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Multi-centre evaluation of a phenotypic extended spectrum β-lactamase detection guideline in the routine setting

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Abstract

This study aimed to evaluate the routine setting performance of a guideline for phenotypic detection of extended spectrum β-lactamases (ESBLs) in Enterobacteriaceae, recommending ESBL confirmation with Etest or combination disc for isolates with a positive ESBL screen test (i.e. cefotaxime and/or ceftazidime MIC >1 mg/L or an automated system ESBL warning). Twenty laboratories submitted 443 Enterobacteriaceae with a positive ESBL screen test and their confirmation test result (74% *Escherichia coli*, 12% *Enterobacter cloacae*, 8% *Klebsiella pneumoniae*, 3% *Proteus mirabilis*, 2% *Klebsiella oxytoca*). Presence of ESBL genes was used as reference test. Accuracy of local phenotypic ESBL detection was 88%. The positive predictive value (PPV) of local screen tests was 70%, and differed per method (Vitek-2: 69%, Phoenix: 68%, disc diffusion: 92%), and species (95% *K. pneumoniae*-27% *K. oxytoca*). A low PPV (3%) was observed for isolates with automated system alarm but third-generation cephalosporin MICs <2 mg/L. Local ESBL confirmation had a PPV and negative predictive value (NPV) of 93% and 90%, respectively. Compared with centrally performed confirmation tests, 7% of local tests were misinterpreted. Combination disc was more specific than Etest (91% versus 61%). Confirmation tests were not reliable for *P. mirabilis* and *K. oxytoca* (PPV 33% and 38%, respectively, although NPVs were 100%). In conclusion, performance of Etests could be enhanced by education of technicians to improve their interpretation, by genotypic ESBL confirmation of *P. mirabilis* and *K. oxytoca* isolates with positive phenotypic ESBL confirmation, and by interpreting isolates with a positive ESBL alarm but an MIC <2 mg/L for cefotaxime and ceftazidime as ESBL-negative.

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Introduction

The prevalence of extended spectrum β-lactamases (ESBLs) in Enterobacteriaceae is increasing worldwide [1]. Accurate detection of ESBLs is necessary for adequate antibiotic therapy, infection control precautions and surveillance purposes. In 2008, the Dutch Society for Medical Microbiology issued a guideline for phenotypic screening and confirmation of ESBLs in Enterobacteriaceae to standardize the method and to improve the accuracy. In contrast to the CLSI guideline for ESBL detection, the Dutch guideline also provides methods for phenotypic ESBL confirmation in Enterobacteriaceae with inducible chromosomal AmpC β-lactamases, using an Etest or combination disc with cefepime and cefepime plus clavulanic acid.

The objectives of this study were to determine the accuracy of phenotypic ESBL detection in Dutch clinical laboratories using this guideline and to compare the performances of Etest and combination discs as ESBL confirmation tests in the clinical setting.

Materials and Methods

Guideline

The ESBL detection strategy in the Dutch guideline recommends a screening step and a confirmation step (Fig. 1). Iso-

