An Improved Extended-Spectrum- β -Lactamase Detection Test Utilizing Aztreonam plus Clavulanate

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ABSTRACT Clinical laboratories test for extended-spectrum β -lactamases (ESBLs) for epidemiological and infection control purposes and also for the potential of cephalosporins to cause therapeutic failures. Testing can be problematic, because the CLSI does not recommend the testing of all producers of ESBLs and also falsely negative results may occur with isolates that coproduce AmpC. Boronic acid-supplemented tests can enhance ESBL detection in AmpC producers. Because aztreonam inhibits AmpCs, a study was designed to compare ESBL detection by the CLSI disk test (CLSI), a boronic acid-supplemented CLSI disk test (CLSI plus BA), and an aztreonam plus clavulanate disk test (ATM plus CA). The study tested 100 well-characterized *Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* isolates. Seventy produced TEM, SHV, or CTX-M ESBLs, with 15 coproducing an AmpC and 11 coproducing a metallo- β -lactamase. Thirty ESBL-negative isolates were also tested. Tests were inoculated by CLSI methodology and interpreted as positive if an inhibitor caused a zone diameter increase of ≥ 5 mm. The percentages of ESBL producers detected were as follows: ATM plus CA, 95.7%; CLSI plus BA, 88.6%; and CLSI, 78.6%. When AmpC was coproduced, the sensitivities of the tests were as follows: ATM plus CA, 100%; CLSI plus BA, 93.3%; and CLSI, 60%. ATM plus CA also detected an ESBL in 90.1% of isolates that coproduced a metallo- β -lactamase. Falsely positive tests occurred only with the CLSI and CLSI plus BA tests. Overall, the ATM plus CA test detected ESBLs more accurately than the CLSI and CLSI plus BA tests, especially with isolates coproducing an AmpC or metallo- β -lactamase.

KEYWORDS AmpC, CLSI, ESBL

Special tests for the detection of extended-spectrum β -lactamases (ESBLs) have been performed since ESBLs were first reported in the early 1980s (1, 2). The tests were designed to identify patient infections at risk of therapeutic failures with certain β -lactam drugs and also for infection control (3). Recently, some laboratories abandoned ESBL testing for therapeutic purposes, in the belief that lowered breakpoints of certain cephalosporins and aztreonam eliminated the risk of therapeutic failures with these drugs (4, 5). This has created controversy over whether ESBL testing is really warranted (6–14). Irrespective of the debate about the value of ESBL testing, laboratories that continue to test must cope with some unresolved issues. If the revised CLSI breakpoints have not been implemented, ESBL testing is required, and for ESBL-positive isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis*, susceptibility to any penicillins, cephalosporins, or aztreonam should be discounted and the isolates should be reported as resistant. In contrast, for ESBL-positive organisms other than these, susceptibility to these drugs should be reported (4, 15–20). This implies that ESBLs are only clinically significant in the organisms covered by the CLSI recommendations (21). Another issue is that the CLSI tests sometimes yield falsely negative results with isolates that coproduce an AmpC β -lactamase (8, 15–18, 22–25).

Received 15 August 2017 **Returned for modification** 10 September 2017 **Accepted** 4 October 2017

Accepted manuscript posted online 18 October 2017

Citation Thomson GK, Ayaz M, Lutes K, Thomson KS. 2018. An improved extended-spectrum- β -lactamase detection test utilizing aztreonam plus clavulanate. J Clin Microbiol 56:e01309-17. <https://doi.org/10.1128/JCM.01309-17>.

Editor Daniel J. Diekema, University of Iowa College of Medicine

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As a consequence, organisms not included in the CLSI recommendations and AmpC producers can be hidden reservoirs of ESBLs (26).

