Evaluation of Oxoid combination discs for detection of extended-spectrum β-lactamases

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Objectives: We evaluated the reliability of cefpirome/clavulanate (CD04) compared with ceftazidime/clavulanate (CD02) and cefotaxime/clavulanate (CD03) Oxoid combination discs for the detection of extended-spectrum β-lactamases (ESBL) in several Enterobacteriaceae isolates, including Enterobacter spp.

Methods: Overall, a total of 105 ESBL-positive [positive double-disc synergy test (DDST)] and 94 ESBL-negative (negative DDST) Gram-negative isolates were evaluated. Ninety-eight isolates were confirmed as ESBL-positive on the basis of the sequence alignments of the blaTEM and/or blaSHV gene amplification products, which matched with previously identified ESBLs. The phenotypic detection of ESBLs was performed by the three combination discs according to the NCCLS and BSAC methods. The CD02 disc was evaluated with the manufacturer’s recommended zone size difference breakpoint of ≥4 mm.

Results: In Escherichia coli and Klebsiella spp., the sensitivities (%)/specificities (%) of CD02, CD03 and CD04 discs, and the combination of CD02 or CD04 discs, were, respectively, 88/92, 90/92, 95/84 and 100/82, while the corresponding figures were 94/100, 4/100, 94/100 and 100/100 in Enterobacter aerogenes. NCCLS and BSAC methods yielded concordant results in 99% of the isolates.

Conclusions: CD04 and CD02 discs were the best combination for detection of ESBLs in our collection of Enterobacteriaceae isolates, including E. aerogenes.

Keywords: Enterobacter aerogenes, ESBL detection, PCR sequencing, TEM, SHV

Introduction

Extended-spectrum β-lactamases (ESBLs) are β-lactamases produced by a variety of Gram-negative bacilli. The distinguishing feature of these enzymes is that compared with the broad-spectrum β-lactamases, such as TEM-1, TEM-2, SHV-1 and others, ESBLs have extended substrate profiles that confer resistance to the expanded-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepime and others) and aztreonam. More than 150 different ESBLs have been characterized, and most of these are derived from point mutations affecting the TEM-1, TEM-2 and SHV-1 enzymes, though a few others have different ancestries. While ESBLs were initially reported mainly in Klebsiella pneumoniae and to a lesser extent in Escherichia coli, a wide range of organisms (Enterobacter, Serratia, Proteus, Morganella, Salmonella and Citrobacter spp.) have been reported worldwide to harbour such a resistance mechanism. In particular, studies from Belgium and France have revealed a sharp increase in the prevalence and proportion of ESBL producers among Enterobacter aerogenes isolates, and a marked propensity of this organism to cause local and country-wide outbreaks among hospitalized patients. Laboratory detection can be problematic, because some ESBLs do not confer obvious resistance to all their substrates in vitro and up to 30% of ESBL producers continue to be reported as susceptible to ceftriaxone and ceftaxime in Europe. Detection of ESBLs can also be difficult in organisms that display multiple resistance mechanisms, such as Enterobacter spp.

Accurate detection is nevertheless considered important, because patients infected with ESBL-producing organisms may have a higher overall fatality rate and a less favourable clinical outcome than patients infected with non-ESBL producing isolates. Moreover, clinical failures may arise even when the MICs of expanded-spectrum cephalosporins for ESBL producers fall in the susceptible range (as low as 1 mg/L). Two different detection strategies for ESBLs are now commonly used. The first uses aztreonam, ceftriaxone, ceftazidime, cefotaxime or cefpodoxime as an indicator drug, and considers Klebsiella and E. coli with reduced susceptibilities to these drugs to be resistant to all β-lactams.
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expanded-spectrum cephalosporins and aztreonam.24,28 The second approach screens for ESBL on the basis of the presence of a synergy between expanded-spectrum cephalosporins and clavulanic acid.29 Synergy can be detected by double-disc tests, but the optimum separation of the discs is strain variable. Alternatively, commercial systems such as Etest, Etest or Phoenix can be used.22,23,30–36

