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Evaluation of the Vitek-2 extended-spectrum β-lactamase test against non-duplicate strains of Enterobacteriaceae producing a broad diversity of well-characterised β-lactamases

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ABSTRACT

The Vitek-2 extended-spectrum β-lactamase (ESBL) test was assessed using a collection of 94 ESBL-positive and 71 ESBL-negative non-duplicate isolates of Enterobacteriaceae. These isolates produced a wide diversity of well-characterised β-lactamases, including 61 different ESBLs, two class A carbapenemases and various species-specific β-lactamases. ESBL detection was performed using (i) the conventional synergy test as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie, (ii) the CLSI phenotypic confirmatory test for ESBLs, and (iii) the Vitek-2 ESBL test. For *Escherichia coli* and klebsiellae, the sensitivity/specificity values were 97.3%/96.9% for the synergy test, 91.8%/100% for the CLSI phenotypic confirmatory test, and 91.8%/100% for the Vitek-2 ESBL test. For other organisms, the sensitivity/specificity values were 100%/97.4% for the synergy test, 90.5%/100% for the CLSI phenotypic confirmatory test, and 90.5%/100% for the Vitek-2 ESBL test. The Vitek-2 ESBL test seemed to be an efficient method for routine detection of ESBL-producing isolates of Enterobacteriaceae, including isolates producing AmpC-type enzymes.

Keywords Detection, Enterobacteriaceae, extended-spectrum β-lactamases, phenotypic test, synergy test, Vitek-2 ESBL test

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INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a large group (>150) of β-lactamases that confer resistance to the oxyimino-cephalosporins and monobactams [1,2]. The first ESBLs described were derived from TEM and SHV penicillinases following a few amino-acid substitutions [2]. More recently, non-TEM and non-SHV ESBLs have been reported, including ceftazidimases of the PER, VEB and GES/IBC types, and cefotaximases of the BES-1 and CTX-M types. ESBL production was originally detected in *Klebsiella pneumoniae*, but is now reported in all Enterobacteriaceae isolated commonly from clinical infections [1,3].

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Some ESBL-producing organisms are susceptible to cephalosporins according to conventional breakpoints; indeed, it has been reported that *c.* 40% of ESBL-producing organisms are susceptible to at least one oxyimino-β-lactam, and that 20% are susceptible to all oxyimino-β-lactams according to CLSI breakpoints [4]. However, treatment failure has occurred with oxyimino-cephalosporins even when MICs are within the susceptible range [4]. Various tests have been developed to improve ESBL detection, all of which are based on the activity of ESBLs against oxyimino-cephalosporins and their susceptibility to inhibitors such as clavulanate. According to CLSI recommendations, *Escherichia coli*, *K. pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis* isolates are ESBL producers if the MIC of cefotaxime or ceftazidime is decreased by at least three two-fold dilutions in the presence of clavulanate, or if the zone diameter around a disk of one of these cephalosporins is increased by at least 5 mm in the presence of clavulanate [5]. This

synergy between oxyimino-cephalosporins and clavulanate is also used in the double-disk synergy test recommended for use in France. An extension of the edge of the inhibition zone of a disk containing ceftazidime, cefotaxime, ceftriaxone, aztreonam, cefpirome or cefepime towards the disk containing clavulanate is considered to indicate synergy (http://www.sfm.asso.fr).

The Vitek-2 ESBL test is based on a comparison of the inhibitory effects of ceftazidime, cefotaxime and cefepime, alone and in combination with clavulanate. The final interpretation of results by the automated Vitek-2 system is based on the Vitek-2 ESBL test results and an analysis of MIC distributions of several β-lactam antibiotics. The aim of the present study was to evaluate the ability of the Vitek-2 ESBL test to detect ESBL production in a large collection of non-duplicate isolates of Enterobacteriaceae, including non-*E. coli* and non-*Klebsiella* spp., that were resistant to oxyimino-cephalosporins. The collection of isolates produced a wide diversity of well-characterised β-lactamases, including 61 different ESBLs, two class A carbapenemases, and various species-specific β-lactamases, particularly AmpC-type enzymes.

