European Journal of Clinical Microbiology & Infectious Diseases (2022) 41:407–413
https://doi.org/10.1007/s10096-021-04385-1

ORIGINAL ARTICLE

Direct detection of extended-spectrum-β-lactamase-producers in Enterobacterales from blood cultures: a comparative analysis

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Received: 17 October 2021 / Accepted: 19 November 2021 / Published online: 25 November 2021

Abstract

Accurate detection of extended-spectrum-β-lactamase (ESBL)-producing Enterobacterales from bloodstream infection (BSI) is of paramount importance for both epidemiological and clinical purposes, especially for optimization of antibiotic stewardship interventions. Three phenotypic methods for the detection of ESBL phenotype in *Klebsiella pneumoniae* and *Escherichia coli* BSI were compared over a 4-month period (May–August 2021) in a main University Hospital from Northern Italy. The methods were the biochemical Rapid ESBL NP®, the immunological NG-Test CTX-M MULTI®, and the E-test technique based on ESBL E-test®. One hundred forty-two blood cultures (BCs) positive for *K. pneumoniae* or *E. coli* were included. ESBL and carbapenemase phenotype were detected in 26.1% ($n=37$) and 16.9% ($n=24$), respectively. The Rapid ESBL NP®, NG-Test CTX-M MULTI®, and direct ESBL E-test® positive and negative predictive values with 95% confidence intervals were 1 (0.87–1) and 0.97 (0.92–0.99), 1 (0.87–1) and 0.97 (0.92–0.99), and 1 (0.88–1) and 1 (0.96–1), respectively. The three phenotypic methods evaluated showed good performance in the detection of ESBL phenotype from *K. pneumoniae*– or *E. coli*–positive BCs. Rapid ESBL NP® and NG-test CTX-M® offer the important advantage of a turnaround time of 15 to 45 min, and the Rapid ESBL NP test in addition detects any type of ESBL producers.

Keywords ESBL · CTX-M · Bloodstream infection · Sepsis · Gram-negatives · Rapid diagnostic test

Introduction

Extended-spectrum-β-lactamase (ESBL)-producing Enterobacterales (EB) bloodstream infections (BSI) represent a worldwide clinical issue, especially given their association with multidrug resistance, severity of illness, poor outcomes, and growing number in the community [1–3]. The spread of plasmids carrying CTX-M-type genes in the community beginning mostly in the 2000s is the main driver of ESBL dissemination in EB and replaced other ESBL enzymes (i.e., mostly TEM, SHV derivatives) that were mostly identified in hospital-acquired EB infections [1, 4, 5]. However, given the heterogeneity of ESBL family, other-than-CTX-M-type enzymes have been also reported as a source of outbreaks of ESBL producers [6]. Therefore, accurate detection of ESBLs is of paramount importance for both epidemiological and clinical purposes, given the ability of ESBLs of hydrolyzing penicillins, monobactams, and cephalosporins. Several phenotypic tests are available for ESBL detection [7–9]. They are mostly performed on bacterial colonies and associated with non-negligible turnaround time (TAT) contributing

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to delay obtention of results in critical scenarios as that of management of BSI patients. Various phenotypic tests that can detect ESBLs have been evaluated directly from EB-positive blood cultures (BCs) to provide result on the same day of positive BC processing with variable specificity and sensitivity [10–23]. Although the benefit in reducing TAT is recognized [12, 23], evidence of the detection of ESBL producers directly from EB-positive BCs in clinical routine are limited. Therefore, this study was aimed at evaluating the performance of three methods for direct detection of ESBL producers from BSI, none of them being molecular based and all of them being very recently commercialized.

