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The β-Lacta Test for Direct Detection of Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* in Urine

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With the β-Lacta test, production of extended-spectrum β-lactamases (ESBLs) was assayed in 200 urine samples showing Gram-negative bacilli during direct microscopic examination. While 168 samples tested negative, all samples yielding ESBL-producing *Enterobacteriaceae* after culture gave positive ($n = 30$) or uninterpretable ($n = 2$) results. The sensitivity and specificity of ESBL detection were 94% and 100%, respectively.

A survey conducted from 2002 to 2010 by the French national infection control program demonstrated an increase of 282% in the incidence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBLE), particularly ESBL-producing *Escherichia coli* (1). The increasing importance of multiresistant ESBL-producing *E. coli* strains in the community should make clinicians aware of potential treatment failures associated with serious and potentially life-threatening infections (2). A recent study by Peralta et al., conducted in 19 Spanish hospitals, found that the ESBLE causing bacteremia were mainly from the urinary (55.3%) and biliary (12.7%) tracts. *E. coli* accounted for 89% of all ESBLE strains, and 45.7% of these were multidrug resistant (e.g., resistant to β-lactam–β-lactamase inhibitor combinations, cephalosporins, quinolones, and aminoglycosides) (3). Furthermore, empirical antibiotic treatment was adequate in only 48.8% of the cases, and the in-hospital mortality was 20.9% (3). ESBL-producing *E. coli* isolates, particularly those producing CTX-M enzymes, account for a significant number of cases of bacteremia in hospitalized and nonhospitalized patients (4). Moreover, Livermore has reported that, since 2003, 90% of ESBL-producing *E. coli* isolates in the United Kingdom produce CTX-M-15 (5).

Urinary tract infections (UTI) are among the most frequent bacterial infections in the community and in the health care setting (6). Broad-spectrum cephalosporins, β-lactam–β-lactamase inhibitor combinations, or fluoroquinolones are recommended for first-line empirical therapy of sepsis arising from the urinary tract in community-acquired and nosocomially acquired cases (4, 7). These recommendations might be difficult to apply if a significant proportion of such infections is caused by ESBLE (4).

A new chromogenic test (β-Lacta test [BLT]; Bio-Rad, Marnes-La-Coquette, France) was developed for the rapid detection (in less than 15 min) of strains of *Enterobacteriaceae* with decreased susceptibility or resistance to third-generation cephalosporins (3GC) conferred by enzymes such as ESBLs and carbapenemases. This test provides useful information to guide antibiotic treatment before full results of antimicrobiotic susceptibility testing are available (8). The aim of this study was to highlight the time saved in cases of UTI when the BLT is performed directly with urine rather than bacterial colonies.

Urine samples. All urine samples received over a 3-month period and showing Gram-negative bacilli (GNB) upon direct microscopic examination and on Gram stains of uncentrifuged urine were prospectively included. Urine samples with hematuria which would interfere with the chromogenic BLT were excluded.

TABLE 1 Phenotypes of Gram-negative bacilli isolated from urine samples subjected to the β-Lacta test

Organism(s) or test result	No. of strains with indicated phenotype				
	Wild	BSBL[a]	ESBL	AmpC-type β-lactamases[b]	Total
Escherichia coli	79	48	16	4	147
Klebsiella pneumoniae	9	0	14	0	23
Enterobacter spp.	13	0	3	5	21
Proteus mirabilis	3	4	0	1	8
Citrobacter koseri	3	0	0	0	3
Morganella morganii	3	1	0	0	4
Proteus vulgaris	1	0	0	0	1
Obligate aerobes[c]	9	0	0	5	14
Total	120	53	33	15	221
No. β-Lacta test positive	0	0	31	0	31
No. β-Lacta test uninterpretable	0	0	2	0	0

[a] BSBL, broad-spectrum β-lactamases, including TEM-1, TEM-2, OXA-1, and IRT-2.
[b] AmpC-type β-lactamases conferring resistance to ceftazidime or cefotaxime (chromosome or plasmid mediated).
[c] Ten *Pseudomonas aeruginosa* strains, one *Acinetobacter baumannii* strain, and three *Stenotrophomonas maltophilia* strains.