MATERIALS AND METHODS

Bacterial isolates

The study used a collection of 165 non-duplicate clinical isolates of Enterobacteriaceae producing well-characterised β-lactamases (Table 1). The collection comprised 94 ESBL-producing isolates and 71 ESBL-negative isolates that were resistant to oxyimino-cephalosporins. The ESBL-producing isolates were chosen to include a wide diversity of ESBLs produced by different species, with each isolate producing only one ESBL. The ESBL-negative isolates included isolates that overproduced the intrinsic AmpC and OXY enzymes. The different β-lactamases were identified using PCR methods described previously [6–9]. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used as quality control strains. All the tests were performed blind, with the test inoculum for each isolate being prepared from a plate culture on the same day.

Synergy test

The double-disk-diffusion test, also called the synergy test, was performed as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) (http://www.sfm.asso.fr). Antibiotic disks containing ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg) or aztreonam (30 μg) were placed on a plate, 30 mm (centre to centre) from an amoxycillin–clavulanate (20 μg/10 μg) disk. The inter-disk distance was increased to 45 mm for *Proteus* and *Providencia* isolates, as recommended by the CA-SFM. After overnight incubation at 37°C, an extension of the edge of an antimicrobial inhibition zone towards the disk containing clavulanate was considered to indicate synergy.

CLSI phenotypic confirmatory test

The CLSI disk-diffusion confirmatory test was performed by comparing the inhibition zone diameters given by 30-μg cefotaxime vs. 30-μg cefotaxime plus 10-μg clavulanate disks, and 30-μg ceftazidime vs. 30-μg ceftazidime plus 10-μg clavulanate disks. A ≥5 mm increase between the zone diameters for the cephalosporin disks and their respective cephalosporin–clavulanate disks confirms ESBL production [5]. This CLSI phenotypic confirmatory test is currently validated only for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis*.

Vitek-2 ESBL test

The Vitek-2 ESBL test, included in the AST-N041 card (bioMérieux, Marcy l'Etoile, France), was performed for each isolate according to the manufacturer's recommendations. The test comprised a panel of six wells containing ceftazidime 0.5 mg/L, cefotaxime 0.5 mg/L and cefepime 1.0 mg/L, alone and in combination with clavulanic acid (4, 4 and 10 mg/L, respectively). Growth was assessed quantitatively using an optical reader. A reduction of growth in wells containing a cephalosporin–clavulanate combination compared with that containing the cephalosporin alone was considered to be indicative of ESBL production. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were included as quality control strains in each run. The Vitek-2 ESBL test is currently validated only for *E. coli*, *K. pneumoniae* and *K. oxytoca*. For isolates belonging to other species, the identification was changed to *E. coli* to allow the reading of the Vitek-2 ESBL test. This change was possible because the Vitek-2 growth algorithm was the same for all species of Enterobacteriaceae.

Statistical analysis

Sensitivity was calculated as the number of ESBL-producing isolates showing a positive test result × 100, divided by the number of ESBL-producing isolates. Specificity was calculated as the number of non-ESBL-producing isolates showing a negative test result × 100, divided by the number of non-ESBL-producing isolates. Chi-square and Fisher's exact tests were used to compare proportions (Epi-Info v.6 statistical software; CDC, Atlanta, GA, USA).

RESULTS

Double-disk synergy test

The synergy test identified 92 of the 94 ESBL-producing isolates correctly (sensitivity 97.9%). Among *E. coli* and *Klebsiella* spp., 71 of 73 isolates were identified correctly as ESBL producers (sensitivity 97.3%). False-negative results were observed with an *E. coli* isolate producing SHV-2, and a *K. pneumoniae* isolate producing TEM-93.