Methods

Conventional blood culture routine

Our laboratory based at the Microbiology and Virology Unit (University Hospital Città della Salute e della Scienza di Torino, Turin, Italy) is open 7 days per week from 8 a.m. to 6 p.m. BACT/ALERT FA and FN Plus BC bottles (bioMérieux, Marcy l'Étoile, France) are incubated in the BACT/ALERT Virtuo (bioMérieux, Marcy l'Étoile, France) at various times each day. Positive BCs are subjected to Gram staining and subculture on appropriate solid culture media. Pathogen identification is performed on overnight subcultures using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker DALTONIK GmbH, Bremen, Germany). Antimicrobial susceptibility testing is performed on overnight subcultures using the MicroscanWalkAway plus system according to the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). Antimicrobial susceptibilities are interpreted according to EUCAST breakpoints as updated in 2021 [24]. A disc-based phenotypic method evaluating inhibitory activity of clavulanate or cloxacillin on broad-spectrum-β-lactamases (total ESBL + AmpC Confirm kit, Rosco, Taastrup, Denmark) is used to identify ESBL and AmpC producers if cefotaxime and/or ceftazidime minimum inhibitory concentrations (MICs) were > 1 mg/L. The Mastdiscs combi Carba plus disc system (Mast Group Ltd, Bootle, UK) is used to assess carbapenemase producers when meropenem MIC value was > 0.125 mg/L. Xpert Carba-R assay (Cepheid, Sunnyvale, CA) was also carried out to detect the main carbapenem resistance genes when meropenem MIC value was > 0.125 mg/L and/or ceftazidime-avibactam (CZA) MIC value was ≥ 8 mg/L.

Study design

The bacterial pellets of positive BCs with Gram-negative bacilli obtained using the MBT Sepsityper IVD Kit (Bruker DALTONIK GmbH, Bremen, Germany) from 1 mL of positive BC broth underwent MALDI-TOF MS analysis using the MALDI BioTyper system in accordance with the manufacturer's instructions (Bruker DALTONIK GmbH, Bremen, Germany). In case of identification of *Klebsiella pneumoniae* or *Escherichia coli*, three phenotypic methods were used in parallel for the detection of ESBL phenotype and results were compared with conventional culture-based result over a 4-month period (May–August 2021).

The Rapid ESBL NP® (Liofilchem, Roseto degli Abbruzzi, Italy) is a colorimetric cefotaxime hydrolysis-based assay able to detect the presence/absence of any type of ESBLs or the presence of an enzyme or combination of enzymes that can hydrolyze cefotaxime, but which is not inhibited by the addition of tazobactam (i.e., cephalosporinase, ESBL + cephalosporinase, carbapenemase with or without an ESBL) [25, 26]. Rapid ESBL NP® test was performed using the bacterial pellet recovered with MBT Sepsityper IVD Kit from 1 mL of positive BC broth, as previously described [11, 12, 27]. Briefly, after keeping test panel at room temperature for 10 min, 400 µL of lysis buffer was added to the bacterial pellet and vortexed for 5 s. After 15 min, 100 µL of the solution obtained was dispensed into each well (A, B, and C) of the test cassette. Then, the panel was covered with the lid provided and incubated at 36 ± 2 °C for 20 min in ambient air. Rapid ESBL NP test results were read within 20 min. Positive result for ESBL phenotype was considered if wells A and C remained red and well B turned orange/yellow (Fig. 1a).

The NG CTX-M MULTI® assay (NG Biotech, Guipry, France) is a lateral flow immunoassay exploiting monoclonal antibodies specific for the specific detection of CTX-M-type (groups 1, 2, 8, 9, and 25) ESBL enzymes only [10–12]. As opposed to the Rapid ESBL NP test, this technique is not aimed to identify all types of ESBL producers. NG CTX-M MULTI® assay was also performed using the bacterial pellet recovered with MBT Sepsityper IVD Kit from 1 mL of positive BC broth. Briefly, five drops of lysis buffer provided with the kit were added to bacterial pellet and vortexed for 5 s. One hundred microliters was added to the sample well of the test cassette. Assay results were read within 15 min. Positive result for ESBL phenotype was based on the presence of visible line specific for CTX-M ESBL enzyme (Fig. 1c).

