Characterization of extended-spectrum β -lactamase (ESBL)-producing Kuwait and UK strains identified by the Vitek system, and subsequent comparison of the Vitek system with other commercial ESBL-testing systems using these strains

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Two hundred and fifty-one unique patient isolates of *Klebsiella pneumoniae* (123), *Escherichia coli* (114), *Klebsiella oxytoca* (7), *Enterobacter cloacae* (5) and *Citrobacter freundii* (2), flagged as extended-spectrum β -lactamase (ESBL) positive by the Vitek system (GNS-526 card), were collected. These strains were isolated from a variety of clinical specimens submitted to the clinical bacteriology laboratories of the Royal Infirmary of Edinburgh (RIE), Edinburgh, UK (and associated GP practices), Hairmyers Hospital, Glasgow, UK, and the Amiri and Farwania Hospitals, Kuwait. Of the 101 RIE strains tested, 15 *E. coli* strains were found to be ESBL negative by Etest ESBL strips. On retesting the 15 *E. coli* strains with the Vitek GNS-532 card, 14 were found to be ESBL negative, despite being originally flagged as ESBL positive. The remaining 236 ESBL-producing strains were also subjected to the double disc-diffusion (DDD) technique for the detection of ESBLs. Of these, two were false negatives by Etest ESBL test strips (using both cefotaxime and ceftazidime strips), and 38 were false negatives by the DDD method. The Etest false-negative ESBL-producing strains of *K. pneumoniae* were positive by DDD. Technically, the Vitek method was the least demanding method to perform, as it was an integral part of the routine susceptibility test card. Etest strips were reliable, but were the most expensive of all the techniques used. The DDD test, while relatively inexpensive, was technically subjective, and in our hands, seven of the ESBL-positive strains that were confirmed by the other two techniques were not detected. Despite the false-positive ESBL-producing *E. coli* strains, the Vitek susceptibility card with its integral ESBL test offers the clinical laboratory a valuable and quick option to screen for ESBL-producing *Klebsiella* spp. and *E. coli* as part of the routine laboratory methodology.

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INTRODUCTION

Since their introduction into clinical practice, oxyimino cephalosporins have been excessively used in hospitals (Du Bois *et al.*, 1995; Heritage *et al.*, 1999). The effectiveness of these β -lactam antibiotics has been diminished by *Klebsiella* spp. which have become resistant to their mode of action. This resistance has spread to strains of *Escherichia coli* and to other Gram-negative bacteria as well (Sanders *et al.*, 1996). Investigations into the mechanism responsible for this resistance have revealed the presence of extended-spectrum β -lactamase (ESBL) enzymes derived from the widespread

TEM-1/2 and SHV-1 family. To date, there are over 110 derivatives of TEM β -lactamases and more than 63 derivatives of SHV β -lactamases (Bush *et al.*, 1995; Bradford *et al.*, 1995; Bradford, 2001; Hawkey & Munday, 2004; Sanders *et al.*, 1996). These enzymes are usually less efficient at hydrolysis than their parent enzymes, and consequently their detection by currently used routine susceptibility tests is difficult. Therefore, ESBL-producing *Klebsiella* spp. and *E. coli* may falsely appear to be susceptible to newer cephalosporins (Gibb & Crichton, 2000). Because current breakpoints for cephalosporin sensitivity are set for clinical efficacy, they are too high to detect ESBL mutations; therefore, there is clearly a requirement to detect the resistance mechanism itself rather than to rely on *in vitro* susceptibility testing. The Vitek system (bioMérieux) has an ESBL

Abbreviations: DDD, double disc-diffusion; ESBL, extended-spectrum β -lactamase; RIE, Royal Infirmary of Edinburgh.

detection test as an integral part of the routine susceptibility test card. There have been several studies of the efficacy on the Vitek system with known ESBL-producing strains (Gibb & Crichton, 2000; Sanders *et al.*, 1996). The current study was performed to assess the behaviour of ESBLs in clinical strains detected by the Vitek system, in comparison with two other commercial ESBL detection methods. Screening and confirmation of the test were done following National Committee for Clinical Laboratory Standards (NCCLS) rules (National Committee for Clinical Laboratory Standards, 1999).