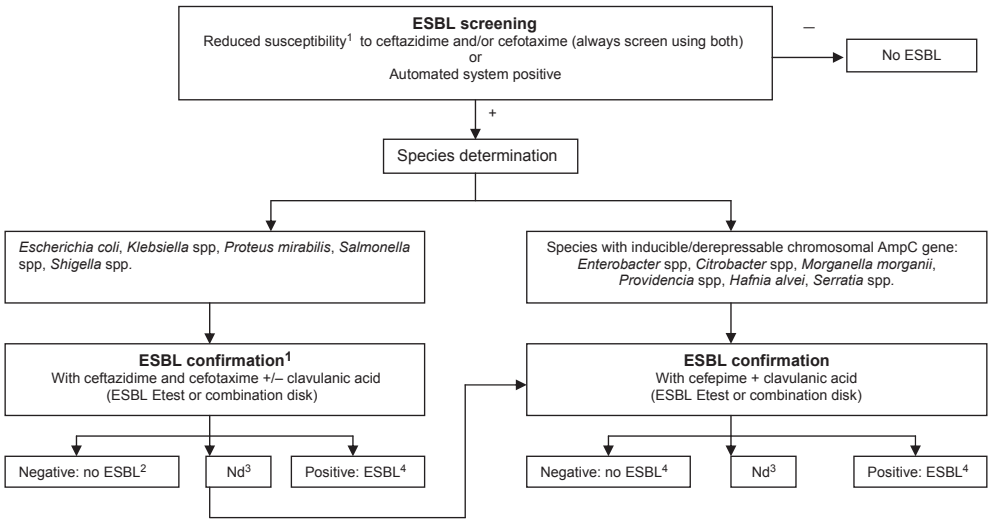

FIG. 1. Extended spectrum β-lactamase (ESBL) detection algorithm for Enterobacteriaceae according to the Dutch guideline for phenotypic ESBL detection. (1) If cefoxitin resistant, perform cefepime confirmation test. (2) Inhibitor resistant ESBL not excluded. (3) Nd = non-determinable = out of range (MIC >Etest strip or no inhibition zone). (4)The ESBL confirmation test may generate false-positive results in K1 β-lactamase hyperproducing *Klebsiella oxytoca*. Exceptions are *K. oxytoca* isolates with high-grade resistance to ceftazidime and synergy between ceftazidime and clavulanic acid, which is indicative of ESBL production.

lates with a ceftazidime MIC >1 mg/L and/or a cefotaxime MIC >1 mg/L determined by any MIC method fulfilling quality control criteria, or an ESBL alert in Phoenix (BD Diagnostics, Sparks, MD, USA) or Vitek-2 (BioMérieux Marcy l'Etoile, France) are considered screen positive. In centres using the disc diffusion method isolates were selected for ESBL confirmation in case of zones $\leq$ 20 mm with the Oxoid discs for ceftazidime or cefotaxime.

Confirmation of ESBL production is based on the detection of synergy between clavulanic acid and third-generation cephalosporins. The recommended methods are the ESBL Etest (BioMérieux) or combination discs. The synergy between cephalosporins and clavulanic acid may be masked in isolates co-expressing an ESBL and AmpC β-lactamase; therefore, the Enterobacteriaceae are divided into two groups, with a specific strategy for ESBL confirmation for each. Group I comprises species without inducible chromosomal AmpC β-lactamases (*Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella* spp. and *Shigella* spp.). ESBL production in these species is confirmed by demonstrating synergy between ceftazidime and/or cefotaxime and clavulanic acid. Group II comprises Enterobacteriaceae with inducible chromosomal AmpC β-lactamases (*Enterobacter* spp., *Serratia* spp., *Providencia* spp., *Citrobacter freundii*, *Morganella morganii* and *Hafnia alvei*). ESBL confirmation in this group is based on synergy between clavulanic acid and cefepime, a fourth-generation cephalosporin that is hydrolysed by ESBLs, but generally not by AmpC β-lactamases [2–4]. The results of the confirmation test are classified as positive, negative or out-of-range.

Isolates

From 1 February 2009 until 1 May 2009, 20 Dutch laboratories submitted all *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *P. mirabilis* and *Enterobacter* spp. with a positive ESBL screen test. For each isolate, participating laboratories provided information on the method and results of screening (Vitek, Phoenix, disc diffusion), ESBL confirmation results (combination disc or Etest), and the MICs of third-generation cephalosporins from automated systems for isolates with an ESBL alarm but an MIC of ceftazidime and cefotaxime <2 mg/L as determined in the reference laboratory. The first 25 non-repeat isolates of each laboratory (if available) were selected for further analysis. Isolates were excluded when: (i) there was evidence that another isolate was submitted than originally tested by the participating laboratory (defined as an eight-fold or higher difference in MICs of the indicator-cephalosporins reported by the participating and tested by the central laboratory), or (ii) the phenotypic test results of the participating laboratory were lacking.

ESBL detection

Phenotypic detection in the reference laboratory was performed by ESBL Etest (BioMérieux) or combination disc

(ROSCO, Taastrup, Denmark). The MICs for ceftazidime and cefotaxime were determined using micro-broth dilution (Sensititre, TREK Diagnostic Systems, East Grinstead, UK).

The presence of an ESBL gene was determined using microarray analysis (Check-KPC ESBL, Check-Points B.V., Wageningen, the Netherlands), which detects the most prevalent CTX-M, TEM and SHV ESBL gene groups [5]. PCR and sequencing, using the same DNA batch as used for the microarray, was performed to determine the exact CTX-M, TEM and SHV genes. Additional PCRs were performed to detect the presence of rare ESBL families such as PER, GES and VEB β-lactamase genes, as well as plasmid-borne AmpCs [6].

