

# Rapid Detection of Extended-Spectrum- $\beta$ -Lactamase-Producing *Enterobacteriaceae*

Patrice Nordmann, Laurent Dortet, and Laurent Poirel

Service de Bactériologie-Virologie, INSERM U914 Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, K-Bicêtre, France

**Enterobacterial strains producing clavulanic-acid-inhibited extended-spectrum  $\beta$ -lactamases (ESBLs) are increasingly reported worldwide. Conventional detection of ESBL production remains time-consuming (24 to 48 h). Therefore, the ESBL NDP (Nordmann/Dortet/Poirel) test was developed for a rapid identification of ESBLs in *Enterobacteriaceae*. This biochemical test was based on the *in vitro* detection of a cephalosporin (cefotaxime) hydrolysis that is inhibited by tazobactam addition. The ESBL activity was evidenced by a color change (red to yellow) of a pH indicator (red phenol) due to carboxyl-acid formation resulting from cefotaxime hydrolysis that was reversed by addition of tazobactam (positive test). The ESBL NDP test was applied to cultured strains (215 ESBL producers and 40 ESBL nonproducers). Its sensitivity and specificity were 92.6% and 100%, respectively. Its sensitivity (100%) was excellent for detection of CTX-M producers. A few ESBL producers ( $n = 16$ ) that remained susceptible to cefotaxime were not detected. The test was also evaluated on spiked blood cultures and showed excellent sensitivity and specificity (100% for both). The test was rapid (less than 1 h) and cost-effective. It can be implemented in any health care facility and is well adapted for infection control purposes in particular.**

Multidrug resistance is now emerging worldwide at an alarming rate among Gram negatives, causing both community-acquired and nosocomial infections. One of the most important emerging resistance traits in *Enterobacteriaceae* corresponds to resistance to broad-spectrum  $\beta$ -lactams, which is mainly associated with production of clavulanic-acid-inhibited extended-spectrum  $\beta$ -lactamases (ESBLs) (3, 7, 9, 10, 13). An ESBL is a  $\beta$ -lactamase that may confer resistance or reduced susceptibility to the oxymino-cephalosporins (i.e., cefotaxime, ceftriaxone, ceftazidime) and monobactams (i.e., aztreonam) (1). However, ESBLs do not hydrolyze the cephamycins (e.g., cefoxitin and cefotetan), and the carbapenems (imipenem, meropenem) and their hydrolytic activity can be inhibited by several  $\beta$ -lactamase inhibitors such as clavulanic acid and tazobactam (1). ESBL producers are mostly *Escherichia coli* and *Klebsiella pneumoniae*, being the main source of community- and hospital-acquired infections (9).

A variety of ESBLs, mostly of the CTX-M, TEM, and SHV types, have been reported in *Enterobacteriaceae* (1, 10). This detection is necessary in order to screen patients and subsequently improve hospital infection control practices, to curb inappropriate antibiotic use, and therefore to prolong the efficacy of the currently available antibiotics (12, 13).

Current techniques for detecting ESBL producers are based on the determination of susceptibility to expanded-spectrum cephalosporins followed by the inhibition of the ESBL activity, mostly by the use of clavulanic acid or tazobactam (5, 6). The double-disk synergy test and the ESBL “Etest” were proposed for that purpose. Sensitivities and specificities of the double-disk test and of the Etest are good, ranging from 80% to 95% (6). Automated methods for bacterial identification and susceptibility testing are also used in the detection of ESBL-producing organisms (5, 6). The performances of those systems differ depending on the species investigated, with much higher sensitivity (80% to 99%) than specificity (50% to 80%) (5, 6).

Those tests require overnight growth, meaning that up to 24 to 48 h can elapse before ESBL production is detected once the

isolate has grown (5, 6). This may conduce to a delay in the initiation of appropriate antibiotic therapy (12). Molecular detection of ESBL genes (PCR and sequencing) is an interesting alternative but remains costly and requires a certain degree of expertise that is not accessible to nonspecialized laboratories (5, 6). Recently, real-time PCR and DNA microarray assays (Check-Points) to detect ESBL gene variants have become commercially available (4). In any case, however, those PCR-based techniques require isolation of bacteria from clinical samples prior to susceptibility testing and phenotypic identification. Therefore, those results cannot be obtained until at least 48 h after obtaining the clinical samples. The assays are usually not performed in a routine laboratory but restricted to epidemiological purposes. Therefore, a simple and efficient technique for detection of ESBL producers is needed.

Here, we propose a novel test, based on a technique designed to identify the hydrolysis of the  $\beta$ -lactam ring of a cephalosporin (cefotaxime), which generates a carboxyl group, by acidifying a culture medium. It uses 96-well microtiter plates but is also adaptable to single-tube assays. The acidity resulting from this hydrolysis is identified by the color change generated using a pH indicator (red phenol). Inhibition of ESBL activity is evidenced by adding tazobactam in a complementary well. We demonstrated that this test, applied either to bacterial colonies or directly to blood cultures, possessed excellent sensitivity and specificity.

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Address correspondence to Patrice Nordmann, nordmann.patrice@bct.aphp.fr.

