Revised Version

Rapid ESBL NP® test for rapid detection of expanded-spectrum ß-lactamase producers in Enterobacterales

Anthony Demord,[1,2] Laurent Poirel,[1,2,3] Fernando D'Emidio,[4] Stefano Pomponio,[4] and Patrice Nordmann[1,2,3,5*]

[1]Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; [2]Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg; [3]INSERM European Unit (IAME), University of Fribourg, Fribourg; [4]Lioficlhem, Roseto degli Abruzzi, Italy, and [5]Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

Keywords: ESBL, Enterobacterales, Gram-negatives, rapid diagnostic

*Corresponding author. Mailing address: Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Chemin du Musée 18, CH-1700 Fribourg, Switzerland. Phone: 41-26-300-9581. E-mail: patrice.nordmann@unifr.ch

ABSTRACT

Objectives: To evaluate the biochemical Rapid ESBL NP® (Liofilchem, Italy) for the rapid detection of extended-spectrum ß-lactamases (ESBL) in Enterobacterales.

Methods: A total of 100 clinical Gram-negative strains (40 ESBL producers with or without cephalosporinase, 43 carbapenemase producers with or without additional ESBL, eight AmpC-type producers, six penicillinase producers and three non-β-lactamase producers) were tested using this colorimetric technique.

Results: The overall sensitivity and specificity of the test were found to be 93.9% and 98.5%, respectively. This test is rapid (turn-around-time of 40-45 min), easy to perform, and reliable for identification of ESBL producers.

Conclusion: The Rapid ESBL NP® test allows differentiation between ESBL producers on one hand, and non ESBL-producers or isolates expressing combined mechanisms of resistance on the other hand.

Multidrug resistance in Enterobacterales represents a serious threat to public health, since the accumulation of resistance determinants may be the source of difficult-to-treat infections in humans. One of the most important resistance traits is plasmid-mediated resistance to broad-spectrum cephalosporins through production of extended-spectrum β-lactamases (ESBL). ESBLs hydrolyze oxyimino-cephalosporins and aztreonam, while being inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam.[1] The most frequent ESBLs are of CTX-Ms, followed by TEMs and SHVs, and to a lesser extent PER, VEB, GES variants.[2] ESBL-producing Enterobacterales constitute a major source of hospital-acquired infections (bacteremia mainly caused by *Klebsiella pneumoniae*) and community infections (urinary tract infections mainly caused by *Escherichia coli*).[3] Therefore, the rapid identification of this resistance trait is important to optimize the treatment of infections caused by ESBL producers and to prevent the spread of ESBL producers in nosocomial settings.

Several techniques have been developed to identify ESBL producers, such as phenotypic techniques based on the inhibition of ESBL activity by clavulanic acid or tazobactam.[4] Those techniques require a preliminary growth step of 24 to 48 h.[5] Molecular detection of ESBL-encoding genes is also interesting but remains costly, requires expertise, and detects only known ESBL-encoding genes.[5] Other techniques, such as those adapted from the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are being developed, but they do require additional material and a significant degree of expertise.[5] The **ß-lacta test** (Bio-Rad) has been developped and is based on detection of hydrolysis of a chromogenic cephalosporin, HMRZ-86.[6] Indeed, the ß-Lacta test turns positive when either ESBL, cephalosporinase or carbapenemase enzymes are produced, considering that all those enzymes actually hydrolyze HMRZ-86. The recently introduced immunological detection of ESBLs is rapid, sensitive and specific (NG- CTX-M [NG Biotech, France] for example), but detects only ESBLs of the CTX-M types.[7]

Therefore, developing tests based on biochemical detection of any type of ESBL activity is quite interesting. For that purpose, the Rapid ESBL NDP had been developed based on the detection of hydrolysis of the cefotaxime ß-lactam ring revealing the production of a broad-spectrum ß-lactamase, coupled with a tube containing tazobactam that inhibits the ESBL activity, signaling ESBL production.[8-10] This colorimetric test is based on the change of color of the pH indicator by acidification of the medium. The hydrolysis of the cephalosporin, here cefotaxime, gives a carboxyl group, which decreases the pH. The red phenol present in the reagent of the test subsequently turns yellow upon acidification of the pH.