The E-test® (bioMérieux, Marcy l'Étoile, France) is an antimicrobial gradient method that combines the principle of dilution methods with that of diffusion methods to determine the MIC value. Both ESBL E-test® ceftazidime ± clavulanic acid and cefotaxime ± clavulanic acid (CTX/CTXL) are the antimicrobial agents required for ESBL confirmation. To confirm the presence of ESBLs in isolates with high-level expression of AmpC β-lactamases, it is recommended that an additional ESBL confirmation test is performed with cefepime as the indicator

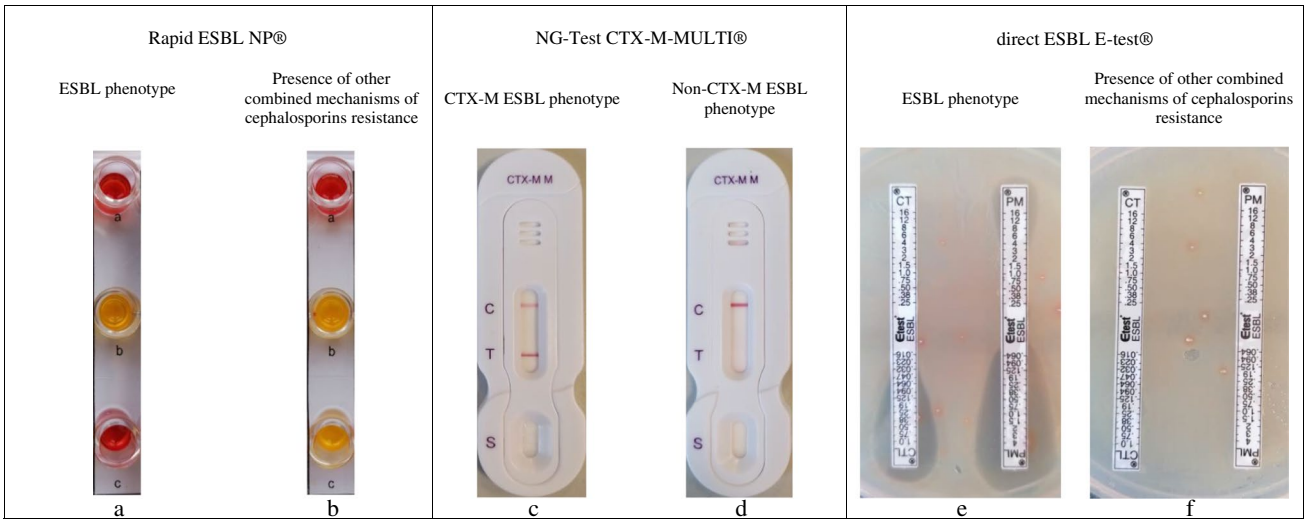

Fig. 1 Several phenotypic test results obtained directly from *K. pneumoniae*– or *E. coli*–positive blood cultures. Rapid ESBL NP®: **a** ESBL phenotype, **b** presence of other combined mechanisms of cephalosporins resistance. NG-Test CTX-M-MULTI®: **c** CTX-M ESBL phenotype, **d** non-CTX-M ESBL phenotype. Direct ESBL E-test®: **e** ESBL phenotype, **f** presence of other combined mechanisms of cephalosporin resistance

cephalosporin, as cefepime is usually not hydrolyzed by AmpC β-lactamases. Direct ESBL E-test® was performed modifying EUCAST inoculum recommendations for rapid AST from positive BCs (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/RAST/EUCAST_RAST_methodology_v1.1_Final.pdf). In this modified procedure of E-test®, 200 µL of positive BC broth was mixed to 200 µL of normal saline and the total suspension was inoculated and spread gently on a cation-adjusted MHA plate. MHA surface was allowed to dry under the fume hood for 2 min. Then, a CTX/CTXL and cefepime ± clavulanic acid (PM/PML) strips were deposited on the agar surface and the plate was incubated at 37 °C in 5% CO_2. Direct ESBL E-test® results were read after 5 h of incubation only if the growth was confluent and zone edges were clearly visible. Growth of microcolonies inside the entire inhibition zone was ignored. The test was considered positive for ESBL phenotype if ≥ eightfold reduction was observed in the MIC of CTX and/or PM combined with clavulanic acid compared with the MIC of the CTX and/or PM alone or if a phantom zone or deformed ellipse was present (Fig. 1e).

All identified isolates were further analyzed with a multiplex real-time polymerase chain reaction assay specific for *bla*CTX-M-like genes (ESBL ELITe MGB Kits, ELITechGroup Molecular Diagnostics, Turin, Italy) as previously described [28].