ESBL confirmation

The reference method for determining the presence of ESBL genes consisted of a step-wise procedure.

Step 1. If the results of the phenotypic confirmation tests reported by the participating laboratory, and the ESBL microarray results were both positive, the isolate was defined as ESBL-positive. If both were negative the isolate was defined as ESBL-negative.

Step 2. If the phenotypic result of the participating laboratory and the result of the microarray were discrepant, the phenotypic confirmation tests (Etest or combination disc, identical to the test performed in the participating laboratory) were repeated in the reference laboratory. If the result of the repeated phenotypic test confirmed the microarray result, the isolate was considered accordingly: either ESBL-positive or ESBL-negative.

Step 3. If there was a discrepancy between the array results and the repeated phenotypic confirmation test the isolates were tested with PCR and, if indicated, DNA sequencing. The results of the PCR and sequencing were considered the gold standard for the presence of an ESBL gene.

Statistics

Frequency data were analysed with the chi-squared test or Fisher's exact if appropriate, using SPSS 15.0

Results

Among the 443 isolates included, *E. coli* were the most prevalent (n = 326; 74%), followed by *Enterobacter cloacae* (n = 54; 12%), *K. pneumoniae* (n = 37; 8%), *P. mirabilis* (n = 15; 3%) and *K. oxytoca* (n = 11; 2%). Based on microarray and/or sequencing 312 (70%) isolates contained ESBL genes: 79% CTX-M (66% CTXM-1 group and 12% CTX-M-9 group, <1% other CTX-M groups), 6% TEM (5% TEM-3 group, 1% other TEM groups), 7% SHV (6% SHV-4 group, 1% other SHV groups), 6% a combination of these and 1% PER or GES.

ESBL screening

Vitek-2 was used for ESBL screening for 350 isolates (79%) in 16 laboratories, Phoenix was used for 68 isolates (15%) in three laboratories and the disc diffusion method (Oxoid, Basingstoke, UK) for 25 isolates (6%) in one laboratory. The overall positive predictive value (PPV) of local ESBL screening methods was 70% (312/443). The PPV of the Vitek-2 was 69% (243/350; 95% CI 64–74%), of the Phoenix 68% (46/68; 95% CI 56–78%) and of the disc diffusion method 92% (23/25; 95% CI 74–99%).

The PPV of ESBL screening—as locally performed—varied per species: 95% (35/37; 95% CI 81–99%) for *K. pneumoniae*, 76% (248/326; 95% CI 71–80%) for *E. coli*, 44% (24/54; 95% CI 32–58%) for *E. cloacae*, 13% (2/15; 95% CI 2–39%) for *P. mirabilis*, and 27% (3/11; 95% CI 9–57%) for *K. oxytoca* (p <0.01 for each species compared with *K. pneumoniae*). Per species, the false-positive rate did not depend on the screening method (data not shown).

The PPV of the ESBL screen tests was influenced by the MIC of isolates to ceftazidime and cefotaxime. For the 40 isolates with cefotaxime and ceftazidime MIC <2mg/L, as determined by broth microdilution in the reference laboratory, but an ESBL alarm of the automated system (*K. pneumoniae* 3%, *E. coli* 10%, *E. cloacae* 2%, *P. mirabilis* 33%, *K. oxytoca* 36%), the PPV was 3% (1/40), and the rate of false-positive confirmation tests in those isolates was 30% (12/40). If isolates had an MIC <2 mg/L for ceftazidime and cefotaxime in the reference laboratory, this was also the case in the automated system of the participating laboratory, except for two of the 40 cases. Only one isolate (an *E. coli* with a cefotaxime MIC of 0.25 and ceftazidime MIC of 1 mg/L) contained an ESBL gene (CTX-M-1 group). For 403 isolates with a cefotaxime and/or ceftazidime MIC ≥2 mg/L, the PPV of ESBL screening was 77% (311/403) and the rate of false-positive confirmation tests was only 3% (12/403). The odds ratio was 131.8 (95% CI 17.9–972.7) for comparison of PPVs and 0.07 (95% CI 0.03–0.17%) for false-positive rate.

