Laboratory detection of extended-spectrum beta-lactamase by an automated system

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SUMMARY

This study aims to evaluate the positive predictive value (PPV) and the negative predictive value (NPV) of Vitek2 in detecting extended-spectrum beta-lactamase (ESBL) phenotypes when compared to a manual confirmatory test as gold standard.

A sample of *Escherichia coli, Klebsiella spp* and *Proteus mirabilis* isolates were collected by 5 laboratories in the Emilia-Romagna Region (Italy).

Vitek2 appears to be an accurate tool to detect ESBL phenotypes of *E. coli* isolates; some concern remains about its performance with the other bacterial species, especially *P. mirabilis*.

KEY WORDS: ESBL detection, Diagnostic tests, Enterobacteriaceae, Antimicrobial resistance, Predictive value of tests

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A worrying increase in resistance to cephalosporins among enterobacteriaceae, due mainly to the spreading of extended-spectrum beta-lactamase (ESBL), has been observed in many countries around the world (EARSS Management Team, 2006; Paterson and Bonomo, 2005; Woodford *et al.*, 2007). Bacteria with ESBL resistance phenotypes, which were previously typical of hospital acquired infections, are now isolated even from community-acquired infections. Moreover, some ESBL genotypes, such as CTX-M group, show a particular ability to spread in the community (Livermore, 2005; Pitout *et al.,* 2005). Although the confirmation of ESBL phenotypes is classically based on manual tests, which are the combination disk (Clinical and Laboratory Standards Institute (CLSI), 2007), the double disk synergy and the Etest (Paterson and Bonomo, 2005), there is growing consideration of automated systems (Hope *et al.,* 2007; Pfaller and Segreti, 2006). These systems, which proved accurate in detecting ESBL, have been proposed as screening and also as confirmatory methods (Pfaller and Segreti, 2006).

In Emilia-Romagna (a northern Italy region of about four million inhabitants) a rapid increase in antimicrobial resistance was observed in *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and other enterobacteriaceae isolates during the period 2003-2005. This increase in resistance, involving penicillins, cephalosporins, fluoroquinolones and aminoglycosides was detected through the regional surveillance system of antimicrobial resistance, which includes the main laboratories of the region. The prevalence of resistance to third generation cephalosporin in *E. coli* isolates from blood cultures increased from 4.8% in 2003 to 11.7% in 2005 (Gagliotti *et al.*, 2006). Regional laboratories lacked a stan-

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dard approach to ESBL detection, which, in most cases, was based on Vitek2 (bioMérieux Italia S.p.A.) (the most commonly used automated system at regional level) without further confirmation tests. In most laboratories, the routine use of manual confirmatory tests for ESBL phenotypes detection was considered not compatible with the work-load due to time and personnel constraints. Therefore, based on previous reports of good performance and accuracy of an automated system in detecting ESBL phenotypes, a protocol based on a pragmatic principle was disseminated throughout the region in March 2006. This protocol recommended accepting the output of Vitek2 advanced expert system (AES) for ESBL detection when an ESBL resistance phenotype was the unique phenotype detected for beta-lactamic antimicrobials in *E. coli, K. pneumoniae, Klebsiella oxytoca* and *P. mirabilis*. Conversely, when an ESBL phenotype was detected as one among two or more phenotypes or when it was detected in bacteria species other than the group of four, further confirmatory testing was recommended (double disk synergy, combination disk or Etest).

The present study aims to evaluate the positive predictive value (PPV) and the negative predictive value (NPV) of Vitek2 in detecting ESBL phenotypes when compared to a manual confirmatory test as gold standard.

Five laboratories, among the 17 included in the regional surveillance system of antimicrobial resistance, were recruited on a voluntary basis to participate to the study. These laboratories (4 hospital laboratories and 1 teaching hospital laboratory) were requested to collect data for all the consecutive clinical isolates of *E. coli, K. pneumoniae, K. oxytoca and P. mirabilis,* identified between May and the first half of June 2006, for which Vitek2 AES detected the ESBL phenotype (either as unique phenotype or as one among two or more); no specific indications were given on Vitek2 cards to be used for the study. Each centre provided a minimum participation period of 15 consecutive days in the defined time interval. A further sample of 15-20 consecutive isolates (among the four listed species), identified in the same period and not detected as ESBL producers by Vitek2, were collected by each participating laboratory. All included isolates were tested for

TABLE 1 - *ESBL phenotype: comparison between the results of the Vitek2 advanced expert system and the confirmation test.*