Multiple methods of overcoming AmpC interference in ESBL tests have been advocated. These include supplementing ESBL tests with AmpC inhibitors such as boronic acid (15, 22, 23) or cloxacillin (21), performing clavulanate- or tazobactam-based ESBL confirmatory tests with cefepime, which is not a substrate for AmpCs (18, 21, 25, 27, 28), or using modifications of the original double disk test (2), in which disks containing ESBL substrate drugs are placed in proximity to a disk containing a β -lactamase inhibitor (25, 27, 29). The boronic acid supplementation approach has shown high sensitivity in tests with isolates coproducing AmpC and ESBL enzymes, but may yield falsely positive results in isolates producing only a plasmid-mediated AmpC (22) or with isolates producing a class A carbapenemase (15). The cloxacillin method is inconvenient because it requires in-house incorporation of cloxacillin in the test medium. In our experience, while cefepime-based confirmatory tests improve ESBL detection in AmpC-producing isolates, they sometimes yield falsely positive results with highly susceptible ESBL-negative isolates (data not shown). The various modifications of the double disk test have been used with mixed success (2, 25, 27, 29). They typically utilize two to four ESBL substrates.

Because aztreonam is a substrate of ESBLs and also an inhibitor of AmpCs (30), a study was designed to compare the accuracy of a disk test requiring a single substrate, aztreonam, tested alone and also with clavulanate supplementation (here designated the ATM plus CA test). The comparator tests for the study were the two-substrate CLSI confirmatory disk test (4) (designated CLSI) and the CLSI test with boronic acid supplementation (designated CLSI plus BA).

MATERIALS AND METHODS

Isolates. The isolates comprised 100 *Enterobacteriaceae* and *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates that were previously characterized by molecular, phenotypic, and chemical tests for the types of β -lactamase production. Seventy isolates were ESBL positive and 30 were ESBL negative. The ESBL-positive isolates produced the following enzymes: SHV-2, SHV-3, SHV-4, SHV-5, SHV-7, SHV-18, TEM-3, TEM-7, TEM-8, TEM-10, TEM-12, TEM-16, TEM-52, CTX-M-1, CTX-M-2, CTX-M-3, CTX-M-5, CTX-M-9, CTX-M-12, CTX-M-14, CTX-M-15, CTX-M-16, CTX-M-17, CTX-M-18, CTX-M-30, and CTX-M-45. Fifteen of these isolates coproduced an AmpC β -lactamase. These included high- and low-level chromosomal AmpC production and plasmid-mediated AmpCs of the FOX, ACT, CMY, DHA, and LAT families. Eleven ESBL-positive isolates coproduced a metallo- β -lactamase (MBL) carbapenemase of the NDM or VIM families. The β -lactamases of the negative-control isolates included the limited-spectrum enzymes TEM-1, TEM-2, SHV-1, PSE, OXA-1, and OXA-9, the chromosomal K1 enzyme of *Klebsiella oxytoca*, class A carbapenemases of the SME family and NMC-A, and members of the AmpC family. Table S1 in the supplemental material provides information about the characteristics and study results for each of the individual isolates.

ESBL tests. The CLSI ESBL confirmatory test (4) utilized disks containing cefotaxime, cefotaxime plus clavulanate, ceftazidime, and ceftazidime plus clavulanate (BD Diagnostic Systems, Sparks, MD). These were also used with and without 300 μ g boronic acid (catalog no. 410705-1G; Sigma-Aldrich, St. Louis, MO) supplementation for the CLSI plus BA test. Aztreonam disks (BD Diagnostic Systems, Sparks, MD) with and without 10 μ g clavulanate (USP, Rockville, MD) supplementation were used for the ATM plus CA test. For the latter, 5 mg of clavulanate was dissolved in 10 ml phosphate buffer (0.1 mol/liter, pH 6.0) and filter sterilized. Twenty microliters of this solution was added to each disk as required. The remaining clavulanate solution was stored at -20°C in 1-ml aliquots. Tests were inoculated as lawn cultures by CLSI disk test methodology (4) and interpreted as positive if any test exhibited an increased zone diameter of ≥ 5 mm. Results were reported as uninterpretable if inhibition zones were absent or too small to interpret.