METHODS

Bacterial isolates. One hundred and forty-one clinical isolates were collected in Scotland. One hundred and one isolates consisted of *Klebsiella pneumoniae* (69) and *E. coli* (32) obtained from various clinical samples sent to the clinical bacteriology laboratories of the Royal Infirmary of Edinburgh (RIE), Edinburgh, UK, and 40 Enterobacteriaceae clinical isolates [*K. pneumoniae* (16), *E. coli* (10), *Klebsiella oxytoca* (7), *Ent. cloacae* (5) and *C. freundii* (2)] were obtained from various clinical samples sent to the clinical bacteriology laboratories of the Hairmyers Hospital, Glasgow, UK. The 101 RIE isolates had previously been identified and reported as ESBL positive by the Vitek system. From Kuwait, 110 isolates, consisting of *K. pneumoniae* (38) and *E. coli* (63) were collected from Al-Amiri hospital, and nine *E. coli* isolates were from Farwania hospital. Isolates were collected from various clinical samples and submitted to the clinical laboratories of the Al-Amiri hospital between June and December 2003. All isolates were stored on nutrient agar slopes at room temperature until tested.

ESBL detection methods

Vitek system. The Vitek is an automated system for identification and/or susceptibility testing. Vitek susceptibility test results are expressed as MIC values, and interpreted as susceptible, intermediate or resistant by reference to a National Committee for Clinical Laboratory Standards breakpoint (National Committee for Clinical Laboratory Standards, 1999).

This system is able to detect ESBL production by an automated version of the synergy test, which is based on detection of the inhibitory effect of clavulanic acid on ESBLs in the presence of either cefotaxime or ceftazidime. The test is interpreted by measuring the difference in bacterial growth with or without inhibitor. All isolates were originally tested with Gram-negative susceptibility (GNS-526) cards. Etest-negative strains were retested with GNS-532 cards. In the course of the study, GNS-532 cards superseded GNS-526 cards, with a subsequent software upgrade.

Etest ESBL strips. Etest ESBL strips (AB Biodisk) are double-ended strips with antibiotic and antibiotic/inhibitor gradients. These strips yield the MIC as well as the MIC ratio, which determines the presence of an ESBL. The strips used in this study were ceftazidime + clavulanic acid (TZ/TZL) and cefotaxime + clavulanic acid (CT/CTL), for which the recommended ratio value indicates the presence of an ESBL. Interpretation of the result was as recommended by the manufacturer.

Double disc-diffusion (DDD) method. This method was used to reconfirm the strains that were ESBL positive by Etest and/or Vitek GNS-532 card. The DDD method that was used here employed six discs, three containing ceftazidime (30 µg), cefotaxime (30 µg) or cefpodoxime (10 µg), and three with a combination of the same

antibiotics (10, 10 and 1 µg, respectively) with the addition of clavulanate (Oxoid). A broth culture of the test organism was adjusted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar (Oxoid). The combination discs and the corresponding standard cephalosporin disc were placed at the recommended distance from each other on the plate. The plates were incubated at 37 °C for 18 h aerobically before the zone sizes were recorded. A positive result was indicated by a zone-size difference of ≥5 mm diameter between the combination disc and the corresponding standard antibiotic disc, as recommended by the manufacturer.

For all ESBL detection methods, the known ESBL-producing *E. coli* strains SA1636 (TEM-3) and SA1652 (SHV-2) were used as positive controls. *E. coli* NCTC 10418 was used as a negative control.

RESULTS

UK strains

Of the 101 strains from the RIE originally tested, 15 were negative for ESBL production by both cefotaxime and ceftazidime Etest strips. These 15 Etest-negative strains (all *E. coli*) were retested with Vitek (GNS-532 card), and 14 of these strains were subsequently found to be ESBL negative. One strain still flagged ESBL positive by Vitek. The 14 retested ESBL-negative strains were not subjected to further study.