Table 1. Summary of 165 isolates of Enterobacteriaceae included in the study

Microorganism (no. of isolates)	Extended-spectrum β-lactamase type (no. of isolates)				Non-ESBL β-lactamase (no. of isolates)
	TEM	SHV	CTX-M	Other	
Citrobacter amalonaticus (1)			CTX-M-8 (1)		
Citrobacter freundii (10)					AmpC (10)
Enterobacter aerogenes (23)	TEM-24 (1)		CTX-M-8 (1)		AmpC
			CTX-M-9 (1)		(20)
Enterobacter cloacae (8)					AmpC
					(8)
Escherichia coli (79)	TEM-3 (1)	SHV-2 (1)	CTX-M-1 (1)	GES-1 (1)	AmpC
	TEM-4 (1)	SHV-3 (1)	CTX-M-2 (1)	GES-2 (1)	(30)
	TEM-6 (1)	SHV-4 (1)	CTX-M-3 (1)	GES-5 (1)	
	TEM-8 (1)	SHV-5 (1)	CTX-M-9 (1)	PER-1 (1)	
	TEM-9 (1)	SHV-6 (1)	CTX-M-14 (1)	BES-1 (1)	
	TEM-10 (1)	SHV-12 (1)	CTX-M-15 (1)		
	TEM-12 (1)		CTX-M-16 (1)		
	TEM-16 (1)		CTX-M-18 (1)		
	TEM-18 (1)		CTX-M-19 (1)		
	TEM-19 (1)		CTX-M-27 (1)		
	TEM-21 (1)		CTX-M (1)		
	TEM-24 (1)				
	TEM-25 (1)				
	TEM-26 (1)				
	TEM-28 (1)				
	TEM-29 (1)				
	TEM-46 (1)				
	TEM-50 (1)				
	TEM-52 (1)				
	TEM-71 (1)				
	TEM-109 (1)				
	TEM-112 (1)				
	TEM-114 (1)				
	TEM-125 (1)				
	TEM-126 (1)				
	TEM-151 (1)				
	TEM-152 (1)				
Klebsiella oxytoca (3)	TEM-3 (1)		CTX-M (1)		OXY (1)
Klebsiella pneumoniae (23)	TEM-4 (1)	SHV-2 (2)	CTX-M-2 (1)		KPC (1)
	TEM-5 (1)	SHV-3 (1)	CTX-M (1)		
	TEM-7 (1)	SHV-4 (1)			
	TEM-8 (1)	SHV-5 (2)			
	TEM-9 (1)				
	TEM-11 (1)				
	TEM-12 (1)				
	TEM-14 (1)				
	TEM-15 (1)				
	TEM-16 (1)				
	TEM-18 (1)				
	TEM-24 (1)				
	TEM-26 (1)				
	TEM-93 (1)				
Proteus mirabilis (8)	TEM-3 (1)		CTX-M-2 (1)		
	TEM-11 (1)				
	TEM-21 (1)				
	TEM-24 (1)				
	TEM-66 (1)				
	TEM-92 (1)				
	TEM-113 (1)				
Proteus rettgeri (1)	TEM-24 (1)				
Providencia stuartii (2)	TEM-24 (1)				
	TEM-92 (1)				
Salmonella spp.(4)	TEM-20 (1)		CTX-M-4 (1)		
	TEM-25 (1)		CTX-M-6 (1)		
Serratia marcescens (3)		SHV-5 (1)			SME-1 (1)
		SHV-12 (1)			

The *E. coli* isolate producing SHV-2 displayed a low level of resistance to oxyimino-cephalosporins (MICs ≤1 mg/L), whereas the *K. pneumoniae* isolate producing TEM-93 displayed a high level of resistance to oxyimino-cephalosporins (MICs ≥64 mg/L). Synergy was only observed for the *K. pneumoniae* isolate when the inter-disk distance was decreased from 30 to 20 mm.