A commercial version of this test, the Rapid ESBL NP® (Liofilchem, Roseto degli Abruzzi, Italy) is now available, based on mostly on the same protocol as the home-made Rapid ESBL NDP test. It can identify any ESBL producer in 40-45 minutes, regardless of the ESBL type. The aim of our study was to evaluate this novel Rapid ESBL NP® test using a collection of ESBL-producing isolates.

A well-characterized panel of 100 clinical enterobacterial strains (40 ESBL producers with or without additional AmpC-type ß-lactamases, 43 carbapenemase producers with or without additional ESBL, eight AmpC-type producers, six penicillinase producers and three non β-lactamase-producers) from the collection of clinical strains of the Medical and Molecular Microbiology Unit, Faculty of Science and Medicine, University of Fribourg, Switzerland, was tested. All strains were characterized for their ß-lactamase content at their molecular level (PCR and sequencing) prior to any testing.

The antimicrobial resistance profiles of the strains were determined by disk diffusion according to EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/); when needed, precise determination of MIC values was performed by using MIC test strip technique (Liofilchem).[11]

Rapid ESBL NP®. The Rapid ESBL NP® assay was performed according to the recommendations of the manufacturer from fresh overnight bacterial colonies grown on selective or non-selective agar plates at 37°C. URISelect™ 4 agar plates (Bio-Rad, Cressier, Switzerland) were used in this study. One to two full-calibrated loop (10-µl loop) of bacterial colonies was suspended in 400 µl of lysis buffer. After 15 min, 100 µl of suspension were dispensed in three wells (A, B and C) of the gallery. The well A does not contain antibiotic. The well B contains cefotaxime, and the well C contains cefotaxime and tazobactam. The results were interpreted according to the recommendations of the manufacturer (Figure 1) as follows. When all wells A, B, and C remain red, no ESBL is produced (negativity). When wells A and C remain red and well B turns orange/yellow, an ESBL is produced (positivity). When well A remains red and wells B and C both turn orange/yellow, different possibilities are possible, namely the production of an AmpC-type ß-lactamase, co-production of ESBL + AmpC, or production of a carbapenemase with or without an additional ESBL and/or AmpC (undetermined result) (Figure 1). Production of an AmpC enzyme may be detected by comparing susceptibility results of ß-lactams obtained on an additional 18 h culture step on media containing or not cloxacillin[4] whereas carbapenemase detection may be performed by using the Rapid Carba NP test. [12]

Reading of the results of the test were done after 20 min of incubation at 37°C. All strains have been tested in duplicate. Around 2 min per isolate were needed for sample preparation, with a total turn-around-time of approximately 40 min to obtain the result.

The Rapid ESBL NP® could detect 31 out of 33 ESBL producers (Table 1). Two *Escherichia coli* strains producing SHV-12 and VEB-1 with a low expression levels of their ESBL were not detected. MIC values of cefotaxime were 1 and 2 µg/ml, respectively for the SHV-12 and the VEB-1 strains, indicating that those strains, despite being ESBL producers, were still susceptible to cefotaxime which is the substrate for ESBL detection of this test. No

carbapenemase producer was detected as an ESBL producer. All the cephalosporinases with or without ESBL did not give a positive result for ESBL production except a single DHA-1 producer that co-produced an ESBL being SHV-11. This strain was cefotaxime susceptible with an MIC value of 0.25 mg/L.

Altogether, the sensitivity and specificity of the Rapid ESBL NP® test were 93.9% and 98.5%, respectively. The main advantage of this test is the ability to detect all types of ESBLs, being either known or unknown, and clearly differentiate isolate that only produce an ESBL from those exhibiting other multidrug resistance patterns. This test is therefore more specific that the ß-Lacta test that detect any strain that may hydrolyze the cephalosporin HMRZ-86, i.e non only ESBL, but also cephalosporinase and carbapenemase producers) .