The cefotaxime ESBL strip detected the presence of ESBL activity in 84 (96.5 %) of the remaining 87 isolates tested. Of these, 19 (22 %) demonstrated phantom phenomena that indicated them to be ESBL positive, as described in the manufacturer's interpretation guide. Three strains (two *E. coli* and one *K. pneumoniae*) were negative for ESBL activity with cefotaxime ESBL strips.

With ceftazidime Etest strips, ESBL activity was detected in 47 (54 %) of the study isolates. Of these, three (3.5 %) were positive by demonstration of phantom phenomena. The use of the combination of both cefotaxime and ceftazidime Etest ESBL strips detected ESBLs in 86 of the 87 test isolates (98.8 %). From these data, it appears that the use of both strips is essential for optimal detection of ESBL activity. Only one strain of *K. pneumoniae* that demonstrated positive ESBL activity with the Vitek system was not detected by Etest ESBL strips. This strain remained positive when retested with Vitek.

Using the DDD method, the combination disc containing cefotaxime detected the presence of ESBL activity in 74 strains (85 %) (64 *K. pneumoniae* and 10 *E. coli*), whilst the cefpodoxime combination disc detected ESBL activity in 64 strains (73.5 %) (54 *K. pneumoniae* and 10 *E. coli*). The ceftazidime combination disc had the poorest sensitivity, detecting the presence of ESBL activity in only 40 strains (46 %) (31 *K. pneumoniae* and 9 *E. coli*). The strain of *K. pneumoniae* that demonstrated no ESBL activity by Etest was ESBL positive with the DDD method. When the results of all three cephalosporin combination discs were taken into

consideration, 80 (92 %) of the 87 ESBL-positive strains were detected.

All 40 strains tested from Hairmyers Hospital, Glasgow, were ESBL positive. ESBL activity in all these isolates was reconfirmed by the Etest ESBL strip and DDD methods. The cefotaxime ESBL strip detected the presence of ESBL activity in 38 (95 %) of the tested isolates. Of these, 19 (48 %) were phantom phenomena ESBL positive, as described in the manufacturer's interpretation guide. Two strains (two *E. coli*) were negative for ESBL activity with cefotaxime ESBL strips. With ceftazidime Etest strips, ESBL activity was detected in 32 (80 %) of the study isolates. Of these, seven (17.5 %) were positive by demonstration of phantom phenomena. The use of the combination of both cefotaxime and ceftazidime Etest ESBL strips detected ESBLs in all of the isolates tested (100 %). From these data, it appears that the use of both strips is essential for optimal detection of ESBL activity.

Using the DDD method, the combination disc containing cefotaxime detected the presence of ESBL activity in 35 strains (87.75 %) (16 *K. pneumoniae*, 10 *E. coli*, six *K. oxytoca* and three *Ent. cloacae*), whilst the cefpodoxime combination disc detected ESBL activity in 31 strains (77.5 %) (15 *K. pneumoniae*, nine *E. coli*, four *K. oxytoca* and three *Ent. cloacae*). The ceftazidime combination disc was the least efficacious, detecting the presence of ESBL activity in only 17 strains (42.5 %) (eight *K. pneumoniae*, four *E. coli*, three *Ent. cloacae* and two *C. freundii*). The strains that demonstrated no ESBL activity by the DDD method were ESBL positive with the Etest method. When the results of all three cephalosporin combination discs were taken into consideration, 37 (92.5 %) of the 40 ESBL-positive strains were detected (see Table 1).

