Evaluation of a lateral flow immunoassay to detect CTX-M extended-spectrum β-lactamases (ESBL) directly from positive blood cultures for its potential use in Antimicrobial Stewardship programs

Emilio Cendejas-Bueno
María del Pilar Romero-Gómez
Iker Falcés-Romero
Alfonso Aranda-Díaz
Diana García-Ballesteros
Jesús Mingorance
Julio García-Rodríguez

Clinical Microbiology Department, Hospital La Paz, IdiPaz, Madrid, Spain

ABSTRACT

Background. Bloodstream infections (BSI) caused by extended-spectrum beta-lactamases Enterobacteriaceae (ESBL-E) are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed. Our aim was to evaluate the anticipation of ESBLs detection and the potential improvement of the time response of the Vitek 2 System (Biomerieux; France).

Methods. We compared this lateral flow immunoassay when used directly on fluid from positive blood cultures to the VITEK2 AST system. We evaluated 80 isolates, 61 tested directly on fluid from positive blood cultures and 19 tested on fluid from blood cultures spiked with known ESBL positive and negative organisms.

Results. The concordance between the CTX-LFIA and the reference method (Vitek 2) had a Cohen’s Kappa coefficient of 0.97, indicating a particularly good correlation between both compared methods.

Conclusion. This lateral flow immunoassay can be more rapid than the Vitek 2 for earlier presumptive identification of CTX-M ESBLs and can be useful to anticipate results and the adjustment of antimicrobial therapy.

Keywords: lateral flow immunoassay; extended-spectrum beta-lactamase; positive blood cultures; VITEK2

INTRODUCTION

The spread of extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-E) is a growing public health threat worldwide. In Spain, since 2000, the percentage of extended-
spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* has been increasing, mostly seen in cases of urinary tract infections [1]. Bloodstream infections (BSI) caused by ESBL-E are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed [2]. An empirically appropriate treatment is important to reduce mortality and complications [3].

To identify resistance mechanisms, such as carbapenemases and ESBLs, new molecular and non-molecular methods are being developed [4–7]. CTX-M MULTI (CTX-LFIA) (NG biotech, France) is a lateral flow immunoassay for detecting CTX-M ESBL producers. The system has been validated for use directly from colonies. Our aim was to find a diagnostic tool than can anticipate de ESBLs detection and improve the time response of the Vitek 2 System (BioMérieux; France) and if this LFIA could be implemented as microbiological tool in antimicrobial stewardship programs.

**MATERIAL AND METHODS**

We tested eighty isolates of *Enterobacteriales* from blood cultures in this study. The isolates were the following: sixty-one consecutive routine positive blood cultures detected in our laboratory between March and June of 2019, and nineteen stored gram-negative blood culture isolates, including both positive and negative ESBLs isolates, which were evaluated from spiked blood cultures. These nineteen isolates (seventeen positive ESBLs and two negative isolates) were included due to the low proportion of positive ESBLs isolates in the routine work of our laboratory. All the isolates were identified by mass spectrometry (MALDI-TOF, Bruker, Germany) following the procedures described before [8].

Susceptibility testing. Susceptibility testing was performed directly from blood cultures by determining MIC values and ESBL screening using the Vitek 2 System (BioMérieux; France) [8].

ESBL CTX-LFIA test using spiked blood cultures. For spiked blood cultures BD BACTEC TM Plus aerobic and anaerobic Culture Vials (Becton Dickinson, Madrid, Spain) were inoculated with 10 ml of blood from healthy volunteers and each bottle was inoculated with 500 μl of a suspension adjusted to 10^5 bacteria/ml in 0.9% sodium chloride and incubated at 35°C with agitation in a BACTEC FX automated blood culture system until bottles flagged positive. For control tests, the bottles were inoculated with 10 ml of blood from healthy volunteers and 100 μl of 0.9% sodium chloride.