ESBL confirmation

ESBL confirmation was performed with Etest in 282 isolates (64%; 13 laboratories), with combination disc in 135 isolates (30%; six laboratories) and with both Etest and combination disc in 26 isolates (6%; one laboratory). Overall sensitivity and specificity of phenotypic ESBL confirmation tests as

performed by the local laboratories was 95% and 70%, respectively (PPV 93% and negative predictive value (NPV) 90%) (Table 1, Fig. 2). The PPV of confirmation tests varied per species and was ≥95% for *E. coli*, *K. pneumoniae* and *E. cloacae*, but <40% for *K. oxytoca* and *P. mirabilis* (Table 2).

Although sensitivity, PPV and NPV of ESBL confirmation with Etest and combination discs, as performed in local participating laboratories, were comparable, specificity of the Etest was 59% (95% CI 48–68%) compared with 92% (95% CI 80–97%) for the combination disc (Table 1). In the one laboratory that confirmed 26 isolates with both confirmation tests, test characteristics were equal (sensitivity 100% (21/21), specificity 80% (4/5), NPV 100% (4/4) and PPV 95% (21/22)).

Discrepancy analysis

For 388 (88%) of 443 isolates the results of phenotypic confirmation tests, as performed by local laboratories, and genotypic confirmation of presence or absence of ESBL genes were concordant. For 32 (7%) of the isolates, phenotypic results of the local laboratory were discrepant from phenotypic confirmation tests repeated in the reference laboratory as well as genotype confirmation (Fig. 2, step 1). In six iso-

TABLE 1. Comparison of extended spectrum β-lactamase (ESBL) confirmation with Etest versus combination disc in the clinical setting

	All isolates *n* = 443[a] (ESBL-positive *n* = 312 ESBL-negative *n* = 131)	Etest *n* = 308 (ESBL-positive *n* = 221 ESBL-negative *n* = 87)	Combination disc *n* = 161 (ESBL-positive *n* = 113 ESBL-negative *n* = 48)	p-value (Etest vs combination disc)
Sensitivity	95% (296/312)	96% (212/221)	93% (105/113)	NS
Specificity	70% (92/131)	59% (51/87)[b]	92% (44/48)	p <0.001
PPV[c]	93% (296/320)	91% (212/233)	96% (105/109)	NS
NPV[c]	90% (92/102)	94% (51/54)	85% (44/52)	NS

[a]*n* = 443 (Etest *n* = 282, combination disc *n* = 135, both Etest and combination disc *n* = 26 isolates).
[b]The participating laboratories reported the ESBL Etest as off-range in 15 of 87 (17%) of the ESBL-negative isolates and as false-positive in 21 of 87 (24%) of the ESBL-negative isolates.
[c]For calculation of the PPV and NPV the off-range Etests were not taken into account.
NPV, negative predictive value; NS, not significant; PPV, positive predictive value.