As an alternative to the double-disc synergy test (DDST), another detection method relies on the comparison of the inhibition zones of cephalosporin discs alone and with the addition of clavulanic acid. This method has been used successfully by several investigators, and comparison of the inhibition zone sizes achieved by cefotaxime (30 µg) and ceftazidime (30 µg) discs, with or without clavulanic acid (10 µg) added, is now advocated by the NCCLS and by the BSAC.24,28,32,33,37 The use of cefepime and cefpirome in DDSTs has also been recommended in order to facilitate the detection of ESBL in inducible bacterial species such as Enterobacter sp. that may simultaneously display drug resistance to a wide range of cephalosporins by virtue of the hyperproduction of AmpC β-lactamases.30

Current international (NCCLS) or national (BSAC) guidelines focus mainly on the detection of ESBLs among Klebsiella spp. and E. coli, while there are currently no definite recommendations concerning the methods to be used, nor the interpretative criteria to be applied, for other species. Because of the rising prevalence of ESBL-producing isolates among species other than E. coli and Klebsiella spp., we aimed to determine whether the commercial ceftazidime and cefotaxime-based combination discs method developed by Oxoid (Basingstoke, UK) would reliably detect ESBLs in a large collection of clinical Gram-negative isolates, including Enterobacteriaceae spp., we aimed to determine whether the commercial ceftazidime and cefotaxime-based combination discs method developed by Oxoid (Basingstoke, UK) would reliably detect ESBLs in a large collection of clinical Gram-negative isolates, including Enterobacteriaceae spp.11,14

We evaluated a set of 105 ESBL-positive and 94 ESBL-negative isolates. Bacterial strains

Materials and methods

Bacterial strains

We evaluated a set of 105 ESBL-positive and 94 ESBL-negative isolates. The ESBL-positive organisms included 84 clinical isolates that displayed a positive DDST performed at routine susceptibility testing using ceftazidime, cefotaxime and cefepime discs placed 30 mm apart (centre to centre) from a co-amoxiclav disc, as well as 21 characterized strains whose ESBLs had previously been identified genotypically.29,38 The DDST was considered positive when synergy was observed with one or more of the stated antimicrobials. The clinical isolates consisted of E. aerogenes (n = 49), Enterobacter cloacae (n = 6), E. coli (n = 12), K. pneumoniae (n = 113), Proteus vulgaris (n = 2), Proteus mirabilis (n = 1) and Providencia stuartii (n = 1) strains. The collection strains [E. coli (n = 12) and K. pneumoniae (n = 9)], kindly provided by H. Goossens (University Hospital Antwerp, Antwerp, Belgium), were chosen in order to provide organisms producing a variety of ESBLs. These included E. coli isolates harbouring TEM-3 (n = 1), TEM-4 (n = 1), TEM-5 (n = 1), TEM-6 (n = 2), TEM-7 (n = 2), TEM-9 (n = 1), SHV-2 (n = 1), SHV-3 (n = 1), SHV-4 (n = 1) and SHV-12 (n = 1) ESBLs. On the other hand, the K. pneumoniae strains analysed harboured TEM-3 (n = 1), TEM-7 (n = 1), SHV-2 (n = 1), SHV-3 (n = 2), SHV-12 (n = 3) and SHV-18 (n = 1) ESBLs.

The proportion of ESBL-positive strains selected among each species was defined according to published national data on the prevalence of ESBL-positive E. coli, K. pneumoniae and E. aerogenes strains and/or according to the availability of other bacterial species.11,14,38 Most clinical isolates were collected from specimens from patients hospitalized in our centre between December 1999 and June 2002 and were selected on the basis of a positive DDST. About one-third of the E. aerogenes isolates originated from a collection of strains gathered during a national epidemiological surveillance programme performed between 2000 and 2001.11,14

Ninety-four ESBL-negative clinical isolates (negative DDST), including challenging isolates with mechanisms of resistance known or anticipated to be different than ESBL production, based on antimicrobial susceptibility testing results [e.g. AmpC hyperproducers, K1-hyperproducing Klebsiella oxytoca, inhibitor resistant (IR) β-lactamases], were included as negative controls. These isolates consisted of E. aerogenes (n = 40), E. cloacae (n = 2), E. coli (n = 20), K. pneumoniae (n = 15), K. oxytoca (n = 8), Serratia marcescens (n = 6), Morganella morganii (n = 2) and Citrobacter freundii (n = 1).