β-Lacta test. The BLT was performed directly on urine sediments according to the manufacturer's recommendations (8). Two collection periods were scheduled to test for possible variations in BLT efficiency; during the first 5 weeks, 1-ml urine samples collected from 100 patients were centrifuged for 2 min at 3,000 × *g* in Eppendorf tubes, and during the following 7 weeks, 1.5-ml samples collected from an additional 100 patients were

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TABLE 2 β-Lacta test results during the first and second collection periods for extended-spectrum β-lactamase-producing *Enterobacteriaceae*

β-Lacta sample collection period	ESBLE	Amt of GNB at microscopic examination	CFU/ml by culture	Initial color	Immediate color change (<1 min)	Color at 15 min	Result	*bla* gene
1st	*Klebsiella pneumoniae*	Several	$>10^5$ and 10^5 *P. aeruginosa*	Yellow	Yes	Purple	Positive	CTX-M-15
	Klebsiella pneumoniae	Plenty	10^5	Yellow	No	Orange	Uninterpretable	CTX-M-15
	Escherichia coli	Abundant	10^5	Yellow	No	Red	Positive	CTX-M-15
	Klebsiella pneumoniae	Abundant	$>10^5$ and $>10^5$ *E. coli*	Yellow	No	Red	Positive	CTX-M-15
	Escherichia coli	Few	$>10^5$	Yellow	No	Red	Positive	CTX-M-64*
	Klebsiella pneumoniae	Several	$>10^5$	Yellow	No	Red	Positive	CTX-M-15
	Klebsiella pneumoniae	Plenty	10^5	Yellow	Yes	Purple	Positive	CTX-M-15
	Escherichia coli	Abundant	10^5	Yellow	No	Red	Positive	CTX-M-15
	Escherichia coli	Plenty	$>10^5$	Yellow	No	Red	Positive	CTX-M-55
	Klebsiella pneumoniae	Several	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15
	Escherichia coli	Few	10^4	Yellow	Yes	Orange	Uninterpretable	CTX-M-1
	Klebsiella pneumoniae	Plenty	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15
2nd	*Enterobacter cloacae*	Abundant	$>10^5$	Orange	Yes	Purple	Positive	CTX-M-15
	Escherichia coli	Abundant	10^5	Yellow	Yes	Purple	Positive	CTX-M-55
	Enterobacter cloacae	Few	10^5	Yellow	No	Purple	Positive	CTX-M-15
	Klebsiella pneumoniae	Abundant	$>10^5$	Yellow	Yes	Purple	Positive	CTX-M-15
	Klebsiella pneumoniae	Several	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15
	Klebsiella pneumoniae	Few	$>10^5$	Yellow	No	Purple	Positive	CTX-M-14
	Escherichia coli	Several	$>10^5$ and $>10^5$ *Enterococcus* species	Yellow	No	Purple	Positive	CTX-M-15
	Escherichia coli	Abundant	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15
	Escherichia coli	Several	10^5	Yellow	No	Purple	Positive	CTX-M-55
	Escherichia coli	Several	10^5	Yellow	Yes	Purple	Positive	CTX-M-15
	Escherichia coli	Few	$>10^5$ and $>10^5$ *E. cloacae*	Yellow	No	Red	Positive	CTX-M-64**[a]
	Escherichia coli	Abundant	10^5	Yellow	No	Purple	Positive	CTX-M-15
	Escherichia coli	Several	10^5	Yellow	No	Red	Positive	CTX-M-14
	Escherichia coli, *Klebsiella pneumoniae*	Few	10^5, 10^5, and 10^5 *A. baumannii*	Yellow	No	Red	Positive	CTX-M-15
	Klebsiella pneumoniae	Abundant	10^5	Yellow	No	Red	Positive	CTX-M-27
	Escherichia coli	Plenty	$>10^5$	Yellow	No	Red	Positive	CTX-M-1
	Escherichia coli	Several	$>10^5$	Yellow	Yes	Purple	Positive	CTX-M-55
	Klebsiella pneumoniae	Plenty	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15
	Enterobacter cloacae	Several	10^5	Yellow	Yes	Purple	Positive	CTX-M-15
	Klebsiella pneumoniae	Several	$>10^5$	Yellow	No	Purple	Positive	CTX-M-15

[a] CTX-M-64** was found twice in the same patient in two independently collected urine samples.

centrifuged for 5 min, also at 3,000 × *g*. After elimination of the supernatant, the BLT was performed in the sediment-containing tube. The sediment was completely homogenized in one drop (ca. 50 μl) of each reagent, and after incubation for 15 min at room temperature, the test was interpreted visually as follows: no change in color indicated a negative result, a color change to red or purple indicated a positive result, and a color change to orange was uninterpretable. In some cases, an immediate color change was observed (in less than 1 min).