Kuwait strains

Sixty-three strains were obtained from non-repeat isolates from Amiri Hospital patients, and nine strains were isolated from neonates in Farwania Hospital in 1994. The Amiri isolates originated from many departments, and 27 of the 62 patients were >60 years old. Etest ceftazidime and cefotaxime/clavulanate strips detected ESBLs in 71 of 72

isolates (98.6 %), whereas cefpodoxime, which performed best in the DDD test, showed clear results in 46 of 72 isolates (64 %). Of the 38 *K. pneumoniae* strains tested, one showed no synergy with both cefotaxime and ceftazidime Etest strips. The cefotaxime ESBL strips detected the presence of ESBL activity in 29 (76.3 %) of the tested isolates, and nine strains were ESBL negative by cefotaxime strips. With ceftazidime Etest strips, ESBL activity was detected in 32 (84.4 %) of the isolates. The use of a combination of both cefotaxime and ceftazidime Etest ESBL strips detected the enzyme in 37 of the isolates (97.4 %) (see Table 2). From these data it appears that the use of both strips is essential for optimal detection of ESBL activity, particularly as the ceftazidime Etest strip appeared less sensitive in detecting ESBL activity. Only one isolate that was reported ESBL positive by the Vitek method was not detected by Etest ESBL strips; however, this strain remained positive when retested with Vitek.

DISCUSSION

There is currently a great need for reliable and efficient tests to detect ESBLs in clinical isolates of Enterobacteriaceae. Conventional susceptibility testing methods, on their own, fail to offer reliable susceptibility results for β -lactam antibiotics when testing those species that harbour ESBLs.

Currently, most UK clinical laboratories do not use a standard method for the detection of ESBLs, and many clinical laboratories do not routinely identify Enterobacteriaceae to genus and species level. The Vitek system addresses this issue. It will only validate a susceptibility result once the organism has been identified to species level. If the system detects the presence of an ESBL resistance mechanism in strains of *Klebsiella* spp. and *E. coli*, it then utilizes its expert software and applies it to the final susceptibility results. If β -lactams are found to be susceptible to ESBL activity, the strain is then flagged as resistant, regardless of whether the *in vitro* test indicates susceptibility.

This study has shown that the Vitek system, in our hands, whilst easy to perform and without any subjective interpretation of results, reported false-positive detection of ESBL activity with 14 strains of *E. coli*. On retesting these

Table 1. Results obtained with Etest ESBL strips and the DDD method for detecting the presence of ESBLs in 127 strains of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Ent. cloacae* and *C. freundii* isolated from UK hospitals

Combination disc/strip	No. of positive ESBL isolates (%)	
	DDD method	Etest ESBL strip
Cefotaxime	109 (85.8)	122 (96)
Ceftazidime	57 (44.8)	79 (62.2)
Cefpodoxime	95 (74.8)	Not tested
Total ESBL positive (%)	117 (92.1)	126 (99.2)

Table 2. Results obtained with Etest ESBL strips and the DDD method for detecting the presence of ESBLs in 110 strains of *E. coli* and *K. pneumoniae* isolated from Kuwait hospitals

Combination disc/strip	No. of positive ESBL isolates (%)	
	DDD method	Etest ESBL strip
Cefotaxime	43 (39)	95 (86)
Ceftazidime	35 (31.8)	84 (76.4)
Cefpodoxime	54 (49)	Not tested
Total ESBL positive (%)	82 (75)	108 (98.2)

strains with Vitek (GNS-532 card), they were correctly reported as ESBL negative. One of the retested *E. coli* isolates was found to be ESBL positive by the Vitek system and by the DDD method; however, ESBL activity could not be detected by Etest. All these strains were initially tested with the same batch number of Vitek (GNS-526 card). When retested, the GNS-532 card was used; this card has superseded the GNS-526, with accompanying software upgrades. Although internal quality control (QC) is performed on these cards, an ESBL-producing *E. coli* is not used as part of the QC battery. It was initially thought that the 14 false-positive ESBL *E. coli* results might have been the result of a faulty batch of GNS-526 cards. However, recent work in this laboratory, with ten recently isolated Vitek ESBL-positive *E. coli* has shown two of these ten strains to be ESBL negative by Etest. On retesting with the same batch of Vitek GNS-532, these two strains were subsequently reported as ESBL negative. Although the percentage of false-positive *E. coli* ESBL strains fell from approximately 50 to 20 % with the new GNS-532 card, it was still a concern that these strains were misreported as ESBL producers with subsequent expert rules for antibiotic susceptibility to β -lactam antibiotics applied. There appears to be no obvious reason for these results. They are unlikely to arise from a technical error, as the Vitek is a highly standardized system. These strains have been forwarded to bioMérieux for further studies.