In case of discordant result between the direct phenotypic methods and conventional culture-based diagnostics, phenotypic tests were performed on bacterial colony.

Statistical analysis

Descriptive data are shown as absolute (*n*) and relative (%) frequencies for categorical data. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the three phenotypic methods for direct detection of ESBL phenotype from positive BCs with 95% confidence interval (95% CI) were computed using the online calculator at http://vassarstats.net/clin1.html.

Results

Over the study period, 242 positive BCs deemed representative of a single Gram-negative bacilli BSI event were processed. Of these, 58.7% ($n=142$) were associated with *K. pneumoniae* ($n=62$) or *E. coli* ($n=80$) and were included in the study (Table 1). ESBL and carbapenemase phenotype were detected in 26.1% ($n=37$) and 16.9% ($n=24$), respectively. All the isolates displaying a carbapenemase phenotype were *K. pneumoniae*, being KPC (95.8%, $n=23$) the most prevalent enzyme followed by VIM type (4.2%, $n=1$). Among KPC producers, 21.7% ($n=5$) were co-producers of CTX-M.

Among the three methods for direct detection of ESBL phenotype, the best performance was obtained by direct ESBL E-test® that showed no false-negative/positive results when combining the results of the CTX/CTXL and PM/PML strips (Table 2). Of note, CTX/CTXL strips test

Table 1 Characterization of *Klebsiella pneumoniae* and *Escherichia coli* isolates recovered in this study

	ESBL phenotype 26.1 (37)	Carbapenemase phenotype 16.9 (24)	Non-ESBL and/or carbapenemase phenotype 57 (81)	Total 100 (142)
Klebsiella pneumoniae	25.8 (16)	38.7 (24)	35.5 (22)	43.7 (62)
Escherichia coli	26.3 (21)	0	73.7 (59)	56.3 (80)

Table 2 Performance of rapid methods for direct detection of ESBL phenotype in *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infections

Direct detection of ESBL phenotype		Conventional phenotypic results $n = 142$		Sensitivity	Specificity	PPV	NPV
		Positive	Negative				
Rapid ESBL NP®	Positive	34	0	0.92 (0.77–0.98)	1 (0.96–1)	1 (0.87–1)	0.97 (0.92–0.99)
	Negative	3	105				
NG CTX-M MULTI®	Positive	34	0	0.92 (0.77–0.98)	1 (0.96–1)	1 (0.87–1)	0.97 (0.92–0.99)
	Negative	3	105				
Direct ESBL E-test®	Positive	37	0	1 (0.88–1)	1 (0.96–1)	1 (0.88–1)	1 (0.96–1)
	Negative	0	105				

Abbreviations: *PPV* positive predictive value, *NPV* negative predictive value. 95% confidence intervals are shown in parentheses

failed to detect one isolate with an ESBL phenotype and with low MIC (≤ 1 mg/L) for CTX. Similarly, PM/PML strip test failed to detect one ESBL isolate with low MIC value (2 mg/L) for PM.

The Rapid ESBL NP® and NG CTX-M MULTI® showed similar performance (Table 2). In fact, for both the tests, sensitivity, specificity, and positive and negative predictive values with 95% confidence intervals were 0.92 (0.77–0.98), 1 (0.96–1), 1 (0.87–1), and 0.97 (0.92–0.99). The false-negative results obtained with the Rapid ESBL NP® were as follows: two were positive for CTX-M according to the PCR results while one presented with both bacterial pellets and colony with hypermucoid phenotype and PM, CTX, and CAZ MICs of 4 mg/L, > 32 mg/L, and > 32 mg/L, respectively. By repeating the tests from overnight subcultures, one of the isolates carrying CTX-M tested positive and showed PM, CTX, and CAZ MICs of 2 mg/L, 32 mg/L, and 2 mg/L, respectively, while the other two isolates tested negative. Moreover, the Rapid ESBL NP® failed to provide indications on the presence of other combined mechanisms of cephalosporin resistance in four cases: one KPC-producing *K. pneumoniae* isolate; one CZA-resistant KPC–producing *K. pneumoniae* isolate with PM, CTX, and CAZ MICs of 8 mg/L, 4 mg/L, and > 32 mg/L, respectively; one VIM–producing *K. pneumoniae*; and one AmpC–producing *E. coli* with PM, CTX, and CAZ MICs of ≤ 0.5 mg/L, 4 mg/L, and 32 mg/L, respectively. However, by repeating the Rapid ESBL NP® tests from overnight subcultures, only the CZA-resistant KPC–producing *K. pneumoniae* isolate remained negative.