By testing different culture media (Drigalski, Columbia blood agar and the screening medium for ESBL, ChromID ESBL (bioMérieux) with thirty strains producing or not an ESBL, we found that ESBL detection is optimal after culture on Columbia blood agar medium and on ChromID ESBL selective medium. Prior culture on Drigalski medium before testing for the Rapid ESBL NP test gave totally inconsistant (data not shown).

Use of the Rapid ESBL NP will be of clinical value in particular for antibiotic stewardship in the context of bacteremia in order to identify patients deserving a carbapenem-containing therapy.[13]

The Rapid ESBL NP may be also interesting for immediate isolation of the patient's carriers of ESBL producers before waiting the results of antibiogram (24-h delay). The excellent positive predictive value of the Rapid ESBL NP is also a crucial feature. Actually, strains that co-produced a CTX-M enzyme along with a carbapenemase (for example KPC) may be misidentified as a CTX-M-only producing isolate by using the NG-CTX-M test. Similarly, the use of molecular techniques for identification of ESBL encoding genes are limited to screening of few ESBL genes, thus leading to false-negative results when some minor ESBL enzymes not

included in the screening pool are produced. Therefore, use of molecular techniques for detection of ESBL producers shall gather not only ESBL encoding genes but also carbapenemase genes, to eventually prevent mistaken interpretations when an ESBL is actually produced together with an additional carbapenemase. In addition ,the immunological and molecular tests cannot detect the never-ending population of ESBL genes that are emerging worldwide.

In conclusion, the Rapid ESBL NP® test is a rapid, easy to perform, reliable and low-cost technique for detection of any type of ESBL producer with good sensitivity and specificity.

ACKNOWLEDGMENTS

The work was funded by the National Reference Center for Emerging Antibiotic Resistance for Switzerland (NARA).

DISCLOSURE STATEMENT

The Rapid ESBL NP® kits used in this evaluation were kindly provided by Liofilchem, Italy.

REFERENCES

1. Peirano, G., and J.D.D. Pitout. 2019. Extended spectrum ß-lactamase-producing *Enterobacteriaceae*: update on molecular epidemiology and treatment options. Drug 2019:1529-1541.

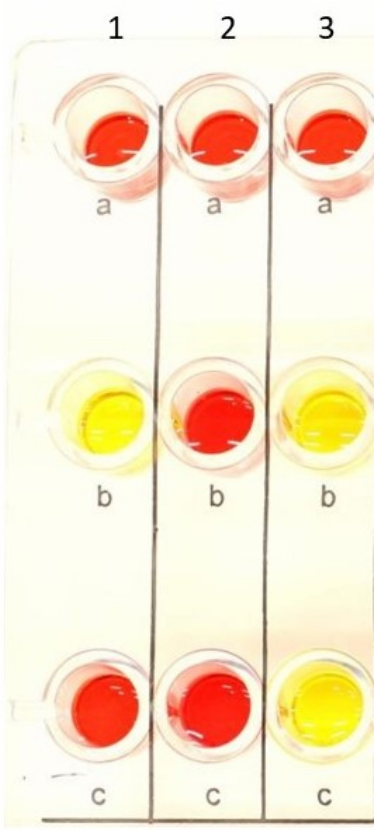
2. Zahar, J.R., O. Lortholary, C. Martin, G. Potel, P. Plésiat, and P. Nordmann. 2009. Addressing the challenge of extended-spectrum ß-lactamases. Curr. Opin. Investig. Drug 10:172-180.

3. Coque, T.M., F. Baquero, and R. Canton. 2008. Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe. Eurosurveillance 13:19044.

