Laboratory diagnosis of bloodstream infections caused by extended-spectrum ß-lactamase-producing *E. coli* and *Klebsiella* species

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ABSTRACT

Extended-spectrum ß-lactamase (ESBL)-producing organisms are resistant to the third-generation cephalosporins commonly used as empirical therapy for a wide range of serious infections. It is therefore important for laboratories to offer reliable ESBL detection methods. This study compares two combination disc methods (Oxoid and Mast Diagnostics) containing cepodoxime with and without clavulanate with Vitek 2 for routine detection of ESBLs in *Escherichia coli* and *Klebsiella* spp. isolated from blood cultures. From December 2003 to April 2005, a total of 58 potential ESBL-producing isolates (resistant to cefotaxime and/or ceftazidime) by BSAC disc susceptibility were tested by the combination discs and Vitek 2. The Advanced Expert System, a feature of Vitek 2 reports possible mechanisms of resistance, based on interpretive reading of MICs. This study detected 7.4% more ESBL-producing isolates by Vitek 2 than by Oxoid disc testing (95% CI: 0.15–14.7%; $P<0.2$) and 31.6% more ESBL-producing isolates were detected by Vitek 2 than by Mast disc testing, (95% CI: 16.2–46.96%; $P<0.001$). Batch-to-batch variation was evident in disc performance for both disc types. Thus, use of appropriate controls is recommended when testing by the combination disc methods. Although no phenotypic test is 100% sensitive and specific, the Vitek 2 was a reliable system for ESBL detection; however, it is expensive and interpretation of results can be confusing to inexperienced users. Further studies to compare Vitek 2 with cefotaxime and ceftazidime combination discs may reveal disc methodology for ESBL detection to be a more reliable alternative than using cefpodoxime combination discs alone.

KEY WORDS: Antibiotics. Lactams. Septicemia.

Introduction

Organisms producing extended-spectrum ß-lactamases (ESBLs) are able to hydrolyse the third-generation cephalosporins. These antibiotics are among the most widely prescribed ß-lactam antibiotics worldwide.[1,2] The mechanism of resistance mediated by ESBLs is complex. Gram-negative organisms that demonstrated resistance to the early ß-lactam antibiotics usually possessed either SHV enzymes (which indicate a variable response to sulphydryl inhibitors), derived from *Klebsiella* species, or TEM enzymes (so-called because they were first detected in *Escherichia coli* isolated from the blood culture of a Greek patient called Temoneira),[3] found in the Enterobacteriaceae. At least 130 TEM-type and 50 SHV-type ESBLs have been recognised.[4] More recently, CTX-M-type ESBLs have been detected that hydrolyse cefotaxime (CTX) preferentially, although mutation can confer ceftazidime (CAZ) activity. These enzymes may sometimes be referred to as cefotaximases.

Cephalosporins form part of the empiric antibiotic therapy for a wide range of serious infections, including intra-abdominal infections, community-acquired pneumonias and Gram-negative septicaemias.[5] The consequences of not identifying an organism correctly as an ESBL producer and subsequently not applying the rule of reporting all cephalosporins (except cephamycins) as resistant can be treatment failure or death of the infected patient.[5]

There is an urgent need for microbiology laboratories offering a diagnostic service to offer reliable ESBL detection methods.[6] The NHS National Standard Methods Guidance Note QSOP 51, available through the Health Protection Agency (HPA), outlines current strategies for the detection of ESBLs.[5] Briefly, this document recommends that any ESBL detection method should screen all Enterobacteriaceae against an indicator cephalosporin, the best agent being cefpodoxime, as all ESBL-producing organisms are resistant to it. If cefpodoxime is unavailable, testing with both cefotaxime and ceftazidime is required to include the CTX-M enzymes that show variable resistance to ceftazidime, and may be missed if this agent is used alone. Organisms conferring resistance to the indicator cephalosporin(s) should be subjected to an ESBL confirmatory test.

The aims of this study are to compare ESBL combination discs from two manufacturers with a semi-automated method such as the Vitek 2 for routine detection of ESBLs in *E. coli* and *Klebsiella* species.

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Materials and methods

The study was conducted at the Department of Clinical Microbiology and HPA Collaborating Centre, University College London Hospital (UCLH) NHS Trust. All potential ESBL-producing *E. coli* and *Klebsiella* spp. (n=58) obtained from blood cultures during the period December 2003 to April 2005 were selected for further testing. An isolate was labelled as a potential ESBL producer if it was resistant to cefotaxime and/or ceftazidime by BSAC disc sensitivity testing methods.

Identification and selection of isolates

The Becton Dickinson Bactec 9240 system was used to detect the presence of organisms from blood cultures. *E. coli* and *Klebsiella* spp. were identified biochemically using the API20E strip (bioMérieux, Basingstoke, UK). Identification and antibiotic sensitivities (BSAC disc method) were performed directly from the positive blood culture broths. Isolates that met the criteria for a potential ESBL producer were subjected to further tests.