Genotypic detection of ESBLs

PCR amplification of blaTEM genes and blaSHV genes. Genomic DNA was extracted by using the Chelex method (Bio-Rad, Nazareth, Belgium) as described previously.39 The blaTEM genes were detected by PCR using a forward (5′-TGGGGAATGTCGGC-3′) and a reverse primer (5′-TGTAACTATCGGAGCCACC-3′) (described previously), which amplified a 966 bp fragment corresponding to the entire coding region of the blaTEM gene.39 The blaSHV genes were detected by PCR using a forward (5′-CCCGGTATTCTCTATTGCG-3′) and a reverse (5′-ATGCGCGCGCCAGTGCA-3′) primer, designed to flank the start and stop codons of the blaSHV gene to amplify the entire coding region of 1007 bp. For the PCR amplification, ~500 µg of genomic DNA was added to a 50 µL mixture containing 200 µM of dNTPs, 0.4 µM of each primer and 2.5 U of Taq polymerase (Roche Diagnostics) in the appropriate buffer. The reactions were performed in a 2400 thermal cycler (PE-ABI) under the following conditions: 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 72°C and 1 min at 72°C, with a final extension at 72°C for 7 min. PCR amplification products were detected by ethidium bromide fluorescence after electrophoresis in 1.5% agarose gel and the amplicons were purified by the QIAquick purification kit (Qiagen) following the manufacturer’s instructions.

Direct DNA sequencing. The ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used for the sequencing of the PCR product. The sequencing reaction and template preparation were performed in accordance with the manufacturer’s instructions (Applied Biosystems; Warrington, Cheshire, UK). Sequencing reactions were performed with corresponding primers specific for the blaTEM and blaSHV genes used for the previous amplification. The sequencing product was purified by ethanol/sodium acetate precipitation (Applied Biosystems) and sequence analysis was realized with a 3100 Automatic Sequencer (Applied Biosystems). Each sequence of blaTEM and blaSHV genes was identified by comparison with known ESBL sequences available in the GenBank and EMBL databases by multiple sequence alignment using the BLAST program.

Phenotypic detection of ESBL production

All isolates were tested for the presence of ESBLs by disc diffusion method with ceftazidime (30 µg), cefotaxime (30 µg) or cefpirome (30 µg) discs alone, and with the same three compounds in combination with clavulanic acid (10 µg) (Oxoid). The disc tests were performed both (i) with confluent growth on Mueller–Hinton agar (Oxoid) in accordance with the NCCLS recommendations for non-fastidious bacteria, and (ii) with semi-confluent growth on IsoSensitest agar (Oxoid), according to BSAC recommendations.24,28 Zone diameters were measured manually with a calliper to the nearest millimetre. A difference of ≥5 mm between the inhibition zones of the CD02 (ceftazidime 30 µg plus 10 µg clavulanic acid) and ceftazidime 30 µg disc alone, or between zones of
Table 1. Sensitivities (%) of combination discs tests

<table>
<thead>
<tr>
<th>Organisms (no. of strains)</th>
<th>Genotypic detection of ESBL</th>
<th>Clinical detection of ESBL</th>
<th>NCCLS method</th>
<th>BSAC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (199)</td>
<td>-</td>
<td>593</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>E. coli</td>
<td>11 (85)</td>
<td>76 (4)</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>E. aerogenes (89)</td>
<td>38 (43)</td>
<td>76 (4)</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>16 (43)</td>
<td>76 (4)</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>Other (13)</td>
<td>49 (55)</td>
<td>76 (4)</td>
<td>76</td>
<td>84</td>
</tr>
</tbody>
</table>

CD02: ceftazidime 30 µg plus 10 µg clavulanic acid and cefotaxime 30 µg disc alone, was taken to indicate an ESBL, as recommended by NCCLS and BSAC criteria. For ceftipime, in the absence of formal recommendations, different cut-offs (≥24 and ≥5 mm) between zone sizes observed with CD04 (ceftipime 30 µg and clavulanic acid 10 µg) and cefpirome 30 µg disc alone were considered to indicate of the presence of ESBLs.

Gold standard definition of an ESBL-positive strain

Isolates were considered ESBL-positive when the sequence alignment of either the blaTEM and/or blaSHV gene amplification products matched previously identified ESBLs. Isolates with genotypic detection of blaTEM and/or blaSHV genes encoding β-lactamases enzymes other than ESBLs (i.e. broad-spectrum β-lactamases or IRTs) were disregarded.