The DDD test requires careful spacing of discs for accurate results and careful interpretation of zone sizes. It is therefore technically demanding. In previous studies, the DDD test was able to detect 82 and 88 % of ESBL-positive strains, respectively (Gibb & Crichton, 2000; Thomson & Sanders, 1992). The limitations of this test have been described elsewhere (Bush, 1996; Thomson & Sanders, 1992). A recent study has reported that cepodoxime achieves a 100 % sensitivity rate in detecting ESBLs in tested isolates, cefotaxime 92 %, and ceftazidime 82 % (Appleton & Hall, 2000). In contrast, the results of isolates from the UK used in this study showed that cefotaxime was the most efficient cephalosporin for the detection of ESBLs, with a sensitivity rate of 85.8 %; the values for cepodoxime and ceftazidime were 74.8 % and 44.8 %, respectively. The results from Kuwait hospital isolates showed that cepodoxime was the most efficient cephalosporin for the detection of ESBLs, with a sensitivity rate of 49 %; the values for cefotaxime and ceftazidime were 39 % and 31.8 %, respectively. Overall, the DDD method was able to detect ESBL activity in 75 % of the Kuwait isolates and 92.1 % of the UK isolates. This may reflect the type of ESBLs in our isolates, and emphasizes the importance of testing more than one cephalosporin.

The commercially available ESBL Etest strip is a quantitative technique, and is widely regarded as the 'gold standard' for detection in clinical laboratories of ESBL production (M'Zali *et al.*, 2000). In our hands, it detected 99.2 % of the UK test isolates and 98 % of the Kuwait isolates, but only if both cefotaxime and ceftazidime strips were used in conjunction. As noted above, the ceftazidime Etest strip was less sensitive;

the reason for this was not obvious, but it is possible that there were other ceftazidime-hydrolysing β -lactamases in some strains that were not sensitive to clavulanic acid, which could have reduced the sensitivity of the test. Thus, Etests with both cephalosporins are recommended; however, this makes the technique expensive, and most clinical laboratories would use it only for confirmation rather than as a routine test.

The majority of clinical laboratories do not have the resources to identify ESBL-producing strains by molecular methods; therefore, it is important to have an easy-to-perform methodology that can be used in the routine laboratory. This study shows the importance of identification of Enterobacteriaceae to species level and the usefulness of the Vitek system for routine detection of ESBLs if accurate and consistent results are to be reported to clinicians. This has been clearly demonstrated in a recent report that describes the retrospective detection of ESBLs in five of 20 strains of *K. pneumoniae* isolated from blood cultures. In some of these cases, patients were treated with an inappropriate third-generation cephalosporin, and treatment in these patients was changed after clinical failure (Crowley, 2001). If an ESBL detection test had been available at the time of the original susceptibility testing, appropriate results would have reported to the clinician.

It should be noted that in our hands, some *E. coli* were falsely reported as ESBL positive. For ESBL-positive *K. pneumoniae* in this study, the Vitek test was accurate, but it should be noted that this study only included Vitek ESBL-positive strains, and was therefore not an evaluation of the Vitek ESBL test itself.

In conclusion, the Vitek ESBL test was cost-effective as an ESBL screen in as much as the ESBL test is an integral part of the susceptibility card and is performed simultaneously with the susceptibility tests. In addition, the Vitek test is interpreted by the system itself, which removes any errors of subjectivity. No additional outlay of resources is required. We would recommend that strains of *E. coli* be confirmed as ESBL positive by an additional method, such as Etest.

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