ESBL CTX-LFIA test. The operating procedure to perform the CTX-LFIA test directly on fluid from blood cultures was the following: sample preparation followed the MALDI-TOF direct identification protocol described by Romero-Gómez et al [8]. The following procedure for inoculating the LFIA cards directly from positive blood culture bottles was done: a 4-ml aliquot was centrifuged at 140 g for 5 min. The supernatant was removed and transferred to a new tube, and then centrifuged at 16,000g for 10 min. The supernatant was discarded, and the sediment was used to make a bacterial suspension adjusted to a McFarland standard of 0.7–1. After this, one hundred microliters of the mixture were deposited in the CTX-LFIA cassette, and the result was read fifteen minutes after sample deposition as the manufacturer’s instructions describe. The test was read in the following manner: fifteen minutes after sample deposition the test line is checked versus the control line. This LFIA test has the CE marking that authorizes its marketing for in vitro diagnostics. The discordant results were corroborated by an in-house PCR to detect CTX-M.

**DISCUSSION**

In this study, the performance of CTX-LFIA rapid diagnostic tests for the detection of ESBL directly on fluid from blood cultures was evaluated. These results compare favorably with the 100% of correct CTX-M identifications published recently by Bianco et al. and Bernabeu et al. [9,10]. However, we had one discordant result. We studied this isolate to ensure that it was a CTX-M isolate. The molecular analysis from colonies was positive for the CTX-M ESBL type. Therefore, this CTX-LFIA result was a false negative. We evaluated four species of *Enterobacteriaceae* that include the vast majority of our ESBL isolates (near the 90 percent of the total). CTX-M β-lactamasases are predominant in Spain, and are one of the main causes of healthcare-associated ESBL-producing *E. coli* bacteremia of urinary origin in Spain [11,12]. We did not observe any additional resistance mechanisms in the routine isolates, although the CTX-LFIA can detect the CTX-M enzymes in combination with other ESBLs and other antimicrobial resistance mechanisms [9].

The performance of this CTX-LFIA directly on fluid from blood cultures offers a fast identification of ESBL-E. This tool may
be useful in elderly patients with bacteremia/sepsis/septic shock after a urinary tract infection to adjust the treatment as soon as the blood culture flags positive. It is described in the literature that elderly people in nursing homes had a risk around 40% higher than their community-dwelling peers of having antibiotic-resistant Enterobacteriaceae cultured from their urine samples [13] and almost one in five long term care facilities residents is colonized with ESBL-E [14]. Based on the results obtained in this evaluation the implementation of the CTX-LFIA in our workflow would anticipate the ESBL screening of CTX-M type at least 12 to 24 hours respect to the routine workflow implemented currently [8]. In addition, we did not observe interferences in the interpretation of the results when the CTX-LFIA is performed directly from the pellet. The additional time in the sample processing in our routine work is only the fifteen minutes of the CTX-LFIA.

As we have observed with our clinical isolates, this CTX-LFIA can be also especially useful in positive blood cultures in patients admitted in the emergency room. Reports have also described ESBL-producing E. coli as a cause of bloodstream infections associated with community-onset urinary tract infections [12,15]. Different prevalence of ESBL-producing bacteria has been found in many studies [16–20]. Bloodstream infections caused by ESBL-E are associated with high rates of treatment failure and increased mortality, especially when appropriate antimicrobial therapy is delayed [2]. This CTX-LFIA can be useful in sepsis/bacteremia cases in which the patients are treated empirically with a third-generation cephalosporin. It allows escalation of the treatment 24 hours sooner than antimicrobial susceptibility testing used in our institution for positive blood cultures. This CTX-LFIA can help to ensure an appropriate antimicrobial treatment for these ESBL microorganisms sooner, which is important to reduce mortality and complications [3]. Negative results should be managed carefully, due to other ESBLs (SHV, TEM...) that can be present. In case of negative result of this LFIA, targeted or de-escalation of antimicrobial therapy must be guided by antibiogram results and not by the result of this CTX-LFIA.