Statistical methods

The sensitivity (%) was calculated as follows: number of isolates showing a combination discs positive result) × 100/number of isolates with a positive genotypic identification of ESBL production. The specificity (%) was estimated as follows: number of isolates showing a negative result using the combination discs) × 100/number of isolates that failed to produce any ESBLs according to the PCR-based technique.

Comparison of rates and proportions were performed using the χ² and Fisher’s exact tests using the Epi-Info (version 6) statistical software (CDC, Atlanta, GA, USA).

Results

Genotypic detection of ESBLs

ESBLs were detected in 96 (91%) of the 105 DDST-positive strains and absence of ESBL production was confirmed in 92 (98%) of the 94 DDST-negative strains. Among a total of 98 ESBL-producing strains (Tables 1 and 2), TEM-type ESBLs were recovered in 71 (72%) isolates, and SHV-type ESBLs in 31 (32%) isolates. In four (4%) isolates, both TEM- and SHV-type enzymes were present simultaneously.

Genotypic PCR sequence-based identification of ESBLs

All TEM- and SHV-type β-lactamases produced by the well-characterized E. coli and K. pneumoniae collection strains were correctly identified by the PCR sequencing assay.

Among the clinical isolates, TEM-24 appeared as the most frequent enzyme, present in 52 of 61 (85%) isolates producing TEM-type ESBLs (Table 3). In six isolates, the sequence alignments of the amplified blaTEM fragment showed three nucleic acid differences with the blaTEM.1 gene and did not match with previously described TEM-type ESBLs. The corresponding ESBLs from these isolates were consequently arbitrarily called TEM-A, TEM-B and TEM-C, and were present in K. pneumoniae and E. coli isolates. Further characterization of these enzymes would be necessary to establish whether these represent new enzymes. SHV-3, -4 and -12 enzymes were the most frequent SHV-type enzymes found in the clinical strains.

Overall, TEM-24 was found predominantly in E. aerogenes isolates (86% of ESBL-producing E. aerogenes strains), but it was also present in all the species that produced ESBLs. On the other hand, SHV-3 predominated in K. pneumoniae strains, and SHV-4 and SHV-12 were exclusively found in E. aerogenes and E. cloacae strains.

Two different IRT β-lactamases, TEM-35 (IRT-4) and TEM-79, were identified among E. aerogenes, E. coli, K. pneumoniae and K.
Table 2. Specificities of Oxoid combination discs to detect ESBL according to species

<table>
<thead>
<tr>
<th>Organisms (no. of strains)</th>
<th>Genotypic detection of ESBL</th>
<th>Specificities (%) of combination discs tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCCLS method</td>
<td>BSAC method</td>
</tr>
<tr>
<td></td>
<td>CD02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CD03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>≥5 mm</td>
<td>≥5 mm</td>
</tr>
<tr>
<td>All (199)</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>E. aerogenes (89)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter spp. (97)</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>E. coli (44)</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>K. pneumoniae (37)</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>E. coli and Klebsiella spp (89)</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Others (13)</td>
<td>100</td>
<td>91</td>
</tr>
</tbody>
</table>

<sup>a</sup>CD02: cefotaxime 30 µg plus 10 µg clavulanic acid and cefotaxime 30 µg disc alone.

<sup>b</sup>CD03: cefotaxime 30 µg plus 10 µg clavulanic acid and cefotaxime 30 µg disc alone.

<sup>c</sup>CD04: cefpimrome 30 µg and clavulanic acid 10 µg and cefpimrome 30 µg disc alone.