Among Enterobacteriaceae other than *E. coli* and klebsiellae, the synergy test detected all 21 ESBL-producing isolates (sensitivity 100%). Of the 71 ESBL-negative isolates, 69 were identified as non-ESBL producers (specificity 97.2%). False-positive results were observed for a *K. oxytoca* isolate that overproduced OXY, and a *Serratia marcescens* isolate that produced SME-1. These

two enzymes were active against certain oxyimino-cephalosporins and were susceptible to clavulanate. The results obtained using this test are summarised in Table 2.

CLSI phenotypic confirmatory test

The CLSI phenotypic confirmatory test identified 88 of the 94 ESBL-producing isolates correctly (sensitivity 93.6%). Among the *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* isolates, 75 of the 81 ESBL-producing isolates were identified successfully as ESBL producers (sensitivity 92.6%), and all of the remaining 13 Enterobacteriaceae isolates were identified correctly as ESBL producers (sensitivity 100%). False-negative results were observed with *E. coli* isolates producing TEM-19, TEM-25, TEM-125, TEM-151 and SHV-2, and a *K. pneumoniae* isolate producing SHV-2. *E. coli* and *K. pneumoniae* isolates producing SHV-2, and an *E. coli* isolate producing TEM-19, displayed a low level of resistance to oxyimino-cephalosporins (MICs ≤1 mg/L). *E. coli* isolates producing TEM-125 and TEM-151 showed a complex resistance phenotype, with reduced susceptibility to both oxyimino-cephalosporins and to clavulanate–penicillin combinations, which probably explains why they were not detected as ESBL producers. The *E. coli* isolate producing TEM-25 had oxyimino-cephalosporin MICs ranging from 4 to 64 mg/L, and the reason why this isolate was not detected is unclear.

All 71 ESBL-negative strains were identified as non-ESBL producers (specificity 100%). The results obtained using this test are summarised in Table 2.

Vitek-2 ESBL test

The Vitek-2 ESBL test identified 86 of the 94 ESBL-producing isolates correctly (sensitivity 91.5%). Among the *E. coli* and klebsiellae isolates included in the study, 67 of the 73 ESBL-producing isolates were identified correctly as ESBL producers (sensitivity 91.8%), and 19 of the other 21 ESBL-producing Enterobacteriaceae isolates were also identified as ESBL producers (sensitivity 90.5%).

False-negative results were observed for *E. coli* isolates producing TEM-19, TEM-25, TEM-151, TEM-152 and SHV-2, for a *K. pneumoniae* isolate producing TEM-93, for a *Salmonella* isolate producing CTX-M-6, and for a *Providencia stuartii* isolate producing TEM-24. The *E. coli* isolates producing TEM-19 and SHV-2 displayed a low level of resistance to oxyimino-cephalosporins (MICs ≤1 mg/L), while the *E. coli* isolates producing TEM-151 and TEM-152 had a complex resistance phenotype, with resistance to both oxyimino-cephalosporins and clavulanate–penicillin combinations. Such a phenotype could explain the difficulty in detecting ESBL production by these isolates. The *K. pneumoniae* isolate producing TEM-93 displayed a high level of resistance to oxyimino-cephalosporins (MICs ≥64 mg/L), and was also ESBL-negative according to the CA-SFM synergy test. The *Prov. stuartii* isolate had a low level of resistance to cefotaxime and cefepime

Table 2. Comparison of the synergy test (ST), the CLSI phenotypic confirmatory test (CLSI) and the Vitek-2 ESBL test (V2) for detecting extended-spectrum β-lactamase (ESBL) production among 94 ESBL-producing and 71 non-ESBL-producing isolates