We observed a great correlation between our AST system and the CTX-LFIA, although the ESBL epidemiological situation of Spain, in particular our hospital, brought on this good correlation [11]. This fact can make the CTX-LFIA an epidemiological surveillance method of ESBL in our context and can help to detect changes in the ESBL bacteremia distribution in our hospital ecology.

In addition, this assay combined with other rapid carbapenemase detection methods might be especially useful in antimicrobial stewardship programs. The rapid identification of resistance mechanisms is one of the major efforts that the clinical microbiology laboratory should do to implement these rapid tests in the routine work of an antimicrobial stewardship program [21].

This evaluation has two limitations. Due to the low number of isolates evaluated and the low incidence of bacteremia due to Enterobacteriales as Proteus spp., Salmonella spp. or Raoultella spp., we did not evaluate isolates belonging to these species. The second limitation is the absence of other resistance mechanisms as carbapenemases or AmpC in the isolates evaluated. We only had in our evaluated isolates one additional resistance mechanism in a K. pneumoniae (OXA-48).

Overall, the CTX-LFIA showed good correlation with our routine instrument directly from the positive blood cultures. It can be useful to escalate treatment of bacteremia/sepsis and septic shock of community-onset correctly and promptly, although prospective studies should be performed to corroborate this issue and the utility in the real clinical setting.

**FUNDING**

None to declare
CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCE

1. Angel Diaz M, Ramon Hernandez J, Martinez-Martinez L, Rodriguez-Bano J, Pascual A. [Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Spanish hospitals: 2nd multicenter study [GEIH-BLEE project, 2006]]. Enferm. Infec. Microbiol. Clin. 2009;27:503–10. doi: 10.1016/j.eimc.2008.09.006

2. Russo A, Falcone M, Gutierrez-Gutierrez B, Calbo E, Almirante B, Villal PL, et al. Predictors of outcome in patients with severe sepsis or septic shock due to extended-spectrum beta-lactamase-producing Enterobacteriaceae. Int. J. Antimicrob. Agents. 2018;52:577–85. doi: 10.1016/j.ijantimicag.2018.06.018

3. Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro K, et al. A Lateral Flow Immunoassay for the Rapid Identification of CTX-M-Producing Enterobacterales from Culture Plates and Positive Blood Cultures: A Multicenter Study. Diagnostics (Basel, Switzerland) 2020;9:e024879. doi: 10.3390/diagnostics10100764

4. Bloemberg GV, Braun-Kiewnick A, Tedrup J, Meijerink C, Durer E, et al. Evaluation of the AID carbapenemase line probe assay for rapid detection and identification of carbapenemase genes in Gram-negative bacilli. J. Antimicrob. Chemother. 2007;doi: 10.1093/jac/dkx100

5. Ko YJ, Kim J, Kim H-N, Yoon S-Y, Lim CS, Lee CK. Diagnostic performance of the Xpert Carba-R assay for active surveillance of rectal carbapenemase-producing organisms in intensive care unit patients. Antimicrob. Resist. Infect. Control 2019;8:127. doi: 10.1186/s13756-019-0579-2

6. van den Bijlardiard W, Janssens MM, Buiting AG, Muller AE, Mouton JW, Verweij JJ. Extended-spectrum beta-lactamase (ESBL) polymerase chain reaction assay on rectal swabs and enrichment broth for detection of ESBL carriage. J. Hosp. Infect. 2018;98:264–9. doi: 10.1016/j.jhin.2017.10.014

7. Chantemesse B, Betelli L, Solanas S, Vienney F, Bollache L, Hartmann A, et al. A nitrocefin-based amperometric assay for the rapid detection of extended-spectrum beta-lactamase-producing Escherichia coli in wastewaters. Water Res. 2017;109:375–81. doi: 10.1016/j.watres.2016.11.066

8. Romero-Gomez M-P, Gomez-Gil R, Pano-Pardo JR, Mingorance J. Identification and susceptibility testing of microorganisms by direct inoculation from positive blood culture bottles by combining MALDI-TOF and Vitek-2 Compact is rapid and effective. J. Infect. 2012;65:513–20. doi: 10.1016/j.jinf.2012.08.013