The PCR sequence-based technique and the DDST yielded concordant results in 197 of the 199 (99%) isolates tested (data not shown). In two isolates (K. oxytoca and one E. coli strain), the PCR failed to detect any ESBL while the DDST yielded an unequivocal positive result. In all instances, ESBLs were detected with one or more of the discs using either the NCCLS or BSAC methodology. Furthermore, the specificity decreased overall to 89%, except in the Enterobacter spp. group, for which it remained 100%. The combination of ESBLs and IRT-type enzymes was most frequently found mainly in E. coli, K. pneumoniae and P. vulgaris strains. The IRT-4 enzyme was the most frequent, being observed in two isolates, one K. pneumonia and one E. aerogenes strain. The combination of ESBLs and IRT-type enzymes was observed in two isolates, one K. pneumonia and one E. coli strain.
### Laboratory detection of extended-spectrum β-lactamases

**Table 3. TEM- and SHV-type β-lactamases produced by clinical strains (n = 178) and proportions according to the species**

<table>
<thead>
<tr>
<th>TEM- and SHV-type β-lactamases</th>
<th>ESBL</th>
<th>E. aerogenes</th>
<th>E. cloacae</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>K. oxytoca</th>
<th>P. stuartii</th>
<th>P. mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM-1</td>
<td>No</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TEM-3</td>
<td>Yes</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>TEM-24</td>
<td>Yes</td>
<td>44</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TEM-29</td>
<td>Yes</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>TEM-35 (IRT-4)</td>
<td>No</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>TEM-79 (IRT)</td>
<td>No</td>
<td>1</td>
<td></td>
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<tr>
<td>TEM-A</td>
<td>Yes</td>
<td>4</td>
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<tr>
<td>TEM-B</td>
<td>Yes</td>
<td>1</td>
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<tr>
<td>TEM-C</td>
<td>Yes</td>
<td>1</td>
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<tr>
<td>LEN-1 (SHV-1 ancestor)</td>
<td>No</td>
<td>1</td>
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<tr>
<td>SHV-1</td>
<td>No</td>
<td>8</td>
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<tr>
<td>SHV-3</td>
<td>Yes</td>
<td>1</td>
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<tr>
<td>SHV-4</td>
<td>Yes</td>
<td>7</td>
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<tr>
<td>SHV-5</td>
<td>Yes</td>
<td>4</td>
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<tr>
<td>SHV-12</td>
<td>Yes</td>
<td>4</td>
<td></td>
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</table>

*K. pneumoniae*, CD03 showed the highest zone size differences compared with the two other combination discs, which possibly suggests the occurrence of non TEM- or non SHV-type ESBLs, e.g. cefotaxime-hydrolysing enzymes such as the CTX-M ESBLs.

The sensitivities of detecting TEM-type ESBLs by CD02, CD04 and CD03 discs were 94, 94 and 32%, respectively. The corresponding figures specifically for TEM-24 producers were 96, 92 and 13%, respectively. Regarding the detection of SHV-type ESBLs, CD02 discs had a sensitivity of 84%, CD03 discs 77% and CD04 discs 90%.

All the combination discs accurately detected ESBL production among the two isolates concomitantly producing both an ESBL and an IRT enzyme.

### Discussion

In line with previous reports, our study confirmed the higher sensitivity of ceftazidime/clavulanate discs (CD02) compared with cefotaxime/clavulanate discs (CD03) for the detection of TEM- and SHV-type ESBLs among *E. coli* and *Klebsiella* spp. isolates. Moreover, it was also found to yield higher sensitivity for the detection of TEM-related enzymes compared with SHV-related ones.32,33,37 There is only scarce information concerning the detection of ESBLs among Enterobacteriaceae species other than *E. coli* and *Klebsiella*.32,33,37,42 This lack of data can probably be partly explained by the absence of methodological and interpretive guidelines. Moreover, *E. coli* and *Klebsiella* spp. were until recently the most frequently recovered species among ESBL-positive Enterobacteriaceae.

Since *E. aerogenes* has emerged as a predominant ESBL-positive pathogen in different countries in Europe, especially in Belgium and France, we aimed to evaluate the recommended combinations discs within the *Enterobacter* spp. group.2,11-20 We could indeed show that the CD02 discs performed adequately for the detection of ESBLs in this group while the CD03 discs, on the other hand, had a poor performance. In contrast, in a recent study performed in Israel, CD03 showed the highest performance for the detection of ESBL-producing *E. coli*, *Proteus* spp., *Klebsiella* spp. and *Enterobacter* spp.41