RESULTS

The ATM plus CA test was the most sensitive test, confirming 67 of 70 ESBL producers (95.7%) with no falsely positive results (Table 1). The CLSI test was the least sensitive test, confirming 78.6%, with 6.7% falsely positive results. For the CLSI plus BA test, the addition of boronic acid to the CLSI test increased its sensitivity by 10% to 88.6% and reduced falsely positive results to 3.3%. The falsely negative or uninterpretable results are summarized in Table 2. In tests with isolates that coproduced ESBL and AmpC β -lactamases, the sensitivities were as follows: ATM plus CA, 100%; CLSI plus BA, 93.3%, and CLSI, 60%.

TABLE 1 Accuracy of the three ESBL confirmatory tests

		No. (%)		
Isolate type	No. tested	CLSI	CLSI plus BA	ATM plus CA
True positives				
All ESBL producers	70	55 (78.6)	62 (88.6)	67 (95.7)
Producers of ESBL and AmpC	15	9 (60.0)	14 (93.3)	15 (100)
Producers of ESBL and MBL	11	2 (18.2)	3 (27.3)	10 (90.1)
False positives				
Non-ESBL producers	30	2 (6.7)	1 (3.3)	0 (0)

For isolates that coproduced a metallo- β -lactamase, the ATM plus CA test exhibited 90.1% sensitivity (10/11) in detecting ESBLs, whereas the CLSI plus BA and CLSI tests confirmed ESBL production in only three (27.3%) and two (18.2%) isolates, respectively. Figure 1 shows the ability of the ATM plus CA test to detect ESBLs in an isolate that coproduced an ESBL and an MBL. All three ESBL-negative isolates that produced either NMC-A or SME class A carbapenemases were non-ESBL producers and correctly yielded negative results with each ESBL test.

Twenty-one isolates belonged to taxa not covered by the CLSI recommendations. These included *A. baumannii*, *Citrobacter freundii*, *Citrobacter koseri*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *Salmonella enterica*, *Serratia marcescens*, and *P. aeruginosa*. Eleven were ESBL producers and 10 were ESBL negative. The ATM plus CA and CLSI plus BA tests both detected ESBL production by 10 of the 11 (90.1%) ESBL producers. The CLSI test detected an ESBL in seven of the 11 (63.6%) ESBL producers.

Falsely positive tests occurred only with the CLSI and CLSI plus BA tests. Both tests yielded falsely positive results with a VIM-2-producing *A. baumannii* isolate, and the CLSI test yielded a falsely positive result with a LAT-4-producing *E. coli* isolate.

DISCUSSION

The value of ESBL testing is dependent on its accuracy. Tests that can detect ESBLs in only certain types of pathogens are of limited value and have the potential to cause

TABLE 2 Isolates in which ESBLs were not detected

Organism	ESBL	Other β -lactamases	Result ^a		
			CLSI	CLSI plus BA	ATM plus CA
<i>K. pneumoniae</i>	SHV-4-like	FOX-like, PSE-like, SHV-1	—	+	+
<i>K. pneumoniae</i>	SHV-4-like	FOX-5, SHV-1	—	+	+
<i>K. pneumoniae</i>	SHV-5	TEM-1, SHV-1, OXA-9, ACT-1	—	+	+
<i>K. pneumoniae</i>	CTX-M-15	NDM-1, TEM-1, OXA-1, SHV-1	U	U	+
<i>K. pneumoniae</i>	CTX-M-15	NDM-1, TEM-1, CMY-16, SHV-1	U	U	+
<i>K. pneumoniae</i>	SHV-12-like	KPC-3, TEM-1, OXA-9, SHV-1	+	+	U
<i>K. pneumoniae</i>	CTX-M-15	NDM-1, SHV-1	U	U	+
<i>K. pneumoniae</i>	CTX-M-15	NDM-1, SHV-1	U	+	+
<i>K. pneumoniae</i>	CTX-M-15	NDM-1, OXA-181, SHV-1	U	U	+
<i>K. oxytoca</i>	SHV ESBL	DHA-like, TEM-1	—	+	+
<i>C. freundii</i>	CTX-M-15	NDM-1, TEM-1, OXA-1, AmpC	U	U	+
<i>E. cloacae</i>	SHV-5-like	TEM-1, AmpC	—	+	+
<i>E. coli</i>	CTX-M-15	NDM-1, TEM-1	U	U	+
<i>E. coli</i>	CTX-M-5		+	+	—
<i>E. coli</i>	CTX-M-15	NDM-1, OXA-1, TEM-1	U	U	+
<i>S. marcescens</i>	SHV-4-like	AmpC	—	+	+
<i>P. aeruginosa</i>	OXA-45	VIM-7	U	U	U
Total falsely negative (n)			6	0	1
Total uninterpretable (n)			9	8	2
Total not detected (n [%])			15 (21.4)	8 (11.4)	3 (4.3)