9. Bianco G, Boattini M, Iannaccone M, Cavallo R, Costa C. Evaluation of the NG-Test CTX-M MULTI immunochromatographic assay for the rapid detection of CTX-M extended-spectrum beta-lactamase producers from positive blood cultures. J. Hosp. Infect. 2020;doi: 10.1016/j.jhin.2020.02.009

10. Bernabeu S, Ratnam KC, Boutal H, Gonzalez C, Vogel A, Devilliers K, et al. A Lateral Flow Immunoassay for the Rapid Identification of CTX-M-Producing Enterobacterales from Culture Plates and Positive Blood Cultures. Diagnostics (Basel, Switzerland) 2020;10. doi: 10.3390/diagnostics10100764

11. Livermore DM, Canton R, Ouniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBL in Europe. J. Antimicrob. Chemother. 2007;59:169–74. doi: 10.1093/jac/dkl483

12. Merino I, Shaw E, Horcajada JP, Cercenado E, Mirelis B, Pallarés MA, et al. CTX-M-15-H30Rx-ST131 subclone is one of the main causes of healthcare-associated ESBL-producing Escherichia coli bacteraemia of urinary origin in Spain. J. Antimicrob. Chemother. 2016;71:2125–30. doi: 10.1093/jac/dkw133

13. Pulcini C, Clerc-Urms I, Attinsounon CA, Fougnot S, Thilly N. Antibiotic resistance of Enterobacteriaceae causing urinary tract infections in elderly patients living in the community and in the nursing home: a retrospective observational study. J. Antimicrob. Chemother. 2019;74:775–81. doi: 10.1093/jac/dky488

14. Flokas ME, Alevizakos M, Shehadeh F, Andreatos N, Mylonakis E. Extended-spectrum beta-lactamase-producing Enterobacteriaceae colonisation in long-term care facilities: a systematic review and meta-analysis. Int. J. Antimicrob. Agents. 2017;50:649–56. doi: 10.1016/j.ijantimicag.2017.08.003

15. Pitout JDD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet. Infect. Dis. 2008;8:159–66. doi: 10.1016/S1473-3099(08)70041-0

16. Horie A, Nariai A, Katou F, Abe Y, Saito Y, Koike D, et al. Increased community-acquired upper urinary tract infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in children and the efficacy of flomoxef and cefmetazole. Clin. Exp. Nephrol. 2019;23:1306–14. doi: 10.1007/s10157-019-01775-w

17. Latifpour M, Gholipour A, Damavandi MS. Prevalence of Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae Isolates in Nosocomial and Community-Acquired Urinary Tract Infections. Jundishapur J. Microbiol. 2016;9:e31179. doi: 10.5812/ jjm.31179

18. Rodriguez-Bano J, Navarro MD. Extended-spectrum beta-lactamases in ambulatory care: a clinical perspective. Clin. Microbiol. Infect. 2014;18 Suppl 1:104–10. doi: 10.1111/j1469-0691.2007.01866.x

19. Heymann WR. Noninfectious causes of fever and a rash. Int. J. Dermatol. 1989;28:145–56. doi: 10.1111/j.1365-4362.1989.tb02450.x

20. Diaz-Agero Perez C, Lopez-Fresnena N, Rincon Carlavilla AL, Hernandez Garcia M, Ruiz-Garbiapoa P, Aranzad-Andres JM, et al. Local prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in Klebsiella pneumoniae: a prevalence survey in a Spanish University Hospital. BMJ Open 2019;9:e024879. doi: 10.1136/bmjopen-2018-024879

21. Morency-Potvin P, Schwartz DN, Weinstein RA. Antimicrobial Stewardship: How the Microbiology Laboratory Can Right the Ship. Clin. Microbiol. Rev. 2017;30:381–407. doi: 10.1128/CMR.00066-16

287