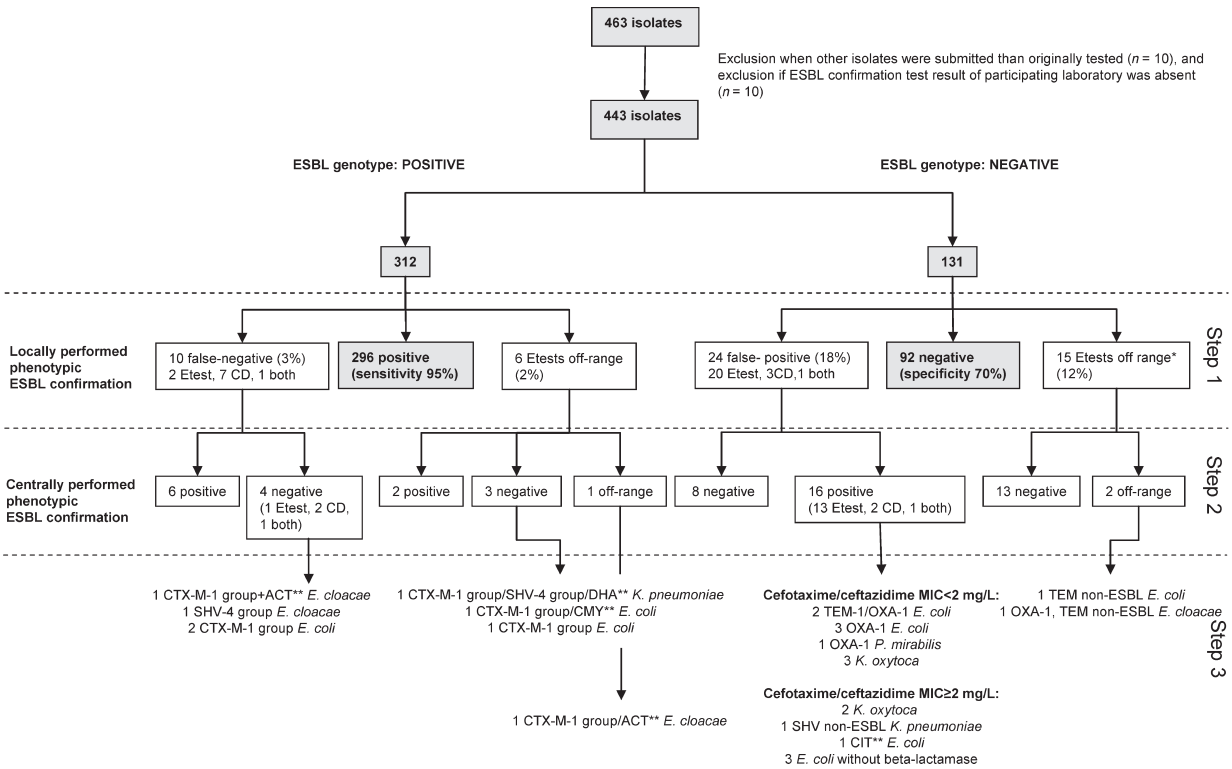

FIG. 2. Diagram of inclusion and test results of isolates. *In five isolates no cefepime/cefepime plus clavulanic acid Etest was performed. **Plasmid-borne AmpC.

TABLE 2. Performance of extended spectrum β-lactamase (ESBL) confirmation in Enterobacteriaceae without (group I) and with inducible chromosomal AmpC β-lactamase (group II) in the clinical setting

Group I (*n* = 389)	*Escherichia coli* / *Klebsiella pneumoniae* / *Proteus mirabilis* / *Klebsiella oxytoca*	*E. coli* *n* = 326 ESBL + *n* = 248 (76%)	*K. pneumoniae* *n* = 37 ESBL + *n* = 35 (95%)	*P. mirabilis* *n* = 15 ESBL + *n* = 2 (13%)	*K. oxytoca* *n* = 11 ESBL + *n* = 3 (27%)	Group II (*n* = 54) *Enterobacter cloacae* ESBL + *n* = 24 (44%)
Sensitivity	96% (276/288)	96% (238/248)	94% (33/35)	100% (2/2)	100% (3/3)	83% (20/24)
Specificity	65% (66/101)	69% (54/78)	1/2[a]	62% (8/13)	38% (3/8)	87% (26/30)
PPV	92% (276/299)	95% (238/251)	97% (33/34)	33% (2/6)	38% (3/8)	95% (20/21)
NPV	90% (66/73)	90% (54/60)	1/2[a]	100% (8/8)	100% (3/3)	90% (26/29)

[a]Only two ESBL-negative *K. pneumoniae* isolates were included, one of which was incorrectly reported as false-positive in the ESBL confirmation test.
NPV, negative predictive value; NS, not significant; PPV, positive predictive value.

lates (in four laboratories) presence of ESBL was missed because of misinterpretations of Etest (n = 1) and combination discs (n = 5). Eight isolates (in eight laboratories) were misclassified as ESBL-positive because of misinterpretations of seven Etests and one combination disc. Three of these eight isolates (38%) had MICs for cefotaxime and/or ceftazidime <2 mg/L. Furthermore, there were 21 isolates (from nine laboratories) with non-determinable Etest results, of which 16 (76%) were ESBL-negative when the Etest was repeated in the central laboratory (Fig. 2). Of those 16 isolates, two isolates harboured a plasmid AmpC, and 12 isolates were probably chromosomal AmpC producers or had decreased permeability, as the cefoxitin MICs were ≥16 mg/L (nine *E. coli*, two *E. cloacae* and one *P. mirabilis*). For the last isolates, the guideline recommends ESBL confirmation with cefepime/cefepime plus clavulanic acid (Fig. 1), but this was not performed in eight isolates (deviation from protocol).

