directly on GNB-positive blood culture broths (Betelli *et al.*, 2018). The main advantage of this new test is the time needed to obtain an objectively measured result (10 minutes) compared to the longer time (2 hours 30 minutes) of the old version. In addition to their simplicity, low cost, speed and sensitivity, electrochemical measurements can be carried out in colored and turbid environments such as blood and allow both objective measurements and the traceability of current signals. In comparison to this new test, previous studies evaluated the rapid detection of ESBL-producing *Enterobacterales* (ESBL-E) in blood cultures using a colorimetric β-lacta test. Dortet *et al.* (Dortet *et al.*, 2015) evaluated the ESBL NDP test directly from blood cultures (n=211) with a sensitivity, a specificity, a PPV and a NPV of 100% for the detection of ESBL-E within 30 minutes. Walewski *et al.* (Walewski *et al.*, 2015) used the β-lacta test® (Bio-Rad, Marnes-la-Coquette, France) for identifying ESBL-E from positive blood cultures (n=132), after treatment with saponin and two washes, with a sensibility of 95.7% and a specificity of 100%. Compain *et al.* (Compain *et al.*, 2015) evaluated the β-lacta test® (Bio-Rad, Marnes-la-Coquette, France) on 3-hour incubated solid medium subcultures from positive blood cultures (n=108) with a sensitivity of 84.8%, a specificity of 100%, a PPV of 100% and a PNV of 94%. Endly, Hasso *et al.* (Hasso *et al.*, 2017) evaluated the β-lacta

Table 1 - *BL-RED test results of the 100 GNB positive blood culture broths.*

Species (n)	*Gold standard*	*BL-RED*		*Index range (electrochemical signal)*	*Interpretation*
		Neg	*Pos*		
E. coli (32)	24 non ESBL (9 wild type, 12 TEM, 3 IRT)	24	0	0	24 TN
	8 ESBL	0	8	1160-3169	8 TP
K. pneumoniae (31)	7 wild type	6	1	0-1360	6 TN – *1 FP*
	18 ESBL	1	17	0-3629	17 TP – *1 FN*
	3 ESBL + OXA-48	0	3	30-2500	3 TP
	2 OXA-48	2	0	0	2 TN
	1 plasmid cephalosporinase	1	0	0	1 TN
K. oxytoca (2)	2 wild type	1	1	0-2500	1 TP – *1 FP*
K. variicola (1)	1 ESBL	0	1	2400	1 TP
E. cloacae (17)	4 wild type	4	0	0	4 TN
	8 overproduced cephalosporinase	5	3	0-299	5 TN – *3 FP*
	5 ESBL	0	5	1370-2920	5 TP
E. aerogenes (6)	6 wild type	6	0	0	6 TN
S. marcescens (3)	2 wild type	2	0	0	2 TN
	1 ESBL	0	1	490	1 TP
C. freundii (1)	1 wild type	1	0	0	1 TN
M. morganii (1)	1 overproduced cephalosporinase	0	1	100	*1 FP*
P. aeruginosa (5)	5 non ESBL	5	0	0	5 TN
A. junii (1)	1 non ESBL	1	0	0	1 TN

IRT = Inhibitor Resistant TEM; TN = True Negative result; TP = True Postive result; FN = False Negative result; FP = False Positive result; OXA-48 = OXA-48 carbapenemase.