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TABLE 1 Detection of  $\beta$ -lactam resistance phenotype, CTX-M-extended-spectrum- $\beta$ -lactamase-producing isolates using the NDP Rapid ESBL test<sup>a</sup>

CTX-M group	$\beta$ -Lactamase content	Species	No. of isolates	Test result		MIC range ( $\mu$ g/ml)			
				CTX	CTX + TZB	CTX	CTX + CLA	CAZ	CAZ + CLA <sup>b</sup>
1	CTX-M-1	<i>E. coli</i>	16	+	–	>16	0.04 to 0.12	0.5 to 2	0.06 to 0.19
		<i>S. enterica</i>	1	+	–	>16	0.06	0.75	0.12
	CTX-M-3	<i>E. coli</i>	1	+	–	>16	>1	4	1
		<i>K. pneumoniae</i>	1	+	–	>16	0.5	1.5	0.19
	CTX-M-15	<i>E. coli</i>	46	+	–	8 to >16	0.06 to 1	0.5 to 24	0.12 to 0.19
		<i>K. pneumoniae</i>	28	+	–	>16	0.03 to 0.12	2 to >32	0.12 to 0.19
		<i>E. cloacae</i>	17	+	–	>16	0.12 to 0.25	2 to 16	0.25 to 0.5
		<i>C. freundii</i>	3	+	–	>16	0.12 to 0.25	8 to >32	0.25 to 0.75
	CTX-M-28	<i>P. mirabilis</i>	1	+	–	>16	0.09	8	0.19
		<i>E. coli</i>	1	+	–	>16	0.06	8	0.09
CTX-M-32	<i>E. coli</i>	1	+	–	>16	0.09	6	0.12	
2	CTX-M-2	<i>E. coli</i>	3	+	–	>16	0.05 to 0.06	1.5 to 4	0.06 to 0.25
	CTX-M-97	<i>E. coli</i>	1	+	–	>16	0.04	2	0.09
9	CTX-M-14	<i>E. coli</i>	15	+	–	>16	0.03 to 0.12	0.5 to 4	0.09 to 0.25
		<i>K. pneumoniae</i>	3	+	–	>16	0.04 to 0.12	0.5 to 6	0.12 to 0.25
		<i>E. cloacae</i>	1	+	–	>16	>1	8	>1
		<i>P. mirabilis</i>	1	+	–	>16	0.09	0.75	0.12
	CTX-M-27	<i>E. coli</i>	6	+	–	>16	0.03 to 0.09	0.5 to 4	0.09 to 0.25
	CTX-M-9	<i>E. coli</i>	1	+	–	>16	0.06	0.19	0.09

<sup>a</sup> CTX, cefotaxime; TZB, tazobactam; CAZ, ceftazidime; CLA, clavulanic acid. +, color change from red to yellow; –, no color change.

<sup>b</sup> Clavulanic acid was added at 10  $\mu$ g/ml.

## MATERIALS AND METHODS

**Strain collection.** A total of 255 strains were used to evaluate the performance of the ESBL NDP (Nordmann/Dortet/Poirel) test. They were from various clinical origins (blood culture, urine, sputum, etc.) and of worldwide origin (Tables 1, 2, 3, and 4). Those strains had previously been characterized for their  $\beta$ -lactamase content at the molecular level. This strain collection included producers of ESBLs ( $n = 215$ ) representing the most common ESBLs (CTX-M, SHV, TEM, GES, and VEB enzymes) identified in clinical isolates (Tables 1 and 2). Negative controls ( $n = 40$ ) were made of strains producing  $\beta$ -lactamases possessing extended-spectrum activity but whose activity is not inhibited by clavulanic acid/tazobactam (Table 3). Negative controls also included strains expressing  $\beta$ -lactamases possessing a narrow spectrum of activity (Table 3).

**Susceptibility testing.** Susceptibility testing was performed by determining MIC values using the Etest (AB bioMérieux; Solna, Sweden) on Mueller-Hinton agar plates at 37°C, and results were recorded according to U.S. guidelines (CLSI), as updated in 2012 (2). The susceptible (S) and resistant (R) breakpoints for cefotaxime are  $\leq 1$   $\mu$ g/ml and  $\geq 4$   $\mu$ g/ml and for ceftazidime are  $\leq 4$  and  $\geq 16$   $\mu$ g/ml, respectively.