For 23 (5%) of the 443 isolates, the discordance between ESBL phenotype and genotype was not the result of inappropriate protocol execution. Repeated testing according to protocol by the reference laboratory failed to confirm phenotypic ESBL-positivity in seven isolates. In six isolates clavulanic acid synergy was not observed with cefotaxime, ceftazidime or cefepime: in all six strains because of interference of AmpC with the synergy tests (three isolates plasmid AmpC, three chromosomal AmpC). Besides, in one isolate presence of ESBL genes was associated with MICs for cephalosporins <2 mg/L.

Sixteen isolates were phenotypically ESBL-positive according to the protocol, but ESBL genes were not detected in the array. Nine of those isolates had an MIC <2 mg/L for cefotaxime and ceftazidime, of which three *K. oxytoca* isolates, which may have been false-positive because of production of the chromosomal OXY (or K1) class A β-lactamase, and six isolates contained an OXA-1 β-lactamase gene. Of the seven remaining isolates, two were *K. oxytoca*, four *E. coli* isolates had no genetic substrate for the false positivity (one CIT positive, the other three contained no β-lactamases), and one *E. coli* isolate was SHV-1 positive, with a susceptibility pattern compatible with SHV-1 hyperproduction (reduced susceptibility to ceftazidime and amoxicillin-clavulanic acid, but susceptible to cefotaxime and cefuroxime) [7].

Therefore, we conclude that the guideline will have a maximum accuracy compared with the used genotyping methods of 94% (95% CI 92–96%) (Fig. 2, step 2). Substitution of the misinterpreted local confirmation results with centrally performed test results yielded sensitivities of the Etest and combination discs of 97% (95% CI 94–99%) and 97% (95% CI 92–99%), respectively, and specificities of 82% (95% CI 72–88%) and 94% (95% CI 82–98%), respectively.

Discussion

Based on the results from 20 clinical microbiology laboratories in the Netherlands we conclude that application of the Dutch national guideline for phenotypic ESBL detection resulted in correct interpretation of the ESBL status in 388 (88%) of 443 isolates with a positive ESBL screen test. Of note, discordance between phenotypic testing and genotypic confirmation remained even after extensive retesting in the reference laboratory for 23 of the incorrect interpretations (5% of all isolates). Based on our findings we provide specific recommendations to further optimize phenotypic ESBL detection in routine microbiology diagnostics. These include genotypic ESBL testing for *P. mirabilis* and *K. oxytoca* isolates with a positive ESBL confirmation test, and to interpret isolates with MIC <2 mg/L for cefotaxime or ceftazidime (but considered as ESBL-positive in automated testing) as ESBL-negative.

The PPV of ESBL screening was 70% and depended on the method, the species, and the third-generation cephalosporin MICs. ESBL screening accuracy was comparable for the Vitek-2 and Phoenix methods. Disc diffusion appeared more specific, but results were based on one laboratory and 6% of all isolates only. Per species, PPV ranged from 95% for

K. pneumoniae to 13% for *P. mirabilis*. As a consequence, the diagnostic yield of ESBL confirmation tests is rather limited for *K. pneumoniae*, but definitely indicated for all other species. The accuracy of ESBL screening with automated systems correlated with the MIC for third-generation cephalosporins, as measured by broth microdilution. An extremely low PPV of screening (3%) was observed in isolates with an ESBL alarm of the automated system but third-generation cephalosporin MICs <2 mg/L.

The accuracy of ESBL confirmation tests also depended on the confirmation method, species, and the third-generation cephalosporin MICs. The test characteristics of combination disc and Etest for ESBL confirmation, as performed by the participating laboratories, were generally comparable, but ESBL Etests were less specific than combination discs (59% vs. 92%). This was because of non-determinable and false-positive Etest results. Most Etest results (86%) reported as off-range could not be confirmed in the reference laboratory, indicating problems with the interpretation of the ESBL Etests and/or lack of expertise in the clinical setting. This problem was not observed in previous experimental studies using the Etests as ESBL confirmation test [8,9]. These data indicate that education of the technicians may improve the accuracy of ESBL confirmation tests with 8% for Etest and 4% for combination disc results. It should be noted that the combination discs are cheaper than the Etest.

The PPV of the ESBL confirmation test in *E. coli*, *K. pneumoniae* and *Enterobacter* spp. was 95–97% versus 33–38% in *P. mirabilis* and *K. oxytoca*. However, a negative confirmation test result excluded ESBL production (NPV 100%, Table 2). Especially the low PPV for *P. mirabilis* is noticeable and, to our knowledge, not reported before. As four out of five false-positive phenotypic test results could not be reproduced in the reference laboratory these false-positive results suggest difficulties with the ESBL confirmation test interpretation, possibly because of swarming of the isolates.