159 4. Drieux, L., F. Brossier, W. Sougakoff, and V. Jarlier. 2008. Phenotypic detection of
160 extended-spectrum β-lactamase production in *Enterobacteriaceae*: review and bench
161 guide. Clin. Microbiol. Infect. 14:90-103.
162 5. Decousser, J.W., L. Poirel, and P. Nordmann. 2017. Recent advances in biochemical and
163 molecular diagnostics for the rapid detection of antibiotic resistant *Enterobacteriaceae*;
164 a focus on ß-lactam resistance. Expert Rev. Mol. Diag. 17:327-350.
165 6. Renvoisé, A., D. Decré, R. Amarsy-Guerle, T.D. Huang, C. Jost, I. Podglajen, L. Raskine,
166 N. Genel, P. Bogaerts, V. Jarlier, and G. Arlet. 2013. Evaluation of the ß-Lacta test, a rapid
167 test detecting resistance to third-generation cephalosporins in clinical strains of
168 *Enterobacteriaceae*. J. Clin. Microbiol. 51:4012–4017.
169 7. Bianco, G., M. Boattini, M. Iannaccone, R. Cavallo, and C. Costa. 2020. Evaluation of
170 the NG-test CTX-M multi immunochromatographic assay for the rapid detection of
171 CTX-M extended-spectrum ß-lactamase producers from positive blood cultures. J. Hosp.
172 Infect. 105:341-343
173 8. Nordmann, P., L. Dortet, and L. Poirel. 2012. Rapid detection of extended-spectrum-β-
174 lactamase-producing *Enterobacteriaceae*. J. Clin. Microbiol. 50:3016-3022.
175 9. Dortet, L., L. Poirel, and P. Nordmann. 2014. Rapid detection of extended-spectrum-ß-
176 lactamase-producing *Enterobacteriaceae* from urine samples by use of the ESBL NDP
177 test. J. Clin. Microbiol. 52:3701–3706.
178 10. Dortet, L., L. Poirel, and P. Nordmann. 2015. Rapid detection of ESBL-producing
179 *Enterobacteriaceae* in blood cultures. Emerg. Infect. Dis. 21:504–507.
180 11. EUCAST. 2020. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/
181 Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf
182 12. Nordmann, P., L. Poirel , and L. Dortet. 2012. Rapid detection of carbapenemase -
183 producing *Enterobacteriaceae*. Emerg. Infect. Dis. 18:1503-1507.

13. Rodríguez-Baño, J., and M.D. Navarro. 2008. Extended-spectrum ß-lactamases in ambulatory care: a clinical perspective. Clin Microbiol Infect 14(Suppl 1):S104-S110.

Figure 1. Rapid ESBL NP test. Positive result (*K. pneumoniae* producing CTX-M-15, roaw 1); Negative result (*E .coli* wild type, roaw 2); undetermined (*K. pneumoniae* producing NDM-1, roaw 3). This is an example of typcial results that are obtained by using the Rapid ESBL NP test



Species (n)	Resistance determinant (n)	Rapid ESBL NP®			
		Results			Interpretation
		Well A (without antibiotic)	Well B (Cefotaxime)	Well C (Cefotaxime + Tazobactam)	
ESBL producers (n=33)					
Klebsiella pneumoniae (10)	CTX-M-2 (1)	-	+	-	+
	CTX-M-3 (1)	-	+	-	+
	CTX-M-14 (1)	-	+	-	+
	CTX-M-15 (2)	-	+	-	+
	CTX-M-18 (1)	-	+	-	+
	CTX-M-19 (1)	-	+	-	+
	CTX-M-37 (1)	-	+	-	+
	GES-1 (1)	-	+	-	+
	SHV-12 (1)	-	+	-	+
Enterobacter cloacae (2)	GES-5 (1)	-	+	-	+
	PER-1 (1)	-	+	-	+
Escherichia coli (20)	CTX-M-1 (1)	-	+	-	+
	CTX-M-3 (1)	-	+	-	+
	CTX-M-14 (1)	-	+	-	+
	CTX-M-15 (10)	-	+	-	+
	VEB-1 (1)	-	+	-	+
	VEB-1 (1)	-	-	-	-
	TEM-52 (3)	-	+	-	+
	SHV-12 (1)	-	+	-	+
	SHV-12 (1)	-	-	-	-
Proteus mirabilis (1)	PER-1 (1)	-	+	-	+
Cephalosporinase producers (n=8)					
Klebsiella pneumoniae (2)	LAT-1 (1)	-	+	+	Other
	DHA-2 (1)	-	+	+	Other