**ESBL NDP test using cultured strains.** Strains were isolated on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) and incubated at 37°C for 16 h to 24 h before the ESBL NDP test was performed as follows. One calibrated inoculated loop (10  $\mu$ l) of the tested strain was resuspended in 150  $\mu$ l of 20 mM Tris-HCl lysis buffer previously distributed in Microbead tubes (Ultraclean Microbial DNA isolation kit; MO BIO Laboratories, Carlsbad, CA). Mechanical lysis of bacteria was performed by strong agitation of Microbead tubes using a vortex adapter (MO BIO Laboratories) for 30 min at room temperature. This bacterial suspension was centrifuged at 10,000  $\times g$  at room temperature for 5 min. A 30- $\mu$ l volume of the supernatant was mixed in a well of a 96-well tray with 100  $\mu$ l of a 1-ml solution made of 3 mg of purified cefotaxime sodium salt (Sigma-Aldrich, Saint-Quentin-Fallavier, France) in a pH 7.8 phenol red solution. Other cefotaxime concentrations, other  $\beta$ -lactam molecules (ceftazidime, ceftriaxone, cefepime, aztreonam), and other pH indicators were tested but gave results that were less clear cut (data not shown). The phenol red solution was made

by taking 2 ml of a concentrated pH 8 phenol solution (made from a mixture of 0.5% phenol red in distilled water) to which 16.6 ml of distilled water was added. The pH value was then adjusted to a 7.8 value by adding drops of a 1 N NaOH solution. A mixture of the phenol red solution and the enzymatic suspension being tested was incubated at 37°C for 30 min. Similarly, culture extracts were analyzed in wells containing cefotaxime and tazobactam (4 mg/ml). Other concentrations of tazobactam and other  $\beta$ -lactamase inhibitors were tested, giving results that were less clear cut (data not shown). A test result was considered positive when the well containing cefotaxime alone turned from red to yellow/orange and the well containing cefotaxime supplemented with tazobactam remained red (unchanged color). This definition of test positivity was applied throughout the study. Results were interpreted in a blinded manner by technicians who did not know which were the ESBL producers.

**ESBL NDP test using spiked blood cultures.** Rapid detection of ESBL producers was attempted from spiked blood cultures whose positivity was assessed using a BactAlert blood culture system (bioMérieux). A preliminary study showed that positivity of the blood cultures using this automated blood culture system corresponded to an inoculum ranging from  $\sim 5 \times 10^7$  to  $\sim 5 \times 10^9$  CFU/ml. This inoculum was reached after 24 to 48 h of incubation in this BactAlert system. Strains were inoculated into the blood culture system in the presence of 10 ml of sterile blood. The panel of strains used for spiking blood cultures included either non-ESBL producers ( $n = 24$ ) or ESBL producers ( $n = 64$ ) (Table 4). Spiked blood cultures were made of  $1 \times 10^3$  CFU of each strain and incubated 24 to 48 h before positivity of the blood culture was detected by the BactAlert system. Then, 15 ml of spiked blood culture was transferred into a sterile tube and centrifuged at 1,500  $\times g$  for 3 min to pellet the red blood cells. The supernatant was recovered and centrifuged for 15 min at 4,000  $\times g$  to pellet the bacteria. The bacterial pellet was then resuspended in 500  $\mu$ l of distilled water, transferred to a 1.5-ml tube, and subjected to a thorough vortex procedure for 15 s to lyse the red blood cell remnant and wash the bacterial pellet. After 5 min of centrifugation at 10,000  $\times g$ , the bacterial pellet was resuspended in 150  $\mu$ l of 20 mM Tris-HCl lysis buffer and distributed

TABLE 2 Detection of  $\beta$ -lactam resistance phenotype, TEM-type-, SHV-type-, GES-type-, VEB-type-, and PER-type-extended-spectrum- $\beta$ -lactamase-producing isolates using the ESBL NDP test<sup>a</sup>

ESBL type	$\beta$ -Lactamase content	Species	No. of isolates	Test result		MIC range ( $\mu$ g/ml)			
				CTX	CTX + TZB	CTX	CTX + CLA	CAZ	CAZ + CLA <sup>b</sup>
TEM	TEM-3	<i>K. pneumoniae</i>	3	+	–	4 to >16	0.06 to 0.09	4 to >32	0.06 to 0.09
		<i>E. cloacae</i>	2	+	–	>16	0.12 to 0.38	>16	0.12 to 0.5
		<i>C. freundii</i>	1	+	–	>16	0.12	>16	0.25
	TEM-12	<i>E. coli</i>	1	–	–	0.5	0.06	8	0.09
	TEM-21	<i>P. mirabilis</i>	1	+	–	0.25	0.06	4	0.12
	TEM-24	<i>E. aerogenes</i>	6	–	–	0.5 to 8	0.5 to 8	8 to >32	0.09 to 8
		<i>P. stuartii</i>	1	–	–	0.5	0.25	8	0.09
	TEM-29	<i>E. coli</i>	2	–	–	0.25	0.06	4	0.12
	TEM-52	<i>E. coli</i>	8	+	–	8 to >16	0.03 to 0.09	8 to >32	0.09
		<i>K. pneumoniae</i>	2	+	–	>16	0.04 to 0.12	>32	0.12 to 0.19
		<i>P. mirabilis</i>	1	+	–	>16	0.09	16	0.12
	TEM-121	<i>E. aerogenes</i>	1	–	–	<0.06	<0.06	>32	32
	TEM-133	<i>E. coli</i>	1	–	–	0.19	0.06	12	0.09
SHV	SHV-2a	<i>K. pneumoniae</i>	4	+	–	8 to >16	0.06 to 0.09	2 to 4	0.06 to 0.19
		<i>K. pneumoniae</i>	2	–	–	1	0.03 to 0.06	1 to 4	0.03 to 0.12
		<i>E. coli</i>	3	+	–	8 to >16	0.06 to 0.09	2 to 4	0.06 to 0.19
	SHV-3	<i>E. coli</i>	1	+	–	12	0.02	>32	0.09
	SHV-5	<i>K. pneumoniae</i>	2	+	–	>16	0.03 to 0.12	16 to 32	0.09 to 0.25
		<i>E. cloacae</i>	1	+	–	>16	0.38	>32	0.5
	SHV-12	<i>E. coli</i>	6	+	–	>16	0.05 to 0.06	8 to >32	0.06 to 0.25
		<i>E. coli</i>	1	–	–	0.75	0.06	8	0.12
		<i>K. pneumoniae</i>	2	+	–	2 to 8	0.03 to 0.06	16 to 32	0.12 to 0.19
		<i>K. pneumoniae</i>	1	–	–	1	0.04	8	0.12
		<i>E. cloacae</i>	6	+	–	8 to >16	0.38 to >1	16 to >32	0.5 to >4
	SHV-28	<i>K. pneumoniae</i>	3	+	–	>16	0.12 to 0.25	>32	0.09 to 0.25
GES	GES-1	<i>K. pneumoniae</i>	1	+	–	0.5	0.06	4	0.5
	GES-5	<i>E. cloacae</i>	1	+	–	>16	>1	>32	>1
VEB	VEB-1	<i>E. coli</i>	1	+	–	8	0.12	>32	0.25
		<i>E. cloacae</i>	1	+	–	16	0.5	>32	0.5
PER	PER-1	<i>P. mirabilis</i>	1	+	–	>16	0.12	>32	0.25
	PER-1	<i>E. cloacae</i>	1	+	–	>16	>1	>32	>1

