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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

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Invasive infections due to extended-spectrum-β-lactamase-producing *Enterobacteriaceae* (ESBL-E) are associated with considerable morbidity and mortality and excess hospital costs (1–4). It is assumed that early identification of ESBL-E bacteremia may lead to optimized treatment, contributing to better outcomes (5). The β-Lacta test (Bio-Rad, Marnes-La-Coquette, France) is a chromogenic assay that can detect resistance to third-generation cephalosporins associated with ESBL production in *Enterobacteriaceae* within 15 min (6). The test has been successfully used for direct detection of ESBL-E in urine samples submitted for culture (7). This study evaluated the accuracy of the β-Lacta test for rapid detection of Ambler class A ESBL-E from smudge plates prepared from positive blood cultures.

The study was conducted at Sunnybrook Health Sciences Centre, a tertiary-care hospital in Toronto, Canada, from August 2016 to July 2017. Blood cultures were collected in Bactec Plus aerobic and anaerobic bottles incubated in the BD Bactec 9240 automated blood culture system (BD Diagnostic Systems, Sparks, MD). Blood cultures that were flagged as positive with Gram-negative bacilli seen in microscopy were included in the study. A 3-ml aliquot aspirated from the blood culture broth was centrifuged, and the bacterial pellet was used to prepare a smudge plate on chocolate agar, as previously described (8). Smudge plates were incubated at 35°C for 2 h, and the resultant bacterial growth was used for organism identification with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the Vitek-MS system (software v2.0; bioMérieux, Durham, NC) (8). If *Escherichia coli* or *Klebsiella* species was identified by the MALDI-TOF MS, the smudge plate growth was used for the β-Lacta test, done in accordance with the manufacturer's instructions. Standard laboratory procedures were used to subculture the positive blood cultures and identify the organisms. Antimicrobial susceptibility testing, including determination of susceptibilities to ceftriaxone and ceftazidime, and confirmation of ESBLs were done in accordance with CLSI guidelines (9). Results obtained with the β-Lacta test from smudge plates were interpreted prior to and in a manner blind to conventional test results. Specimens yielding polymicrobial growth were excluded from the evaluation.

A total of 202 *E. coli*, 60 *Klebsiella pneumoniae*, and 7 *Klebsiella oxytoca* blood culture isolates were included in this evaluation; 39 (19.3%) *E. coli* and 7 (10.4%) *Klebsiella* isolates were confirmed as ESBL producing. All 46 ESBL-E isolates were positive by the β-Lacta test (sensitivity, 100%; 95% confidence interval [CI], 92.3% to 100%) (Table 1).

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TABLE 1 Results of the β-Lacta test compared to conventional antimicrobial susceptibility testing for detection of ESBL-producing *E. coli* and *Klebsiella* spp. using smudge plates prepared from positive blood culture broths

Organism and ESBL result by conventional testing	No. of strains with result by β-Lacta test	
	Negative	Positive
Escherichia coli ($n = 202$)		
Negative	161	2
Positive	0	39
Klebsiella spp. ($n = 67$)		
Negative	57	3
Positive	0	7
Overall ($n = 269$)		
Negative	218	5
Positive	0	46

Five non-ESBL isolates (2 *E. coli*, 2 *K. pneumoniae*, and 1 *K. oxytoca* isolate) were positive by the β-Lacta test (specificity, 97.8%; 95% CI, 94.9% to 99.3%); three of these isolates were resistant to one or more third-generation cephalosporins and two isolates were resistant to cefoxitin, suggesting that AmpC β-lactamase may have accounted for these β-Lacta test results. None of the isolates negative by the β-Lacta test was resistant to any of the third-generation cephalosporins tested, and there were no carbapenemase-producing isolates.

Two previous evaluations of the β-Lacta test from positive blood cultures have been reported (10, 11). One study, using growth from a 3-hour subculture, found that the β-Lacta test detected 28 (84.8%) of 33 blood culture isolates resistant to third-generation cephalosporins (10). Walewski and colleagues determined that the β-Lacta test had 95.7% sensitivity and 100% specificity for identifying ESBL-E from blood cultures after treating bacterial pellets from the blood culture broths with saponin followed by two washes (11). Our evaluation was done in a setting of relatively low rates of carbapenemase-producing organisms, and the assay may perform differently in areas with higher prevalences of carbapenem resistance.

The procedures used in our evaluation of the β-Lacta test using smudge plates prepared from positive blood cultures were technically simple and rapidly yielded highly sensitive and specific results for detection of ESBL-producing *E. coli* and *Klebsiella* spp. This protocol makes it possible to identify Gram-negative bacillus blood culture isolates and to screen them for the presence of ESBLs within 2 to 3 h of the blood culture signaling as positive. The availability of these rapid results may guide appropriate antimicrobial therapy in septic patients and thereby lead to improved outcomes.

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