	No. of ESBL-positive isolates identified as						No. of ESBL-negative isolates identified as						Sensitivity (%)			Specificity (%)		
	ESBL-positive			ESBL-negative			ESBL-positive			ESBL-negative								
Species (no. of isolates)	ST	CLSI	V2	ST	CLSI	V2	ST	CLSI	V2	ST	CLSI	V2	ST	CLSI	V2	ST	CLSI	V2
Escherichia coli (79)	48	44	44	1	5	5	0	0	0	30	30	30	98	89.8	89.8	100	100	100
Klebsiella pneumoniae (23)	21	21	21	1	1	1	0	0	0	1	1	1	95.5	95.5	95.7	100	100	100
Klebsiella oxytoca (3)	2	2	2	0	0	0	1	0	0	0	1	1	100	100	100	0	100	100
Proteus mirabilis (8)	8	8	8	0	0	0	0	0	0	0	0	0	100	100	100			
Citrobacter amalonaticus (1)	1	1	1	0	0	0	0	0	0	0	0	0	100	100	100			
Salmonella spp. (4)	4	4	3	0	0	1	0	0	0	0	0	0	100	100	75			
AmpC-producing species (47) (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Providencia* spp.)	8	8	7	0	0	1	1	0	0	38	39	39	100	100	87.5	97.4	100	100
Total	92	88	86	2	6	8	2	0	0	69	71	71	97.9	93.6	91.5	97.2	100	100

(MICs ≤1 mg/L), but a more typical ESBL-associated ceftazidime MIC of 16 mg/L. However, strong induction of bla_{AmpC} by clavulanate was observed on the synergy test plate. Thus, partially derepressed production of AmpC could explain why ESBL production was not detected in this isolate. The *E. coli* isolate producing TEM-25, and the *Salmonella* isolate producing CTX-M-6, exhibited an ESBL-type level of resistance to oxyimino-cephalosporins, with at least one MIC value in the range 4–16 mg/L. It is not clear why these isolates were not detected by the Vitek-2 test.

All isolates with false-negative results according to the Vitek-2 ESBL test were retested using all methods, but the discrepancies remained in all cases. All of the 71 ESBL-negative isolates were identified as non-ESBL producers (specificity 100%). The results obtained using the Vitek-2 ESBL test are summarised in Table 2.

Statistical comparisons

No significant difference was observed in terms of sensitivity (91.5% vs. 93.6% and 97.9%; p 0.57 and 0.1) and specificity (100% vs. 100% and 97.9%; p non-applicable and 0.49) between the Vitek-2 ESBL test and the CLSI phenotypic confirmatory test or the synergy test, respectively. No significant differences in sensitivity were observed within any subgroup of Enterobacteriaceae: i.e., *E. coli* (89.8% vs. 89.8% and 98%; p 1 and 0.2), *K. pneumoniae* (95.5% vs. 95.5% and 95.5%, p 1) and other species (91.3% vs. 100% and 100%; p 0.49).

DISCUSSION

This study compared the Vitek-2 ESBL test with the synergy test and the CLSI confirmatory test for the detection of ESBL production by a wide variety of previously characterised non-duplicate isolates. Such a study allowed an evaluation of this test against a wide diversity of enzymes, including rare and challenging ESBLs, which could only be obtained during a lengthy multicentre clinical study of consecutive isolates. In the present study of 165 non-duplicate isolates of Enterobacteriaceae, the Vitek-2 ESBL test had a sensitivity level for ESBL detection that was close to that of the synergy test and the CLSI phenotypic confirmatory test, despite the wide variety of β-lactamases produced (n = 68). These results agree with those of a previous study in which the Vitek-2 test was assessed with bacteria producing 21 different ESBLs [10].