Antibiotic susceptibility testing. Standard disk diffusion results were interpreted 48 h after the BLT, and 3GC (i.e., cefotaxime or ceftazidime)-resistant isolates were screened for ESBL production using the double-disk synergy test by following the recommendations of the Comité de l'Antibiogramme of the Société Française de Microbiologie (CA-SFM) (9).

Molecular characterization of β-lactamase genes. All strains suspected of producing one or more acquired broad-spectrum β-lactamases (BSBLs) or extended-spectrum β-lactamases (ESBLs) and strains resistant to ceftazidime or cefotaxime were screened using PCR and sequencing as described previously (10, 11).

Culture characteristics of GNB. In total, 200 urine samples containing GNB, as seen using direct microscopy and Gram staining of uncentrifuged urine, were included in this study. From these, 221 strains grew on Uriselect 4 agar (Bio-Rad) after 16 to 24 h of incubation at a threshold of detection of $\geq 10^4$ CFU/ml. They were mainly *Enterobacteriaceae* ($n = 207$; 94%), including 147 *E. coli* strains (71%), 23 *Klebsiella pneumoniae* strains (11%), 21 *Enterobacter* species strains (10%), 9 *Proteus* species strains (4%), 4 *Morganella morganii* strains, and 3 *Citrobacter koseri* strains (Table 1). Fourteen obligate aerobes (6%) were recovered on the same

medium, including 10 *Pseudomonas* species strains, 3 *Stenotrophomonas maltophilia* strains, and 1 *Acinetobacter baumannii* strain (Table 1).

Performance of the BLT. In total, 30 (15%) of the 200 urine samples were found to be BLT positive, and two (1%) gave a uninterpretable result. During the first collection period, 10 out of 100 samples tested positive with the BLT and two samples gave uninterpretable results (Table 2). During the second collection period, BLT seemed to be somewhat more efficient, with 20 samples out of 100 testing positive and all results being interpretable (Table 2).

Phenotypic characterization of all GNB isolates from urine (Table 1) confirmed that samples giving a positive or an uninterpretable BLT result were ESBL producers, and no negative BLT results were obtained for ESBL producers. All GNB strains producing β-lactamases other than ESBLs, such as broad-spectrum β-lactamases (TEM-1, TEM-2, IRT-2, and OXA-1) and AmpC-type enzymes, gave negative BLT results. Thirty-three ESBLs were isolated from the 32 BLT-positive urine samples, including 16 *E. coli*, 14 *K. pneumoniae*, and 3 *Enterobacter* species strains (Table 1). BLT has shown sensitivities of 87% in the first collection period and of 100% in the second and a specificity of 100% in both periods. The presence of other bacterial morphotypes in the polymorphic flora seen at direct microscopic examination did not interfere with the BLT results.

The molecular results confirmed the phenotypic enzyme characterizations and revealed that all ESBLs belonged to the CTX-M group (Table 2), in apparent keeping with the ongoing CTX-M β-lactamase pandemic (5, 12).

The test yielded very high values for both sensitivity (94%) and specificity (100%). Recently, Renvoisé et al., analyzing isolates grown for 16 to 24 h, demonstrated that the sensitivity and specificity of BLT were 99.6% and 87.7%, respectively, for *Enterobacteriaceae* overexpressing *ampC* and that both were 100% for ESBL producers (8). This demonstrates that the β-lactam ring of the BLT chromogenic substrate (HMRZ-86) is very efficiently hydrolyzed by ESBL but not AmpC-type activities.

All screening tests for rapid detection of ESBL-producing GNB require at least 16 to 24 h, including those that use specific agar media, e.g., ESBL agar (AES Chemunex), ChromID ESBL agar (bioMérieux), or Brilliance ESBL agar (Oxoid). They have sensitivities and specificities of 81.3 to 87.5% and 60.8 to 82.1%, respectively (13). Recently, a biochemical test, ESBL NDP, was proposed by Nordmann et al. (14). Preliminary culturing of the isolate was required, but the test took less than 1 h; it was found to have a specificity of 100% and a sensitivity of 92.6%.

In conclusion, BLT may be considered an efficient test for the detection of ESBL in urine, and due to the rapidity and ease with which it is performed, it is a valuable adjunct in specifying empirical UTI management, including measures to limit cross-transmission. The BLT may be an adequate tool for efficient detection of carbapenemase-producing *Enterobacteriaceae* in countries with an elevated prevalence of theses enzymes (e.g., *Klebsiella pneumoniae* carbapenemase and class B carbapenemases) (15).

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