<sup>a</sup> CTX, cefotaxime; TZB, tazobactam; CAZ, ceftazidime; CLA, clavulanic acid. +, color change from red to yellow; –, no color change.

<sup>b</sup> Clavulanic acid was added at 10  $\mu$ g/ml.

in bead tubes. The ESBL NDP test was then applied to this pellet as described above. Experiments were systematically performed in triplicate.

## RESULTS

**ESBL NDP test and bacterial cultures.** In an assay using the ESBL NDP test with all CTX-M producers ( $n = 147$ ; Table 1), the wells turned from red to yellow in the presence of cefotaxime and remained red when tazobactam was added (Fig. 1). The sensitivity of the test was excellent (100%). In all cases, MIC values of cefotaxime for those strains were high ( $>8 \mu$ g/ml). The test was less sensitive for detecting non-CTX-M ESBL producers, since 17 (25%) of the 68 tested strains among the ESBL producers failed to be detected (Table 2). Negative results were observed in several strains which had MIC values of cefotaxime lower than the resistance breakpoint for that molecule (except for a few *Enterobacter aerogenes* isolates) (Table 2). For all ESBL-producing isolates that hydrolyze cefotaxime (color change from red to yellow in the first well), the second well that contained tazobactam remained red (inhibition of hydrolysis), thus corresponding to a positive test.

That result indicated the *in vitro* inhibition of the ESBL activity by tazobactam. None of the wells containing bacterial extracts obtained from strains that did not produce  $\beta$ -lactamases with extended-spectrum activity had a positive test result (Tables 1, 2, and 3). A few ESBL-negative strains producing  $\beta$ -lactamases hydrolyzing cefotaxime (overproduced cephalosporinases) (Table 2) gave a red-to-yellow color change in wells containing cefotaxime. However, this color change was also observed in wells containing cefotaxime supplemented with tazobactam, indicating that the hydrolytic activity of those enzymes was not inhibited by tazobactam, thus corresponding to a negative test (Table 3). The overall sensitivity and specificity of the ESBL NDP test were 92.6% and 100%, respectively. The ESBL NDP test was able to differentiate ESBL producers from strains resistant to extended-spectrum cephalosporins by other mechanisms or from those susceptible to expanded-spectrum cephalosporins and therefore not producing an ESBL (Table 3). Notably, those isolates combining ESBL production with overproduced (chromosomal or plasmid-mediated) AmpC gave positive results.

TABLE 3 Detection of non-ESBL-producing isolates using the ESBL NDP test<sup>a</sup>