Escherichia coli (4)	LAT-4 (1)	-	+	+	Other
	DHA-1 (1)	-	+	+	Other
	DHA-1 (1)	-	-	-	-
	Overexpressed AmpC (1)	-	-	-	-
Citrobacter freundii (1)	Overexpressed AmpC (1)	-	+	+	Other
Enterobacter cloacae (1)	Overexpressed AmpC (1)	-	+	+	Other
ESBL and cephalosporinase producers (n=7)					
Klebsiella pneumoniae (3)	SHV-11 + DHA-1 (1)	-	+	-	+
	SHV-12 + DHA-1 (1)	-	+	+	Other
	SHV-5 + DHA-1 (1)	-	+	+	Other
Escherichia coli (3)	CTX-M-9 + DHA-1 (1)	-	+	+	Other
	CTX-M-9 + CMY (1)	-	+	+	Other
	CTX-M-15 + CMY (1)	-	+	+	Other
Enterobacter cloacae (1)	CTX-M-15 + overexpressed AmpC (1)	-	+	+	Other
Penicillinase producers (n=6)					
E. coli (1)	TEM-1	-	-	-	-
E. coli (1)	OXA-1	-	-	-	-
K. pneumoniae (1)	TEM-2	-	-	-	-
K. pneumoniae (1)	OXA-1	-	-	-	-
E. cloacae (1)	TEM-1	-	-	-	-
E. cloacae (1)	OXA-10	-	-	-	-
Wild type (n=3)					
Escherichia coli (1)	Wild type (1)	-	-	-	-
K. pneumoniae (1)	Wild type (1)	-	-	-	-
E. cloacae (1)	Wild type (1)	-	-	-	-
ESBL and Carbapenemases producers (n=17)					
Klebsiella pneumoniae (7)	KPC-2 + CTX-M-15 (1)	-	+	+	Other
	KPC-2 + CTX-M-2 + SHV-12 (1)	-	+	+	Other

	NDM-1 + CTX-M-15 + SHV-18 (1)	-	+	+	Other
	NDM-1 + OXA-181 + CTX-M-15 + SHV-11 (1)	-	+	+	Other
	IMP-1 + CTX-M-15 (1)	-	+	+	Other
	OXA-48 + CTX-M-15 (2)	-	+	+	Other
Escherichia coli (9)	NDM-1 + CTX-M-15 (1)	-	+	+	Other
	NDM-4 + CTX-M-15 (1)	-	+	+	Other
	OXA-48 + CTX-M-15 (3)	-	+	+	Other
	OXA-181 + CTX-M-15 (4)	-	+	+	Other
Serratia marcescens (1)	VIM-2 + CTX-M-15 (1)	-	+	+	Other
Carbapenemases producers (n=26)					
Klebsiella pneumoniae (8)	KPC-2 (2)	-	+	+	Other
	KPC-3 (2)	-	+	+	Other
	IMP-1 (1)	-	+	+	Other
	OXA-48 (3)	-	+	+	Other
Escherichia coli (15)	KPC-2 (2)	-	+	+	Other
	NDM-1 (5)	-	+	+	Other
	NDM-4 (1)	-	+	+	Other
	NDM-5 (2)	-	+	+	Other
	OXA-48 (1)	-	+	+	Other
	OXA-181 (5)	-	+	+	Other
Enterobacter cloacae (1)	NDM-1 (1)	-	+	+	Other
Citrobacter freundii (2)	VIM-2 (1)	-	+	+	Other
	OXA-48 (1)	-	+	+	Other

Table 1. The results of the Rapid ESBL NP® with Enterobacterales