The three false-negative results obtained with the NG CTX-M MULTI® were all negative for CTX-M PCR. Of these, one was the same hypermucoid *K. pneumoniae* strain mentioned above.

Technical comparison of the three methods used for direct detection of ESBL phenotype from *K. pneumoniae*– or *E. coli*–positive BCs is summarized in Table 3. ESBL phenotype could be detected within 40 to 45 min (Rapid ESBL NP®), 15 to 20 min (NG CTX-M MULTI®), or 5 h (direct ESBL E-test®).

Discussion

The global CTX-M pandemic underlines the need for rapid ESBL detection to accelerate clinical decision-making and infection control measures and finally contribute to improve patient outcomes. Our data make obvious the high prevalence of ESBL producers in *K. pneumoniae* and *E. coli* BSI in our hospital located in the northern part of Italy, confirming the relevant burden of resistance rates to expanded-spectrum cephalosporins in those species in Italy compared to Europe [29]. Therefore, we compared the performance of two rapid tests, the biochemical-based Rapid ESBL NP® test very recently commercialized and lateral flow immunoassay NG CTX-M MULTI® for the detection of ESBL producers from BSI and compared their results with the direct ESBL E-test® performed also from BC samples. Our results demonstrated that with the

Table 3 Technical comparison of rapid methods used for direct detection of ESBL phenotype in *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infections

	Rapid ESBL NP®	NG CTX-M MULTI®	Direct ESBL E-test®
Pre-procedural steps	Recovery of bacterial pellet using MBT Sepsityper IVD Kit® from 1 mL of positive BC broth	Recovery of bacterial pellet using MBT Sepsityper IVD Kit® from 1 mL of positive BC broth	None
Procedural steps	Leave a panel at room temperature (10 min), and add 400 µL of lysis buffer to one of the empty vials together with the bacterial pellet. After 15 min, dispense 100 µL of the solution obtained into each well (A, B, and C) of the test cassette, cover the panel with the lid provided, and incubate at 36 ± 2 °C for 20 min in ambient air	Add five drops of lysis buffer provided with the kit to bacterial pellet, vortex for 5 s, and add 100 µL to the sample well of the test cassette	Add 200 µL of positive BC broth to 200 µL of normal saline and inoculate on a cation-adjusted MHA plate. Spread it gently over the agar surface. Let it dry under the fume hood for 2 min. Put CTX/CTXL and PM/PML strips on the agar surface, and incubate at 37 °C for 300 min in 5% CO_2
Ease of use	Easy to perform, no special reagents or media necessary beside the test kit	Easy to perform, no special reagents or media necessary beside the test kit	Easy to perform, no special reagents or media necessary
Test reagents and materials	Test kit	Test kit	CTX/CTXL and PM/PML E-test® strips, normal saline (~3.0 mL aliquot), cation-adjusted MHA plate
Interpretation of positive result for ESBL phenotype	If wells A and C remained red and well B turned orange/yellow	Presence of visible line specific for CTX-M ESBL enzymes	≥8-fold reduction in the MIC of the cephalosporin combined with clavulanic acid compared with the MIC of the cephalosporin alone OR presence of a phantom zone OR presence of deformed ellipse
Time to perform test (min)	25–30	5	5
Time to results (min)	40	~15	~300
Estimated cost per test (€)	~3	~7	~10
Strong point	Short TAT; detection of all types of ESBL producers; able to indicate the presence of other combined mechanisms of cephalosporin resistance (cephalosporinases, carbapenemases)	Short TAT	High sensitivity and specificity; able to indicate the presence of other combined mechanisms of cephalosporins resistance (cephalosporinases, carbapenemases)
Main limitation	Low inoculum size may reduce test performance	Unable to detect other-than-CTX-M-type ESBL enzymes; unable to detect other combined mechanisms of cephalosporins resistance (cephalosporinases, carbapenemases)	Long TAT unsuitable for antibiotic stewardship