Results of Vitek2 advanced expert system	*Results of the confirmation test*						
	ESBL–		*ESBL+*		*ESBL undetermined*		*Total*
	n	*%*	*n*	*%*	*n*	*%*	*n*
All organisms							
ESBL -	74	**98.7**	0	0.0	1	1.3	75
ESBL + (unique phenotype)	6	2.3	251	**97.3**	1	0.4	258
ESBL + (with other possible phenotype/s)	7	50.0	6	**42.9**	1	7.1	14
E. coli							
ESBL -	63	**100.0**	0	0.0	0	0.0	63
ESBL + (unique phenotype)	3	1.9	151	**97.4**	1	0.6	155
ESBL + (with other possible phenotype/s)	2	33.3	4	**66.7**	0	0.0	6
K. pneumoniae/oxytoca							
ESBL -	8	**100.0**	0	0.0	0	0.0	8
ESBL + (unique phenotype)	2	5.7	33	**94.3**	0	0.0	35
ESBL + (with other possible phenotype/s)	1	33.3	2	**66.7**	0	0.0	3
P. mirabilis							
ESBL -	3	**75.0**	0	0.0	1	25.0	4
ESBL + (unique phenotype)	1	1.5	67	**98.5**	0	0.0	68
ESBL + (with other possible phenotype/s)	4	80.0	0	**0.0**	1	20.0	5

ESBL with a manual confirmatory test that was either combination disk or Etest; data were collected with an ad hoc form. The PPV and NPV of Vitek2 automated system were calculated using a manual confirmatory test as gold standard after dropping duplicate isolates from the same patient. Data analysis was performed by Stata v.8 (Stata Corp., College Station. TX, USA).

A total of 347 isolates/patients were included; the most frequent species was E. coli. The Vitek2 cards used were the AST-N020 (3 laboratories; 211 isolates) and the AST-N041 (2 laboratories; 136 isolates). Confirmation of ESBL phenotypes was performed by Etest (3 laboratories; 168 isolates) and the combination disk (2 laboratories; 179 isolates). The overall PPV of ESBL detected as unique phenotype by Vitek2 was 97.3% (95% CI 94.5-98.9) with no significant difference among species (p=0.39). The PPV of ESBL detected as one among two or more phenotypes was 42.9% (95% CI 17.7-71.1). The NPV was 98.7% (95% CI 92.8-100) with no false negative and one undetermined result (Table 1).

This study confirms the accuracy of Vitek2 AES in detecting ESBL phenotypes among *E. coli, K. pneumoniae, K. oxytoca* and *P. mirabilis,* as already reported elsewhere (Hope *et al.,* 2007; Livermore *et al.,* 2002; Pfaller and Segreti, 2006; Sanders *et al.,* 2000). A relevant result is the low PPV of Vitek2 when the ESBL phenotype was detected as one among two or more phenotypes. This finding can help in selecting isolates that require further confirmation by manual testing. Isolates with this uncertain output account for only 5.1% (14 out of 272) of all isolates detected by Vitek2 as ESBL producers, but they include 53.8% (7 out of 13) of all false positive results (Table 1).

The main limitation of the present study is the small number of isolates of species other than *E. coli,* which prevents sufficiently precise estimation of the PPV and NPV for these organisms. The sensitivity and NPV of Vitek2 AES in detecting ESBL phenotype for *P. mirabilis* is particularly uncertain. Some reports from regional laboratory that were not included in this study concerned *P. mirabilis* isolates that were not detected as ESBL producers by Vitek2 but were retrieved as producers by a successive manual confirmation test, which was performed because of interpretative concerns. These isolates were interpreted by Vitek2 as not susceptible to cefotaxime (MICs ≥16 µg/ml) but as sensitive to piperacillin-tazobactam (MICs ≤4 µg/ml) and, in most cases, as sensitive also to cefoxitine (MICs ≤8 µg/ml). Further studies are needed to find out if this underdetection of ESBL producers among *P. mirabilis* is a quantitatively relevant problem. Other aspects deserving further investigation are the estimated accuracy of other automated systems in detecting ESBL phenotypes and the genotyping of ESBL producer strains circulating in Emilia-Romagna. Moreover, it is worth to investigate by genotyping the isolates erroneously detected as ESBL producers by Vitek2, because some (or most) of these isolates are likely to be producers of beta-lactamase other than ESBL.

Vitek2 AES appears to be an accurate tool for detecting ESBL phenotypes of *E. coli* isolates and, possibly, also of *K. pneumoniae, K. oxytoca* and *P. mirabilis* isolates in contexts where routine confirmation by manual test is not feasible. This automated system also restricts the need of further confirmation in a small proportion of isolates.

Transparency declarations
No conflict to declare.

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