Phenotype of $\beta$ -lactam resistance	$\beta$ -Lactamase content	Species	No. of isolates	Test		MIC range ( $\mu$ g/ml)			
				CTX	CTX + TZB	CTX	CTX + CLA	CAZ	CAZ + CLA <sup>b</sup>
No resistance	Wild type	<i>E. coli</i>	5	–	–	0.02–0.04	ND	0.05–0.19	ND
	Wild type	<i>S. enterica</i>	1	–	–	0.05	ND	0.19	ND
Penicillinase	TEM-1	<i>E. coli</i>	5	–	–	0.03–0.09	ND	0.09–0.12	ND
	Wild type	<i>K. pneumoniae</i>	2	–	–	0.02–0.03	ND	0.03	ND
	Wild type	<i>K. oxytoca</i>	1	–	–	0.03	ND	0.06	ND
Chromosome- encoded cephalosporinase	Wild type	<i>E. cloacae</i>	5	–	–	0.09–0.5	ND	0.12–0.38	ND
	Wild type	<i>E. aerogenes</i>	1	–	–	0.094	ND	0.25	ND
	Wild type	<i>M. morgani</i>	2	–	–	0.03–0.75	ND	0.09–1	ND
	Wild type	<i>C. freundii</i>	1	–	–	0.12	ND	0.25	ND
	Wild type	<i>C. brakii</i>	1	–	–	0.12	ND	0.25	ND
	Wild type	<i>S. marcescens</i>	2	–	–	0.19–0.75	ND	0.09	ND
	Wild type	<i>S. ficaria</i>	1	–	–	0.12	ND	0.09	ND
Acquired cephalosporinase	DHA-1	<i>E. coli</i>	1	–	–	0.25	1	1	2
	DHA-1	<i>K. pneumoniae</i>	1	–	–	>16	>1	>32	>4
	DHA-2	<i>K. pneumoniae</i>	1	+	+	>16	>1	>32	>4
	ACC-1	<i>E. coli</i>	1	–	–	>16	>1	>32	>4
	ACC-1	<i>E. coli</i>	1	+	+	>16	>1	>32	>4
	ACC-1	<i>P. mirabilis</i>	1	–	–	>16	>1	>32	>4
	CMY-2	<i>E. coli</i>	1	+	+	>16	>1	>32	>4
Overexpressed chromosome-encoded cephalosporinase		<i>E. cloacae</i>	3	+	+	>16	>1	>32	>4
		<i>E. cloacae</i>	2	–	–	>16	>1	>32	>4
		<i>E. sakazakii</i>	1	–	–	>16	>1	>32	>4

<sup>a</sup> CTX, cefotaxime; TZB, tazobactam; CAZ, ceftazidime; CLA, clavulanic acid; ND, not determined. +, color change from red to yellow; –, no color change.

<sup>b</sup> Clavulanic acid was added at 10  $\mu$ g/ml.

The red-to-yellow color change (Fig. 1) was obtained quickly (1 to 5 min after the start of incubation) for most CTX-M producers. Overall, a 15-min incubation time was sufficient to obtain a frank color change for all CTX-M producers ( $n = 147$ ). However, interpretable results were always obtained for all ESBL producers in less than 30 min.

**ESBL NDP test and blood cultures.** The ESBL NDP test was used directly from blood cultures inoculated with ESBL producers ( $n = 64$ ) and non-ESBL producers ( $n = 24$ ) (Table 4, Fig. 2). The sensitivity and specificity of the test were 100% under those conditions. The total amount of time required to obtain results using spiked blood cultures was less than 2 h.

## DISCUSSION

The ESBL NDP test combines multiple advantages. It is cheap, rapid, sensitive, and specific. It is particularly effective for detecting the CTX-M producers that currently account for most of the ESBLs identified worldwide (3, 7, 9, 10). The reasons of the lack of detection of several ESBL producers, in particular of the TEM and SHV series, remain to be elucidated. It might have resulted from weak hydrolysis of cefotaxime but also from low-level production of the ESBL related to low MIC values of cefotaxime. All strains that overproduced an AmpC but were ESBL negative gave negative results. This resulted from either of two possibilities: (i) no (or very poor) hydrolysis of cefotaxime by the AmpC, resulting in two red wells, or (ii) hydrolysis of cefotaxime not inhibited by clavulanic acid or tazobactam in accordance with the AmpC property, resulting in two yellow/orange wells. Notably, several isolates positive for ESBL production and overexpressing their chromosome-

encoded cephalosporinase have been tested. All those strains were positive for the hydrolysis of cefotaxime due to the action of the ESBL, but this hydrolysis was well inhibited by tazobactam, thus giving a positive result. However, we cannot rule out the possibility that some ESBL-positive and AmpC-overproducing isolates could give a positive result for cefotaxime plus tazobactam, if the corresponding AmpC hydrolyzes cefotaxime at high level (leading to a false-negative result).

Interestingly, we showed that this assay can be easily implemented for detection of ESBL-producing isolates from blood cultures. Of note, the overall sensitivity of the ESBL NDP test was even higher (reaching 100%) using this blood culture protocol. These results could be explained by the increased inoculum recovered from blood culture experiments compared to those recovered during pure culture experiments.