Abbreviations: *BC*, blood culture; *CTX/CTXL*, cefotaxime and cefotaxime plus clavulanic acid; *PM/PML*, cefepime and cefepime plus clavulanic acid; *MHA*, Mueller–Hinton agar; *TAT*, turnaround time

three phenotypic tests evaluated, rapid and reliable detection of ESBL phenotype can be achieved directly from *K. pneumoniae*– or *E. coli*–positive BCs. We excluded from this comparison the β-Lacta® test (Bio-Rad, Marnes-La-Coquette, France), based on a chromogenic cephalosporin, that is a rapid technique for identification of any type of cephalosporin resistance since it lacks specificity [15]. Similarly, we excluded the direct β-lactam inactivation method despite its claimed sensitivity and specificity, both being 100% since it remains a homemade technique with a long TAT [18, 19]. We did not also evaluate MALDI-TOF MS-based approach for the detection of ESBL producers (sensitivity 91.1–100% and specificity 91.5–100%) since it required equipment and careful adaptation for its implemetation in routine microbiology [20–23].

The direct ESBL E-test® allows a reliable detection of ESBL phenotype but with a TAT unsuitable for antibiotic management of BSI patients. The Rapid ESBL NP® and NG CTX-M MULTI® also allowed a reliable phenotypic detection of ESBL producers with a TAT ranging from 15 to 45 min compatible with antibiotic stewardship. In addition, the Rapid ESBL NP® test offers the advantage to detect any type of ESBL (and not only CTX-M) and to identify combined mechanisms such as carbapenemase production. This is an important point to consider for implementing a carbapenem-containing therapy.

Based on these results, the following strategy for ESBL phenotype detection from *K. pneumoniae*– and *E. coli*–positive BC is proposed. *Klebsiella* spp.- or *E. coli*–positive BCs should be screened with the NG CTX-M MULTI® assay or the Rapid ESBL NP®. In countries where the prevalence of other-than-CTX-M-type ESBL enzymes is important such as those from Asia, South America, or Middle East, the Rapid ESBL NP® offers the advantage to detect any kind of ESBL producers. In addition, one shall be aware that false detection of ESBL has been noted by using the NG CTX-M MULTI® assay for several *K. oxytoca* strains that possess specific naturally occurring ESBLs of OXY type [30]. All the tests evaluated are low cost and require no specialized equipment or personnel; further studies on cost–benefit analysis are needed however.

In conclusion, the three phenotypic methods evaluated showed good performance for the detection of ESBL phenotype directly from *K. pneumoniae*– and *E. coli*–positive BCs. The rapid ESBL tests directly from BC samples might be implemented worldwide in particular for patients hospitalized in acute care facilities for promptly optimizing their antibiotic therapy.

Author contribution Boattini M., Bianco G., Cavallo R., Nordmann P., and Costa C. designed the study; Boattini M., Bianco G., Comini S., Iannaccone M., and Casale R. acquired the data; Boattini M. and Bianco G. analyzed and interpreted the data; Boattini M. wrote the paper; all authors revised the article critically and approved the final version.

Funding Liofilchem (Italy) kindly provided the Rapid ESBL NP® tests. They were not involved in the study design; the collection, analysis, and interpretation of data; the writing of the paper; and the decision to submit the article for publication.

Availability of data and material The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval This study was conducted in accordance with the Declaration of Helsinki. Formal ethical approval was obtained by our Center's institutional review board (Protocol No. 0029345).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest Patrice Nordmann is the main inventor of the Rapid ESBL NP® test. A patent of this test has been taken on behalf of INSERM, University of Paris XI and Assistance Publique Hôpitaux de Paris (France). All the other authors declare no competing interests.

