Official journal of the Spanish Society of Chemotherapy

Revista Española de Quimioterapia
doi:10.37201/req/164.2021 - Advance Access published March 31, 2022

Brief report

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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

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Article history
Received: 6 December 2021; Revision Requested: 15 January 2022; Revision Received: 15 January 2022; Accepted: 2 February 2022; Published: 31 March 2022

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 (BioMérieux; 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

Evaluación de una inmunocromatografía para detectar beta-lactamasas de espectro extendido CTX-M directamente de hemocultivos positivos para su potencial uso en programas de optimización de antibioterapia

RESUMEN

Antecedentes. Las bacteriemias causadas por *Enterobacteriaceae* productoras beta-lactamasas de espectro extendido (BLEE) están asociadas con altas tasas de fallo de tratamiento y mortalidad, especialmente cuando se retrasa el tratamiento apropiado. Nuestro objetivo ha sido evaluar la anticipación de la detección de estas BLEE y la potencial mejora en el tiempo de respuesta respecto al VITEK2 System (Biomerieux; Francia).

Métodos. Se comparó una inmunocromatografía para su detección con el VITEK2 AST system directamente del hemocultivo. Se evaluaton 80 aislados, 61 evaluados directamente de hemocultivos positivos y 19 de la misma manera pero inoculados con microorganismos productores y no productores de BLEE.

Resultados. La concordancia entre la inmunocromatografía y el VITEK2 AST mostró un coeficiente Kappa de 0,97, indicando una buena correlación entre ambas técnicas.

Conclusión. Esta inmunocromatografía puede ser más rápida que el VITEK2 para una identificación de BLEE tipo CTX-M y puede ser útil para anticipar resultados y ajustar la terapia antimicrobiana.

Palabras clave: inmunocromatografía; beta-lactamasas de espectro extendido, hemocultivos positivos; 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-

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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 EBSLs, 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 *Enterobacterales* 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 19 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 10^3 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.

Statistical analysis. The concordance rate between the CTX-LFIA test and our standard laboratory method was examined using the Cohen´s Kappa coefficient**.**

RESULTS

We tested eighty positive blood culture samples in the study, sixty-one samples were consecutive clinical isolates evaluated directly from positive blood cultures (Table 1), and nineteen by inoculating blood cultures with well-characterized ESBL and non-ESBL bacterial isolates (Table 1). 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. Only one blood culture (*E. coli* involved) was negative in the CTX-LFIA and positive by VITEK 2. This discrepancy was resolved by microdilution Microscan WalkAway (Beckman Coulter, Barcelona, Spain)), confirming the VITEK 2 EBSL positive result. This discordant result was corroborated by an in-house PCR that detected the CTX-M.

DISCUSSION

In this study, the performance of CTX-LFIA rapid diagnostic tests for the detection of EBSL 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 b-lactamases 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

Table 1 **Isolates evaluated by the CTX-LFIA test.**

Gram-negative species (N=80)	Number of isolates tested	Number of ESBL isolates tested	Number of non ESBL isolates tested	Number of ESBL isolates detected by CTX-M MULTI test	Number of negative ESBL isolates detected	Additional resistance mechanisms	Polimicrobial blood culture and additional bacterial species identified
Klebsiella pneumoniae	9	9	0	9	0	OXA 48 (1 strain)	0
Escherichia coli	9	8	1	8	1	0	0
Klebsiella aerogenes	1	0	1	0	1	0	0
Spiked blood cultures clinical isolates							
Klebsiella pneumoniae	17	5	12	5	12	0	0
Escherichia coli	42	5	37	4	37	0	2 (*P. mirabilis, K. pneumoniae*)
Klebsiella oxytoca	2	0	2	0	2	0	0
Routine prospective blood culture isolates							
TOTAL	80	27	53	26	53	0	2

be useful in elderly patients with bacteriemia/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 EBSL screening of CTX-M type at least 12 to 24 hours respect to the routine workflow implemented currently [8]. In addition, we did not observed 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/bacteriemia 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 bacteriemia 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 bacteriemia due to *Enterobacterales* 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.

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