This test can be used to search for ESBL producers among (i) bacteria grown in blood cultures and/or (ii) bacterial colonies grown on selective or nonselective media prior to any antibiotic susceptibility testing. This test may therefore find an excellent application in countries where a high incidence of producers of ESBLs (mostly CTX-M) occurs, such as in many Asian countries (8, 9). Use of this test might significantly improve the outcome for patients infected with ESBL producers by supporting a better antimicrobial stewardship through the rapid and accurate identification of ESBL-producing isolates (12). Interestingly, a positive cefotaxime hydrolysis result (color change from red to yellow in a cefotaxime-containing well) was always associated with expression of either an ESBL or a plasmid-mediated cephalosporinase

TABLE 4 Detection of non-ESBL and ESBL producers in spiked blood cultures using the ESBL NDP test<sup>a</sup>

Strain category	β-Lactamase	Species	Bacterial concn in blood culture (CFU/ml)	ESBL NDP test result from:			
				Blood cultures		Colonies	
				CTX	CTX + TZB	CTX	CTX + TZB
Control	Wild type	<i>E. coli</i> BG 1106 6252	8.0 × 10 <sup>8</sup>	—	—	—	—
		<i>E. coli</i> BG 1106 6367	7.0 × 10 <sup>8</sup>	—	—	—	—
	Penicillinase	<i>E. coli</i> BG 1107 8873	5.2 × 10 <sup>9</sup>	—	—	—	—
		<i>E. coli</i> BG 1106 5698	2.0 × 10 <sup>9</sup>	—	—	—	—
		<i>E. coli</i> BG 1106 7202	1.3 × 10 <sup>9</sup>	—	—	—	—
		<i>E. coli</i> BG 1108 6157	1.2 × 10 <sup>9</sup>	—	—	—	—
		<i>K. pneumoniae</i> BG 1202 5484	4.0 × 10 <sup>8</sup>	—	—	—	—
	Wild type	<i>E. cloacae</i> BG 1106 7746	1.4 × 10 <sup>9</sup>	—	—	—	—
		<i>E. cloacae</i> BG 1106 7725	5.2 × 10 <sup>8</sup>	—	—	—	—
		<i>E. cloacae</i> BG 1107 4118	5.0 × 10 <sup>8</sup>	—	—	—	—
		<i>E. aerogenes</i> BN 1113 0225	1.0 × 10 <sup>9</sup>	—	—	—	—
		<i>M. morgani</i> BG 1106 5902	1.7 × 10 <sup>9</sup>	—	—	—	—
		<i>M. morgani</i> BG 1117 0617	1.4 × 10 <sup>9</sup>	—	—	—	—
		<i>C. freundii</i> BG 1106 7767	8.8 × 10 <sup>8</sup>	—	—	—	—
		<i>S. marcescens</i> BG 1107 4243	8.0 × 10 <sup>8</sup>	—	—	—	—
		<i>S. marcescens</i> BN 1201 0816	3.8 × 10 <sup>9</sup>	—	—	—	—
<i>S. ficaria</i> CIP 104255		2.1 × 10 <sup>9</sup>	—	—	—	—	
Acquired cephalosporinase	DHA-1	<i>E. coli</i> GOU	9.7 × 10 <sup>8</sup>	—	—	—	—
	DHA-1	<i>K. pneumoniae</i> ANG	1.1 × 10 <sup>8</sup>	—	—	—	—
	DHA-2	<i>K. pneumoniae</i> FOR	2.1 × 10 <sup>9</sup>	+	+	+	+
	ACC-1	<i>E. coli</i> (Belgium)	7.8 × 10 <sup>7</sup>	—	—	—	—
	ACC-1	<i>E. coli</i> LOU	2.3 × 10 <sup>9</sup>	+	+	+	+
	ACC-1	<i>P. mirabilis</i> BIK	8.0 × 10 <sup>7</sup>	—	—	—	—
	CMY-2	<i>E. coli</i> Ec13 SYD	1.8 × 10 <sup>9</sup>	+	+	+	+
	TEM producing	TEM-3	<i>K. pneumoniae</i> 09.200	1.5 × 10 <sup>8</sup>	+	—	+
<i>K. pneumoniae</i> 09.138			3.0 × 10 <sup>8</sup>	+	—	+	—
TEM-21		<i>P. mirabilis</i> 09.177	1.2 × 10 <sup>9</sup>	+	—	—	—
TEM-52		<i>E. coli</i> 09.11	2.7 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.40	1.3 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.66	1.8 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.73	1.1 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.92	6.0 × 10 <sup>8</sup>	+	—	+	—
		<i>E. coli</i> 09.124	1.0 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.225	2.0 × 10 <sup>8</sup>	+	—	+	—
		<i>K. pneumoniae</i> 09.162	8.0 × 10 <sup>8</sup>	+	—	+	—
		<i>K. pneumoniae</i> 09.169	1.0 × 10 <sup>9</sup>	+	—	+	—
		<i>P. mirabilis</i> 09.128	5.0 × 10 <sup>8</sup>	+	—	+	—
TEM-121		<i>E. aerogenes</i> E2O15	3.0 × 10 <sup>8</sup>	+	—	—	—
TEM-133		<i>E. coli</i> 09.123	1.7 × 10 <sup>9</sup>	+	—	—	—
SHV producing		SHV-2a	<i>E. coli</i> 09.187	1.3 × 10 <sup>9</sup>	+	—	+
	<i>E. coli</i> 09.207		4.0 × 10 <sup>8</sup>	+	—	+	—
	<i>K. pneumoniae</i> 09.99		1.7 × 10 <sup>9</sup>	+	—	+	—
	<i>K. pneumoniae</i> 09.127		9.0 × 10 <sup>8</sup>	+	—	+	—
	<i>K. pneumoniae</i> 09.181		1.3 × 10 <sup>9</sup>	+	—	+	—
	<i>K. pneumoniae</i> 09.190		1.4 × 10 <sup>9</sup>	+	—	—	—
	SHV-3	<i>E. coli</i> A7R5	1.4 × 10 <sup>8</sup>	+	—	+	—
	SHV-5	<i>K. pneumoniae</i> 09.60	1.6 × 10 <sup>9</sup>	+	—	+	—
		<i>K. pneumoniae</i> 09.217	9.0 × 10 <sup>8</sup>	+	—	+	—
	SHV-12	<i>E. coli</i> 09.41	1.3 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.79	1.4 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.129	1.7 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.173	3.2 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 09.193	2.6 × 10 <sup>9</sup>	+	—	+	—
		<i>K. pneumoniae</i> 09.45	2.6 × 10 <sup>9</sup>	+	—	+	—
		<i>K. pneumoniae</i> 09.158	1.9 × 10 <sup>9</sup>	+	—	+	—
<i>E. cloacae</i> 09.57		1.0 × 10 <sup>9</sup>	+	—	+	—	
CTX-M producing	CTX-M-1	<i>E. coli</i> 10.36	1.8 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 10.38	1.7 × 10 <sup>9</sup>	+	—	+	—
		<i>S. enterica</i> serovar Typimurium 10.81	2.0 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 10.95	1.0 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 10.104	3.3 × 10 <sup>9</sup>	+	—	+	—
	CTX-M-14	<i>E. coli</i> 10.46	7.8 × 10 <sup>8</sup>	+	—	+	—
		<i>E. coli</i> 10.124	5.2 × 10 <sup>8</sup>	+	—	+	—
		<i>E. coli</i> 10.11	1.9 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 10.13	1.6 × 10 <sup>9</sup>	+	—	+	—
		<i>E. coli</i> 10.32	2.7 × 10 <sup>9</sup>	+	—	+	—