ESBL confirmation tests

Potential ESBL producers were tested using Oxoid combination discs (cefpodoxime [10 mg] and cefpodoxime/clavulanate [10 mg/10 mg]) and Mast DD combination discs (cefpodoxime [30 mg] and cefpodoxime/clavulanate [30 mg/10 mg]). An isolate was labelled as an ESBL producer when the difference in the zone diameter was ≥ 5 mm in the presence of clavulanate when tested by the Oxoid disc test. A positive ESBL result by Mast DD disc testing was recorded when the ratio of the zone diameters was ≥ 50% in the presence of clavulanate. A positive control (*E. coli* TEM 3 NCTC 13351) and negative control (*E. coli* NCTC 10418) were included in accordance with the HPA Guidance note QSOP 51.[5]

The isolates were also tested for the presence of ESBLs on Vitek 2 (bioMérieux) using a Gram-negative sensitivity card (AST-N030). The Advanced Expert System (AES), which is a feature of Vitek 2), was utilised. This alerts the user to unlikely sensitivity patterns for a given organism, and mechanisms of resistance are inferred based on interpretive reading of the MICs. An expert summary of resistance mechanisms was printed and kept for analysis. Isolates that were sensitive to all three cephalosporins by BSAC screening and Vitek 2 were considered to be true non-ESBL producing isolates.

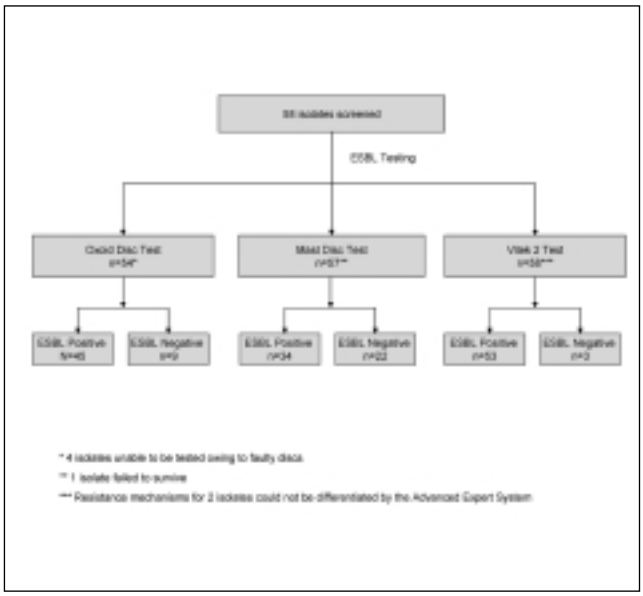

Fig. 1. Comparison of three test methods for ESBL detection.

Statistical analysis

Statistical analysis was performed using the STATA (version 8) software programme. Comparative performance of each disc-based ESBL confirmatory tests with the Vitek 2 was evaluated using McNemar's X^2 test for paired samples. 95% confidence intervals (CI) and tests for significance at the 5% level (P values) were calculated.

Results

During the study period a total of 58 potential ESBL-producing isolates were obtained by screening blood culture BSAC test results. An overview of results of the two disc test methods and the Vitek 2 is illustrated in Figure 1. The variation in numbers tested by each method was due in part to faulty discs, the failure of one isolate to survive, and ambiguous interpretation by the Vitek 2 AES. Vitek 2 detected 53 ESBL-positive isolates compared with 45 and 34 by the Oxoid and Mast disc tests, respectively. The AES was unable to determine the resistance mechanisms in two isolates: the Oxoid disc test found both to be ESBL-negative, whereas the Mast disc test found one to be ESBL-positive.

Comparative performances of each disc test with that of Vitek 2 are described in Tables 1 and 2. McNemar's paired analysis shows that 7.4% more ESBL-producing isolates were detected by Vitek 2 than by Oxoid disc testing (95% CI: 0.15–14.7%; P<0.2). There was no statistically significant difference between the two methods. However, 31.6% more ESBL-producing isolates were detected by Vitek 2 than by Mast disc testing, (95% CI: 16.2–46.96%; P<0.001). This was statistically significant.

Discussion

Reliable diagnosis of ESBL-related infection is a crucial part of the management of infected patients. Laboratories bear the responsibility of being able to offer accurate and timely identification of such organisms.

Extended-spectrum ß-lactamase confirmatory tests currently available to laboratories are the in-house double disc diffusion (DDD) test, commercial combination disc tests with cephalosporin alone and in combination with clavulanate (Oxoid or Becton Dickinson 'Combination Discs' and Mast Diagnostics Mast DD), Etest ESBL strips (AB Biodisk, Bio-Stat and Cambridge Diagnostic Services), Vitek ESBL cards, Vitek 2 AES (bioMérieux) and Phoenix (Becton Dickinson). The Vitek and Phoenix methods are semi-automated systems.

The DDD test is a cheap method for ESBL testing but is no longer recommended because it relies on critical disc

Table 1. Comparative analyses for ESBL detection using Vitek 2 and Oxoid disc tests (n=54).