For *E. coli* and *Klebsiella* spp., there was no significant difference in terms of sensitivity among the Vitek-2 ESBL test, the synergy test and the CLSI phenotypic confirmatory test (91.8% vs. 97.3% and 93.6%; p 0.27 and 1). The Vitek-2 ESBL test was also effective in identifying ESBLs among AmpC-producing Enterobacteriaceae, although its use is not validated for this group of organisms in which ESBL detection seems to be more difficult, especially with automated methods. A study of Greek isolates of *Enterobacter* spp. reported that the first-generation Vitek ESBL card was positive for <10% of ESBL-producing strains [11], and another study has indicated the difficulties of ESBL detection in AmpC-producing isolates using the Microscan ESBL confirmation panels, with sensitivity ranging from 54% to 69% [12]. The use of three oxyimino-cephalosporins, including cefepime, could explain the higher sensitivity (87.5%) of the Vitek-2 ESBL test among this group of Enterobacteriaceae. Cefepime use has also been shown to improve the sensitivity of the synergy test among AmpC producers [13,14]. ESBL detection in the AmpC-producing group of Enterobacteriaceae is particularly useful because of the numerous infections caused by ESBL producers belonging to this group [7,15,16]. However, the present study only tested eight such isolates, and the performance of this test should therefore be evaluated against a larger number of isolates.

Among the wide diversity of enzymes tested, most undetected enzymes belonged to the TEM and SHV families. False-negative results were observed with ESBL-producing isolates that had a low level of resistance to oxyimino-cephalosporins. Certain TEM and SHV enzymes, e.g., SHV-2, are known to confer such a low level of resistance, and similar observations have been made with other detection methods [17–19]. Detection of ESBLs belonging to the Complex Mutant TEM (CMT) subgroup was also problematical using both the Vitek-2 ESBL test and the CLSI phenotypic confirmatory test. Difficulties in detection have also been reported for TEM-121 (CMT-4) and TEM-151 (CMT-7) using the synergy test, and for TEM-125 (CMT-6) using both the synergy test and the CLSI MIC confirmatory test [20–22].

The present study, to the best of our knowledge, is the first to include five CMT-type ESBLs in an evaluation of the ESBL detection test, i.e., TEM-50 (CMT-1), TEM-109 (CMT-5), TEM-125 (CMT-6), TEM-151 (CMT-7) and TEM-152 (CMT-8). The study confirmed the correct identification of TEM-50 as an ESBL [23]. Of the five isolates tested, three were correctly identified as ESBL producers according to the Vitek-2 ESBL test and the CLSI test, whereas all were classified as ESBL producers according to the synergy test. The Vitek-2 ESBL test correctly identified all of the CTX-M-producing isolates as ESBL producers, except for the isolate that produced the uncommon enzyme CTX-M-6. The test also detected the atypical PER-1, BES-1 and GES ESBLs. Overall, the Vitek-2 ESBL test had a detection efficiency close to those of the synergy test and the CLSI test for the wide range of ESBLs tested.

The *K. oxytoca* isolate that overproduced the OXY enzyme, which would often be false-positive using other detection methods, including the synergy test [19,24], was correctly identified as a non-ESBL producer by the Vitek-2 ESBL test. Thomson *et al.* [23] also reported the correct identification of this enzyme using the Vitek-2 ESBL test. However, an evaluation involving a larger number of OXY-overproducing isolates is still required. The Vitek-2 ESBL test also identified the two isolates producing a class A carbapenemase as non-ESBL producers, whereas the synergy test gave a false-positive result for one of these two isolates. A limitation of the present study was that no isolates producing a plasmid-mediated AmpC or metallo-β-lactamase enzyme were included. However, these groups are not known to give false-positive results using ESBL detection tests.

In conclusion, the Vitek-2 ESBL test seems to be an excellent test for routine laboratories that are already using the Vitek-2 system because of its easy integration into the laboratory workflow. It is easier to perform than the double-disk synergy test, the results of which can be reader-dependent and rather subjective [4] . The risk of epidemic outbreaks of infection means that the specific detection of ESBLs is a major challenge for clinical microbiologists [4]. As described above, detection is often difficult, even when using reference testing methods such as the synergy test, because of the wide variety of ESBLs. The Vitek-2 ESBL test is an efficient automated test that allows accurate detection of ESBL production, and it is therefore potentially useful for clinical microbiologists.

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