(Continued on following page)

TABLE 4 (Continued)

Strain category	$\beta$ -Lactamase	Species	Bacterial concn in blood culture (CFU/ml)	ESBL NDP test result from:			
				Blood cultures		Colonies	
				CTX	CTX + TZB	CTX	CTX + TZB
CTX-M-15	CTX-M-15	<i>E. coli</i> 10.16	$2.0 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.33	$3.7 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.43	$3.6 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.49	$1.0 \times 10^7$	+	-	+	-
		<i>E. coli</i> 10.74	$9.6 \times 10^8$	+	-	+	-
		<i>E. cloacae</i> 10.75	$3.8 \times 10^8$	+	-	+	-
		<i>E. cloacae</i> 10.87	$2.2 \times 10^9$	+	-	+	-
		<i>K. pneumoniae</i> SHI	$1.5 \times 10^9$	+	-	+	-
		<i>K. pneumoniae</i> MEK	$3.4 \times 10^8$	+	-	+	-
		CTX-M-2	CTX-M-2	<i>E. coli</i> 10.121	$1.9 \times 10^9$	+	-
<i>E. coli</i> 10.23	$1.2 \times 10^9$			+	-	+	-
<i>E. coli</i> 10.132	$1.4 \times 10^9$			+	-	+	-
CTX-M-27	CTX-M-27	<i>E. coli</i> 10.73	$5.0 \times 10^8$	+	-	+	-
		<i>E. coli</i> 10.178	$1.3 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.198	$2.7 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.204	$1.7 \times 10^9$	+	-	+	-
		<i>E. coli</i> 10.207	$2.3 \times 10^9$	+	-	+	-
CTX-M-3	CTX-M-3	<i>E. coli</i> 10.130	$1.9 \times 10^9$	+	-	+	-
		<i>K. pneumoniae</i> 10.159	$2.1 \times 10^9$	+	-	+	-
CTX-M-32	CTX-M-32	<i>E. coli</i> 10.64	$1.0 \times 10^9$	+	-	+	-
CTX-M-28	CTX-M-28	<i>E. coli</i> C5O52	$1.0 \times 10^9$	+	-	+	-
CTX-M-9	CTX-M-9	<i>E. coli</i> 10.53	$2.2 \times 10^9$	+	-	+	-

<sup>a</sup> CTX, cefotaxime; TZB, tazobactam; CAZ, ceftazidime; CLA, clavulanic acid. +, color change from red to yellow; -, no color change.