test® (Bio-Rad, Marnes-la-Coquette, France) for detection of ESBL-producing organisms using smudge plates prepared from positive blood culture broths (n=269) with a sensitivity of 100% and a specificity of 97.8%. All these studies evaluated a colorimetric test, and even if the authors did not observe uninterpretable color, the interpretation may be subjective when the result is yellow-orange or red-orange. In comparison to these tests, the BL-RED test gives an objective electro-chemical signal allowing reliable interpretation of a positive or negative result. The BL-RED test may be considered an efficient test for detection of ESBL in blood culture, but not for detection of all 3GC-resistant bacteria. Indeed, among the 6 false positive tests, 4 concerned *Enterobacterales* resistant to cephalosporin by other mechanisms such as ampC or overproduction of penicillinase for *K.oxytoca*. Even if no ESBL was detected in these cases, the positive BL-RED test gave interesting information to the clinician regarding probable cephalosporin resistance. On the other hand, the failure to detect an overexpressed cephalosporinase phenotype may be a limit of the BL-RED test: a negative BL-RED test does not ensure that 3GC is efficient. The BL-RED test should be used to allow antibiotic incrementation (use of broader spectrum antibiotic) when it is positive. This rapid diagnostic test will be useful for therapeutic decisions and adjustments in empirical antibiotic therapy in order to rapidly detect sepsis due to ESBL-E, particularly when the ecology is unfavorable, as in intensive care units.

In conclusion, the BL-RED test appears to be an easy, rapid and reliable test to detect the presence of ESBL directly on *Enterobacterales* positive blood culture broths. The test is easy to increment in the workflow of the bacteriological work in a routine laboratory. Depending on the ecology of each hospital, the microbiologist may propose to clinicians the detection of this resistance for certain wards to quickly adapt the antibiotic prescription.

Acknowledgements

BL-RED tests were provided by Coris BioConcept laboratory (Gembloux, Belgium), but the company had no influence on the study design, data collection, evaluation or manuscript preparation.

We thanks Jean-Marie Hoornaert and Michel Lambot for their support thoughout the study.

All authors have no conflicts of interest to declare.

References

Betelli L., Neuwirth C., Solanas S., Chantemesse B., Vienney F., et al. (2018). A voltammetric test for the rapid discrimination of β-lactamase-producing *Enterobacteriaceae* in blood cultures. *Talanta*. **184**, 210-218.

Bush K., Bradford P.A. (2020). Epidemiology of β-Lactamase-Producing Pathogens. *Clin. Microbiol. Rev.* **33**, e00047-19.

Compain F., Bensekhri H., Rostane H., Mainardi J.L., Lavollay M. (2015). β LACTA test for rapid detection of Enterobacteriaceae resistant to third-generation cephalosporins from positive blood cultures using briefly incubated solid medium cultures. *J. Med. Microbiol*. **64**, 1256-1259.

Dortet L., Poirel L., Nordmann P. (2015). Rapid detection of ESBL-producing *Enterobacteriaceae* in blood cultures. *Emerg. Infect. Dis*. **21**, 504-507.

Drieux L., Brossier F., Sougakoff W., Jarlier V. (2008). Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. *Clin. Microbiol. Infect.* **14** (Suppl. 1), 90-103.

Harbarth S., Garbino J., Pugin J., Romand J.A., Lew D. et al. (2003). Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. *Am. J. Med*. **115**, 529-535.

Hasso M., Porter V., Simor A.E. (2017). Evaluation of the β-Lacta Test for detection of Extended-Spectrum-β-Lactamase (ESBL)-producing organisms directly from positive blood cultures by use of smudge plates. *J. Clin. Microbiol.* **55**, 3560-3562.

Hogan C.A., Watz N., Budvytiene I., Banaei N. (2019). Rapid antimicrobial susceptibility testing by VITEK®2 directly from blood cultures in patients with Gram-negative rod bacteremia. *Diagn. Microbiol. Infect. Dis.* **94**, 116-121.

Höring S., Massarani A.S., Löffler B., Rödel J. (2019). Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms. *Eur. J. Clin. Microbiol. Infect. Dis*. **38**, 471-478.

Kollef MH. (2000). Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. *Clin. Infect. Dis.* **31** (Suppl. 4), S131-138.

Nourrisson C., Tan R.N., Hennequin C., Gibold L, Bonnet R., et al. (2015). The MAST® D68C test: an interesting tool for detecting extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. *Eur J Clin Microbiol Infect Dis*. **34**, 975-983.

Walewski V., Podglajen I., Lefeuvre P., Dutasta F., Neuschwander A., et al. (2015). Early detection with the β-LACTA™ test of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in blood cultures. *Diagn. Microbiol. Infect. Dis*. **83**, 216-218.