References

1. Pitout JD, Nordmann P, Laupland KB, Poirel L (2005) Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother 56:52–59. https://doi.org/10.1093/jac/dki166
2. Lee JA, Kang CI, Joo EJ, Ha YE, Kang SJ, Park SY et al (2011) Epidemiology and clinical features of community-onset bacteremia caused by extended-spectrum β-lactamase-producing Klebsiella pneumoniae. Microb Drug Resist 17:267–273. https://doi.org/10.1089/mdr.2010.0134
3. Rodríguez-Baño J, Pascual A (2008) Clinical significance of extended-spectrum beta-lactamases. Expert Rev Anti Infect Ther 6:671–683. https://doi.org/10.1586/14787210.6.5.671
4. Bevan ER, Jones AM, Hawkey PM (2017) Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145–2155. https://doi.org/10.1093/jac/dkx146
5. Kazmierczak KM, de Jonge BLM, Stone GG, Sahm DF (2020) Longitudinal analysis of ESBL and carbapenemase carriage among Enterobacterales and Pseudomonas aeruginosa isolates collected in Europe as part of the International Network for Optimal Resistance Monitoring (INFORM) global surveillance programme, 2013–17. J Antimicrob Chemother 75:1165–1173. https://doi.org/10.1093/jac/dkz571
6. Bush K, Bradford PA (2020) Epidemiology of β-lactamase-producing pathogens. Clin Microbiol Rev 33:e00047-e119. https://doi.org/10.1128/CMR.00047-19
7. Decousser JW, Poirel L, Nordmann P (2017) Recent advances in biochemical and molecular diagnostics for the rapid detection of antibiotic-resistant Enterobacteriaceae: a focus on ß-lactam resistance. Expert Rev Mol Diagn 17:327–350. https://doi.org/10.1080/14737159.2017.1289087