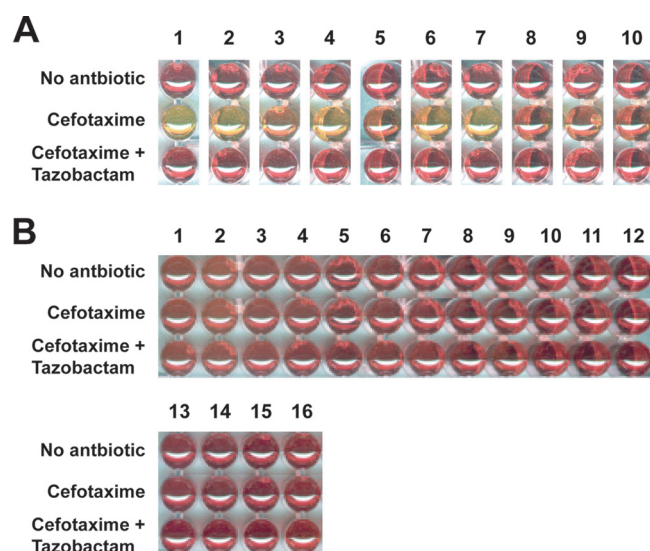


FIG 1 Representative results of the ESBL NDP test using culture isolates. (A) ESBL producers are indicated as follows: *E. coli* FOR CTX-M-15 (column 1), *E. coli* GUE CTX-M-1 (column 2), *E. coli* DEV CTX-M-3 (column 3), *E. coli* GAL CTX-M-14 (column 4), *K. pneumoniae* ISS CTX-M-14 (column 5), *K. pneumoniae* DOU CTX-M-15 (column 6), *E. cloaca* MAZ CTX-M-15 (column 7), *E. coli* SHV-2a (column 8), *E. coli* SHV-12 (column 9), *E. coli* TEM-52 (column 10). (B) The non-ESBL producers are indicated as follows: *E. coli* BG 1106 6175 wild type (column 1), *E. coli* BG 1106 6207 wild type (column 2), *E. coli* BG 1106 6301 wild type (column 3), *Salmonella enterica* BG 1106 6187 wild type (column 4), *Klebsiella oxytoca* BG 1106 6141 wild type (column 5), *K. pneumoniae* BG 1106 7725 wild type (column 6), *K. pneumoniae* BN 1113 0227 wild type (column 7), *E. coli* MAC BG 1106 7257 penicillinase (column 8), *E. coli* BG 1106 5898 penicillinase (column 9), *E. coli* BN 1113 0228 penicillinase (column 10), *E. coli* BN 1113 0231 penicillinase (column 11), *E. cloacae* BG 1106 7746 wild type (column 12), *Citrobacter freundii* BG 1106 7767 wild type (column 13), *E. aerogenes* BN 1113 0225 wild type (column 14), *Morganella morganii* BG 1106 5902 wild type (column 15), *E. cloacae* BG 1106 7725 wild type (column 16).

and high MIC values for cefotaxime that were above the resistance breakpoints (Tables 1, 2, 3, and 4). Although a negative result could not exclude the possibility of the presence of a broad-spectrum cephalosporin-resistant strain resulting from porin deficiency associated with low-level  $\beta$ -lactamase activity, a positive result in the ESBL NDP test may be an important clinical indicator for excluding the choice of expanded-spectrum cephalosporins for treating infected patients and retaining a carbapenem-containing regimen.

It is admitted that ESBL detection is particularly useful for hygiene and infection control (2). A rapid identification of carriage of ESBL producers, in particular when a cephalosporin-re-

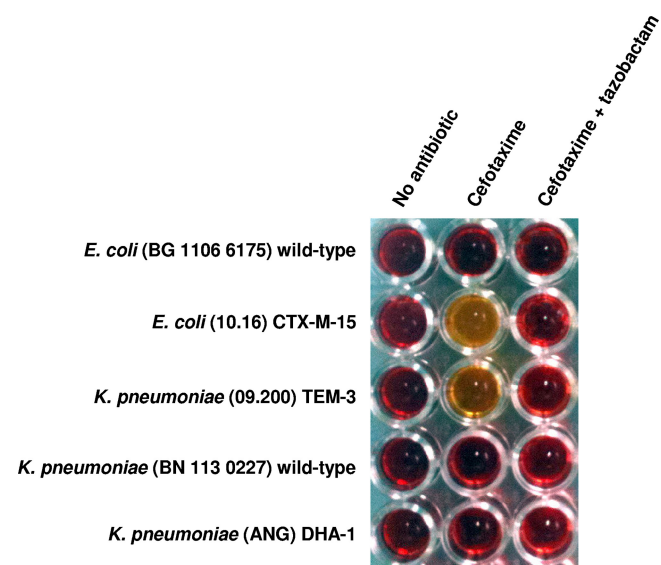


FIG 2 Representative results of the ESBL NDP test using spiked blood cultures.

sistant isolate has been obtained using chromogenic screening media (11), may help to implement adequate hygiene measures rapidly that would further reduce the development of nosocomial outbreaks. Using the ESBL NDP test directly from colonies grown from those screening media would provide a significant gain of time (at least 24 h) compared to phenotypic-based techniques. This may have an important value for prevention of outbreaks, particularly in high-risk units (i.e., intensive care units, etc.), and for cost savings, in particular of those costs resulting from the need for staff reserved for performing tests. In addition, the ESBL NDP test used as a screening test is much cheaper than molecular techniques. This feature is of utmost importance for many developing countries.

The ESBL NDP test would also support novel antibiotic development by facilitating patient enrollment in pivotal clinical trials. Results from the ESBL NDP test can select the strains to be further tested by PCR and/or submitted to sequencing for a detailed identification of the genes at the molecular level.

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