Unfortunately, no characterization of the ESBLs was performed in that study. Owing to the high frequency of AmpC hyperproducers among *E. aerogenes* and *E. cloacae* isolates, we also decided to evaluate the newly described CD04 discs. Our data illustrate that the use of this combination disc with a zone size diameter cut-off value of at least 4 mm indeed may represent an excellent alternative to CD02 discs within this group of organisms, both in terms of sensitivity and specificity. Among the other Gram-negative bacteria, comparable sensitivities were observed between the two combination discs, but CD04 discs appeared clearly inferior to CD02 discs in terms of specificity. Since plasmid-mediated AmpC enzymes have also been shown to disseminate among Enterobacteriaceae, sometimes in combination with ESBLs, it might be highly desirable to develop an ESBL detection test that includes a substrate displaying a higher degree of resistance to such AmpC enzymes.43 Nevertheless, in our experience, cefpirome/clavulanic acid discs (CD04) did not prove clearly superior to ceftazidime/clavulanic acid discs (CD02), despite its theoretical higher degree of resistance to AmpC enzymes. Further evaluation should be performed on a larger range of isolates from different origins to confirm such observations. Indeed, according to Bolmström et al.38 the detection of ESBLs using double Etest of cefepime with and without clavulanic acid was shown to be more sensitive than ceftazidime and cefotaxime for investigating non-determinable ESBL results by NCCLS criteria in species other than *E. coli*, such as *E. aerogenes*, *E. cloacae*, *P. mirabilis* and *S. marcescens*. However, the techniques used, the compounds tested (cefpitome instead of cefpirome) and the antimicrobials concentrations were different in the two studies.

Based on our results, the use of both CD02 and CD04 discs appears to be the preferred combination for the detection of ESBL, whatever the bacterial species involved. However, the specificity slightly decreased in comparison to each compound considered individually.

Cefpodoxime/clavulanate discs (CD01) are also available and this combination has been found to be particularly sensitive and specific (100% each) for detecting ESBL-producing *Klebsiella*.44 However,
in another recent study, which aimed to compare the performance of CD01, CD02 and CD03 for ESBL detection in isolates of Enterobacteriaceae resistant to cefuroxime, CD01 did not prove superior to CD02 and CD03. 42 Furthermore, in Enterobacter spp. isolates, CD01 had a sensitivity of only 34%, compared with 83 and 62% for CD03 and CD02, respectively. Another study also illustrated the inferior sensitivity of CD01 compared with CD02 in E. coli and Klebsiella spp. 32,42 Moreover, in a previous preliminary study, we found that cefpodoxime-based combination discs were not suited for detecting ESBLs in E. aerogenes isolates (sensitivity of 2.5% versus 78% for CD02). 45 Altogether, these data are in line with those of Oliver et al., 46 who showed that cefpodoxime MICs are more heavily influenced by enhanced TEM and AmpC β-lactamase production than are cefotaxime and cefazidime.

Among the discordant results between both methodologies, most false-positive and false-negative results were seen with the NCCLS method. This can mainly be explained by the higher inoculum of bacteria used to perform the tests, resulting in smaller zone sizes around the combinations discs. Consequently, an identical small variation in the zone size will more often lead to an erroneous result in the NCCLS method.

The false-positive results obtained with the combination discs were predominantly obtained among K. oxytoca strains hyperproducing K1 chromosomal enzymes, mainly with the CD04. Detection of ESBL production among such strains is known to be challenging and false-positive results have also been described in other studies. 24,36 In E. cloacaee and P. vulgaris isolates, false-positive results could possibly be explained by the presence of non-SHV- or non-TEM-related ESBLs such as CTX-M β-lactamases, which have been well-documented among epidemic E. cloaece strains in other European countries such as France and Spain. 3,15

Overall, these observations underline the diversity of ESBL types and their variable species distributions, and emphasize the need for regular epidemiological surveillance of the prevalence of these enzymes. Likewise, it is deemed very important to develop and evaluate routinely applicable laboratory techniques that are the most suited for the detection of ESBLs.

In conclusion, our results, based on the analysis of a large number of species and isolates whose ESBLs were characterized by PCR-based techniques, suggest that CD04 discs at a 24 mm threshold value may constitute an accurate alternative to CD02 discs for the detection of ESBL enzymes among Enterobacter spp. We also found that the selection of both CD02 and CD04 discs appears to be the optimal combination for ESBL detection among various Enterobacteriaceae isolates, since it significantly increased the sensitivity of the tests at the expense of only a slight decrease of specificity.

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References

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