8. Noster J, Thelen P, Hamprecht A (2021) Detection of multidrug-resistant Enterobacterales—from ESBLs to carbapenemases. Antibiotics 10:1140. https://doi.org/10.3390/antibiotics10091140
9. Castanheira M, Simner PJ, Bradford PA (2021) Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 3:dlab092. https://doi.org/10.1093/jacamr/dlab092
10. Bernabeu S, Ratnam KC, Boutal H, Gonzalez C, Vogel A, Devilliers K et al (2020) A lateral flow immunoassay for the rapid identification of CTX-M-producing Enterobacterales from culture plates and positive blood cultures. Diagnostics (Basel) 10:764. https://doi.org/10.3390/diagnostics10100764
11. Bianco G, Boattini M, Iannaccone M, Cavallo R, Costa C (2020) Evaluation of the NG-Test CTX-M MULTI immunochromatographic assay for the rapid detection of CTX-M extended-spectrum-β-lactamase producers from positive blood cultures. J Hosp Infect 105:341–343. https://doi.org/10.1016/j.jhin.2020.02.009
12. Boattini M, Bianco G, Iannaccone M, Ghibaudo D, Almeida A, Cavallo R et al (2021) Fast-track identification of CTX-M-extended-spectrum-β-lactamase- and carbapenemase-producing Enterobacterales in bloodstream infections: implications on the likelihood of deduction of antibiotic susceptibility in emergency and internal medicine departments. Eur J Clin Microbiol Infect Dis 40:1495–1501. https://doi.org/10.1007/s10096-021-04192-8
13. Hasso M, Porter V, Simor AE (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. https://doi.org/10.1128/JCM.01354-17
14. Prod'hom G, Durussel C, Blanc D, Croxatto A, Greub G (2015) Early detection of extended-spectrum β-lactamase from blood culture positive for an Enterobacteriaceae using βLACTA test. New Microbes New Infect 8:1–3. https://doi.org/10.1016/j.nmni.2015.05.007
15. Poirel L, Fernández J, Nordmann P (2016) Comparison of three biochemical tests for rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. J Clin Microbiol 54:423–427. https://doi.org/10.1128/JCM.01840-15
16. Nordmann P, Dortet L, Poirel L (2012) Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. J Clin Microbiol 50:3016–3022. https://doi.org/10.1128/JCM.00859-12
17. Dortet L, Poirel L, Nordmann P (2015) Rapid detection of ESBL-producing Enterobacteriaceae in blood cultures. Emerg Infect Dis 21:504–507. https://doi.org/10.3201/eid2103.141277
18. Bianco G, Boattini M, Iannaccone M, Fossati L, Cavallo R, Costa C (2019) Direct β-lactam inactivation method: a new low-cost assay for rapid detection of carbapenemase- or extended-spectrum-β-lactamase-producing Enterobacterales directly from positive blood culture bottles. J Clin Microbiol 58:e01178-e1219. https://doi.org/10.1128/JCM.01178-19
19. Bianco G, Boattini M, Iannaccone M, Zanotto E, Cavallo R, Costa C (2020) Direct Ethylenediaminetetraaceticacid-modified β-lactam inactivation method: an improved method to identify serine-carbapenemase-, metallo-β-lactamase-, and extended-spectrum-β-lactamase-producing Enterobacterales directly from positive blood culture. Microb Drug Resist. https://doi.org/10.1089/mdr.2020.0343
20. Oviaño M, Fernández B, Fernández A, Barba MJ, Mouriño C, Bou G (2014) Rapid detection of Enterobacteriaceae producing extended-spectrum-β-lactamases directly from positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Clin Microbiol Infect 20:1146–1157. https://doi.org/10.1111/1469-0691.12729
21. Jung JS, Popp C, Sparbier K, Lange C, Kostrzewa M, Schubert S (2014) Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid detection of β-lactam resistance in Enterobacteriaceae derived from blood cultures. J Clin Microbiol 52:924–930. https://doi.org/10.1128/JCM.02691-13
22. Torres I, Albert E, Giménez E, Olea B, Valdivia A, Pascual T et al (2021) Performance of a MALDI-TOF mass spectrometry-based method for rapid detection of third-generation oxymino-cephalosporin-resistant Escherichia coli and Klebsiella spp. from blood cultures. Eur J Clin Microbiol Infect Dis 40:1925–32. https://doi.org/10.1007/s10096-021-04251-0
23. Roncarati G, Foschi C, Ambretti S, Re MC (2021) Rapid identification and detection of β-lactamase-producing Enterobacteriaceae from positive blood cultures by MALDI-TOF/MS. J Glob Antimicrob Resist 24:270–274. https://doi.org/10.1016/j.jgar.2020.12.015
24. Available at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf. Accessed 15 Sep 2021
25. Demord A, Poirel L, D'Emidio F, Pomponio S, Nordmann P (2020) Rapid ESBL NP test for rapid detection of expanded-spectrum β-lactamase producers in Enterobacterales. Microb Drug Resist. https://doi.org/10.1089/mdr.2020.0391
26. Blanc DS, Poncet F, Grandbastien B, Greub G, Senn L, Nordmann P (2021) Evaluation of the performance of rapid tests for screening carriers of acquired ESBL-producing Enterobacterales and their impact on turnaround time. J Hosp Infect 108:19–24. https://doi.org/10.1016/j.jhin.2020.10.013
27. Bianco G, Boattini M, van Asten SAV, Iannaccone M, Zanotto E, Zaccaria T et al (2020) RESIST-5 O.O.K.N.V. and NG-Test Carba 5 assays for the rapid detection of carbapenemase-producing Enterobacterales from positive blood cultures: a comparative study. J Hosp Infect 105:162–6. https://doi.org/10.1016/j.jhin.2020.03.022
28. Girlich D, Bernabeu S, Fortineau N, Dortet L, Naas T (2018) Evaluation of the CRE and ESBL ELITe MGB® kits for the accurate detection of carbapenemase- or CTX-M-producing bacteria. Diagn Microbiol Infect Dis 92:1–7. https://doi.org/10.1016/j.diagmicrobio.2018.02.001
29. Rossolini GM, Bochenska M, Fumagalli L, Dowzicky M (2021) Trends of major antimicrobial resistance phenotypes in Enterobacterales and gram-negative non-fermenters from ATLAS and EARS-net surveillance systems: Italian vs. European and global data, 2008–2018. Diagn Microbiol Infect Dis 101:115512. https://doi.org/10.1016/j.diagmicrobio.2021.115512
30. Ortiz de la Rosa JM, Demord A, Poirel L, Greub G, Blanc D, Nordmann P (2021) False immunological detection of CTX-M enzymes *in Klebsiella oxytoca*. J Clin Microbiol 59:e00609-21. https://doi.org/10.1128/JCM.00609-21