^a+, ESBL detected; —, ESBL not detected; U, uninterpretable (inhibition zones absent or too small to interpret).

FIG 1 CTX-M-15 ESBL of this MBL-producing *C. freundii* isolate could be detected only with the ATM plus CA test. The disks with inhibition zones are aztreonam (12-o'clock location) and aztreonam plus clavulanate (11-o'clock position). The other disks are (clockwise around the perimeter beginning at the 1-o'clock position) ceftazidime plus clavulanate, ceftazidime plus clavulanate plus boronic acid, ceftazidime plus boronic acid, cefotaxime plus clavulanate plus boronic acid, cefotaxime plus boronic acid, and cefotaxime plus clavulanate. The two central disks are ceftazidime (upper) and cefotaxime (lower).

harm through failing to detect ESBLs produced by other organisms. The need for improved detection has been advocated for more than a decade (2, 8, 15–25, 27–29, 31–33). The current study, although limited in size, adds to the range of alternative tests that can be performed to increase the accuracy of ESBL detection, especially with isolates that coproduce AmpC and ESBLs and with organisms not included in the CLSI recommendations. Interestingly, it also showed potential for ESBL detection in isolates that coproduce a metallo- β -lactamase. This is a feature that may have value in epidemiological and infection control investigations to identify unsuspected reservoirs of ESBLs.

In this study, both the ATM plus CA and CLSI plus BA tests outperformed the CLSI ESBL confirmatory disk test, with the ATM plus CA test achieving the greatest overall accuracy. The potential benefits of the ATM plus CA test are that it detected ESBLs more accurately than either of the comparator tests and, in requiring only one substrate, it provides greater economy of space than the two-substrate CLSI-based tests. The testing of only two disks instead of four would free up space for testing two additional antibiotic susceptibility disks. An ATM plus CA ESBL detection test could also free up space in other diffusion- or dilution-based susceptibility tests, including automated tests. This would help to meet the clinical need to provide a greater number of potential antibiotic options for therapy of infections by multidrug-resistant Gram-negative pathogens.

The greatest value of the test is its high accuracy in the detection of ESBLs, especially when they are masked by coproduction of an AmpC. This may be relevant to cefepime therapy of infections by AmpC-producing pathogens that harbor an otherwise “hidden” ESBL. It may also provide valuable information about the prevalence of otherwise undetected ESBLs in hospitals and the community, which can be a serious but otherwise unsuspected threat. The test can provide better information about the frequency of ESBLs in organisms not included in CLSI recommendations, such as *Enterobacter* spp., *C. freundii*, etc. Without such information, the true size of the ESBL problem will remain unknown and important information that warrants specific infection control actions may be lacking.

In conclusion, the ATM plus CA test warrants further evaluation as it has the potential to provide a simple and inexpensive solution to two currently unmet needs in providing improved ESBL detection in AmpC-producing isolates and in detecting ESBLs in isolates that are not covered by CLSI ESBL testing recommendations.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.01309-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

ACKNOWLEDGMENTS

We thank the microbiologists who provided the isolates used in this study and Geraldine Kidwell for proof-reading the manuscript and providing editorial suggestions.

The authors have no conflicts of interest.

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