		Vitek 2	
		Pos	Neg
	Pos	45	0
Disc Oxoid			
	Neg	4	5
Total		49	5

spacing,[2,5,7] which is variable depending on the strain tested.[2,5] Commercial combination disc tests offer many advantages. They are relatively inexpensive, sensitive[7] and do not require critical disc spacing.[5]

M'Zali *et al*.[7]achieved greater sensitivity when both cefotaxime (CTX) and ceftazidime (CAZ) were used in combination with clavulanate. The present study utilised cefpodoxime as the sole substrate for ESBL detection in *E. coli* and *Klebsiella* spp. The sensitivity of this method could have been higher if other cephalosporins (e.g., cefotaxime and ceftazidime) were included in the combination panel.

Using cefpodoxime as the indicator cephalosporin for ESBL detection, this study found that the Oxoid combination discs performed better than the Mast DD discs. Although Vitek 2 detected more ESBLs, there was no statistically significant difference between Vitek 2 and the Oxoid disc test for ESBL detection (Table 1). Currently, the Oxoid 'combination disc' tests are undergoing further quality checks.

There was a marked difference between the results obtained with the Mast DD disc test and Vitek 2, and this was statistically significant (Table 2). Further batches tested with the Mast DD showed better concordance with Vitek 2. There may be batch-to-batch variation in commercial combination disc tests; thus, close monitoring and the use of appropriate controls (*E. coli* NCTC 13351, NCTC 13352 and NCTC 13353 [positive] and *E. coli* NCTC 10418 [negative]) is an essential part of using this test method.[5]

Sanders *et al*.[8] found the Vitek ESBL card to be a reliable test for the detection of ESBLs. It was easier to perform than the DDD test and provided excellent sensitivity and specificity. The Vitek 2 AES detects ESBLs by interpretive reading,[9] and automatically analyses the results gained from susceptibility testing at the MIC level and compares that data to its database. The database contains 20,000 MICs, 2500 phenotypes and 200 resistance mechanisms. The AES then proposes the best phenotype match.

Livermore *et al*.[9] evaluated the AES for interpretive reading of antimicrobial resistances. The AES accurately inferred ESBLs among *E. coli* and *Klebsiella* spp. as well as in the more difficult genera of *Enterobacter* and in Citrobacters. In a multicentre trial involving 10 European laboratories, using genotype data as a comparison, Livermore *et al*. reported that ESBL production was accurately inferred in AmpC-inducible species as well as *E. coli* and *Klebsiella* spp. using the Vitek2 AES.[9]

One disadvantage with the Vitek 2, besides that of cost, is that interpretation of results can be confusing for users new to the system. There are two levels of reports that may be printed. A summary indicates all the possible resistance mechanisms for the test isolate. If only one resistance mechanism is inferred, there is no confusion about the result. If there are two or more resistance mechanisms inferred on the summary report, the AES may need to be examined. For example, if a summary reads ESBL, ESBL+Impermeability, Cephalosporinase, Vitek 2 will indicate the antibiotic susceptibilities that need to be reviewed. In most cases, the AES will offer the most likely resistance phenotype for the test isolate, based on its MIC database. Currently, some Vitek cards offer an additional ESBL confirmatory test that can be used to refine the AES.

In the authors' hands the Vitek 2 AES has proved reliable and easy to use. Vitek 2 AES could not determine the resistance mechanism in only two isolates, and the disc tests only served to confuse the issue by providing conflicting results. Under such circumstances, a laboratory will need another reliable ESBL confirmatory test, and, if identification is unresolved, the isolate may need to be sent to a reference laboratory.

Although ESBL genotypes were not actively sought in the present study, the strain typing unit at the HPA Reference Laboratory, Colindale, was able to verify that one isolate of *E. coli* gave an IS26-$bla_{CTX\text{-}M}$ product consistent with the CTX-M 15 strain A that is epidemic in the UK. Vitek 2 identified 3 CTX-M-type ESBLs from the 58 isolates screened, but failed to identify the two CTX-M ESBLs that were confirmed at the HPA Reference Laboratory. These numbers are small and more work is needed, especially with the CTX-M strains, to evaluate the role of Vitek 2 in diagnosing ESBL phenotypes.

This study underscores the fact that none of these phenotypic tests is 100% reliable in ESBL detection, but they do offer laboratories the means to detect the majority of ESBLs. In contrast, molecular tests have the ability to detect ESBL genes, even if they have not been expressed, and can thus identify the type of ESBL by the genotype. Currently, however, such tests are only available as reference laboratory tests.

In conclusion, at the time this study was undertaken automated systems such as Vitek 2 seemed to be more reliable than disc methods for ESBL detection. However, improved quality in the manufacture of ESBL discs may now mean that disc methodology is equally reliable.

Further studies to compare Vitek 2 with cefotaxime and ceftazidime combination discs may enhance the reliability of disc testing, and these may prove to be a better alternative than using cefpodoxime combination discs alone. □

Table 2. Comparative analyses for ESBL detection using Vitek 2 and Mast disc tests (n=57).

		Vitek 2	
		Pos	Neg
	Pos	34	1
Disc Mast			
	Neg	19	3
Total		53	4

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