For *P. mirabilis* and *K. oxytoca* we recommend a genotypic ESBL detection method if the phenotypic ESBL confirmation test is positive.

The accuracy of the confirmation test was low in isolates with an ESBL alarm from the automated system and a third-generation cephalosporin MIC <2 mg/L. False-positive confirmation results were frequently obtained in such isolates. We, therefore, recommend that such isolates be interpreted as ESBL-negative and ESBL confirmation tests should not be performed. According to our findings, this strategy would not significantly reduce the sensitivity, but decrease the rate of false-positive test results with 50%.

Genetic analysis showed that six isolates without an ESBL genotype, but an ESBL-positive confirmation test in the reference laboratory and an MIC <2 mg/L for cefotaxime and ceftazidime, contained an OXA-1 gene (combined with a TEM-1 in two isolates). These six isolates showed decreased susceptibility to cefepime (median MIC 6 mg/L, range 1–8 mg/L) and a positive PM/PML confirmation Etest result, in line with a recent report that expression of OXA-1 may lead to false-positive ESBL test results because of fourth-generation cephalosporin resistance without resistance to cefotaxime and ceftazidime [10].

In contrast to the CLSI guideline for ESBL detection, the Dutch guideline also provides an adequate method for phenotypic ESBL confirmation in Enterobacteriaceae with inducible chromosomal AmpC β-lactamases, using an Etest or combination disc with cefepime and cefepime plus clavulanic acid. Previous studies using cefepime-clavulanate disc combinations for confirmation reported a sensitivity of 88% and a specificity of 91% in a research setting [2,11]. Our findings demonstrate that application of the Dutch guideline is associated with equally high accuracy in daily clinical practice.

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Transparency Declaration

Nothing to declare.

Appendix: Members of the national ESBL surveillance group:

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References

1. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18: 657–686.
2. Cohen Stuart J, Diederen B, Al Naiemi N *et al.* A method for phenotypic detection of extended spectrum β-lactamases in *Enterobacter* species in the routine clinical setting. *J Clin Microbiol* 2011; 7: 2711–2713.
3. Mohanty S, Gaind R, Ranjan R, Deb M. Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum β-lactamases in AmpC co-producing bacteria. *J Infect Dev Ctries* 2009; 4: 24–29.
4. Sturenburg E, Sobottka I, Noor D, Laufs R, Mack D. Evaluation of a new cefepime-clavulanate ESBL Etest to detect extended-spectrum β-lactamases in an Enterobacteriaceae strain collection. *J Antimicrob Chemother* 2004; 54: 134–138.
5. Cohen Stuart J, Dierikx C, Al Naiemi N *et al.* Rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. *J Antimicrob Chemother* 2010; 65: 1377–1381.
6. Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. *Int J Antimicrob Agents* 2011; 4: 134–138.
7. Miro E, del Cuerpo M, Navarro F, Sabate M, Mirelis B, Prats G. Emergence of clinical *Escherichia coli* isolates with decreased susceptibility to ceftazidime and synergic effect with co-amoxiclav due to SHV-1 hyperproduction. *J Antimicrob Chemother* 1998; 42: 535–538.
8. Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of extended-spectrum β-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. *J Clin Microbiol* 2007; 45: 1167–1174.
9. Sturm PD, Bochum ET, van Mook-Vermulst SV, Handgraaf C, Klaassen T, Melchers WJ. Prevalence, molecular characterization, and phenotypic confirmation of extended-spectrum β-lactamases in *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* at the Radboud University Nijmegen Medical Centre in The Netherlands. *Microb Drug Resist* 2010; 16: 55–60.
10. Beceiro A, Maharjan S, Gaulton T *et al.* False extended-spectrum β-lactamase phenotype in clinical isolates of *Escherichia coli* associated with increased expression of OXA-1 or TEM-1 penicillinases and loss of porins. *J Antimicrob Chemother* 2011; 9: 2006–2010.
11. Towne TG, Lewis JS 2nd, Herrera M, Wickes B, Jorgensen JH. Detection of SHV-type extended-spectrum β-lactamase in *Enterobacter* isolates. *J Clin Microbiol* 2010; 48: 298–299.