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Practical Approach for Reliable Detection of AmpC Beta-Lactamase-Producing *Enterobacteriaceae*[∇]

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In this prospective study all *Enterobacteriaceae* isolates ($n = 2,129$) recovered in the clinical microbiology laboratory during October 2009 to April 2010 were analyzed for AmpC production. Clinical and Laboratory Standards Institute (CLSI) cefoxitin and cefotetan susceptibility breakpoints and CLSI critical ESBL diameters were used to screen for potential AmpC producers. In total, 305 isolates (211 potential AmpC producers and 94 AmpC screen-negative isolates as a control group) were further analyzed by multiplex PCR for the detection of plasmid-encoded *ampC* beta-lactamase genes and by *ampC* promoter sequence analysis (considered as the gold standard). Cefoxitin and cefotetan were assessed as primary screening markers. The sensitivities of cefoxitin and cefotetan for the detection of AmpC production were 97.4 and 52.6%, respectively, and the specificities were 78.7 and 99.3%, respectively. As a phenotypic confirmation test, the Etest AmpC and the cefoxitin-cloxacillin double-disk synergy method (CC-DDS) were compared. The sensitivities for the Etest AmpC and the CC-DDS method were 77.4 and 97.2%, respectively, and the specificity was 100% for both methods. The results of the Etest AmpC were inconclusive for 10 isolates. With the CC-DDS method 2 inconclusive results were observed. Based on this study, we propose a comprehensive diagnostic flow chart for the detection of AmpC production consisting of a simple phenotypic screening and a single phenotypic confirmation test with inconclusive results being resolved by molecular analysis. For the proposed flow chart using (i) cefoxitin as a screening marker for AmpC production, (ii) the CC-DDS method as phenotypic confirmation, and (iii) molecular methods in case of inconclusive results, the sensitivity and specificity for AmpC detection would have been 97.4 and 100%, respectively, with respect to the studied isolates. The phenotypic methods used in the AmpC algorithm are simple to perform and easy to implement in the diagnostic laboratory.

In recent years, the prevalence of infections with multidrug-resistant *Enterobacteriaceae* has steadily increased (18). *Enterobacteriaceae* producing AmpC beta-lactamases (AmpCs) have become a major therapeutic challenge. The detection of AmpC-producing *Klebsiella* spp., *Escherichia coli*, *P. mirabilis*, and *Salmonella* spp. is of significant clinical relevance since AmpC producers may appear susceptible to expanded-spectrum cephalosporins when initially tested (13, 27, 28). This may lead to inappropriate antimicrobial regimens and therapeutic failure (24). Thus, a simple and reliable detection procedure for AmpC producers is needed.

Many Gram-negative bacteria harbor chromosomal *ampC* beta-lactamase genes, which are constitutively expressed at low level. In general, the expression of chromosomally located *ampC* genes is inducible by beta-lactam antibiotics, such as cefoxitin, cefotetan, and imipenem, and mediated by the regulator AmpR. Mutations in the repressor gene *ampD* are the most common cause of constitutive (hyper-)production of AmpC beta-lactamases (23). AmpC beta-lactamases degrade penicillins, expanded-spectrum cephalosporins (with the exception of cefepime and ceftipime), cephamycins, monobac-

tams, and beta-lactam inhibitors. In contrast to expanded-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases are inhibited by boronic acid and cloxacillin (2, 9, 25). In *E. coli*, regulation of chromosomal *ampC* expression differs significantly from that of other *Enterobacteriaceae*. In *E. coli* *ampC* is regulated by a weak promoter and a strong attenuator resulting in a constitutive low-level *ampC* expression (11). Diverse mutations in the *ampC* promoter region leading to overexpression have been described (3, 4, 7, 11, 12, 24, 29). In addition to chromosomal *ampC*, *Enterobacteriaceae* can acquire plasmid-encoded *ampC* genes (9). In general, plasmid-encoded AmpC beta-lactamases are expressed constitutively and are readily detected by a multiplex PCR (17).

Different phenotypic AmpC detection tests have been described in the literature (9). A standardized diagnostic approach integrating screening and confirmation tests for the detection of AmpC beta-lactamase-producing *Enterobacteriaceae* has not been established to date. We sought here to develop a comprehensive diagnostic flow chart integrating a simple phenotypic screening and confirmation for implementation in the routine diagnostic laboratory.

MATERIALS AND METHODS

Clinical isolates. In this prospective study all nonduplicate clinical *Enterobacteriaceae* isolates ($n = 2,129$) from the diagnostic laboratory isolated over a period of 7 months from October 2009 until April 2010 were screened for AmpC production (see Fig. 2). Only isolates that were considered clinically relevant were included, i.e., isolates that were considered as normal flora or commensals

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were disregarded. The isolates examined here included *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella enterica*, and *Citrobacter koseri*. With the exception of *E. coli*, we excluded species with known chromosomal AmpC production, e.g., *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Hafnia alvei*, and *Morganella morganii* (9).

Susceptibility testing. For susceptibility testing, the Kirby-Bauer disk diffusion method was used. Antibiotic disks were purchased from Becton Dickinson (Franklin Lakes, NJ), and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2009 guidelines (5). For cefoxitin (30 µg/disk) and cefotetan (30 µg/disk), screening cutoffs of ≤18 and ≤16 mm, respectively, were used (i.e., the CLSI susceptible breakpoints). In addition, the following ESBL CLSI screening cutoff values for expanded-spectrum cephalosporins were used to select for potential AmpC-producing isolates as follows: cefpodoxime (10 µg/disk), ≤17 mm; ceftazidime (30 µg/disk), ≤22 mm; cefotaxime (30 µg/disk), ≤27 mm; and ceftriaxone (30 µg/disk), ≤25 mm. Susceptibility testing was performed on Mueller-Hinton agar (bioMérieux, Marcy l'Etoile, France) using McFarland 0.5 from overnight cultures, followed by incubation at 35°C for 16 to 18 h.

Phenotypic AmpC confirmation testing. The Etest AmpC (AB bioMérieux, Solna, Sweden) was performed according to the manufacturer's instructions. The test principle comprises a strip impregnated with a concentration gradient of cefotetan on one half of the strip and cefotetan with cloxacillin on the other half of the strip. MICs of cefotetan alone and cefotetan with cloxacillin were determined as recommended by the manufacturer. Ratios of cefotetan versus cefotetan/cloxacillin of ≥8 were considered positive for AmpC beta-lactamase production.

The cefoxitin-cloxacillin double disc synergy test (CC-DDS) was performed as described previously (25). This test is based on the inhibitory effect of cloxacillin on AmpC. Disks containing either 30 µg of cefoxitin or 30 µg of cefoxitin plus 200 µg of cloxacillin were manufactured for the present study (Liofilchem, Roseto degli Abruzzi, Italy). The strains were inoculated on Mueller-Hinton agar using McFarland 0.5, followed and incubated at 35°C for 16 to 18 h. A difference in the cefoxitin-cloxacillin inhibition zones minus the cefoxitin alone zones of ≥4 mm was considered indicative for AmpC production.

ampC promoter sequencing (*E. coli* only). DNA was extracted from colonies grown on sheep blood agar medium using the InstaGene Matrix (Bio-Rad, Switzerland) according to the manufacturer's instructions. For the *ampC* promoter mutation analysis, a 271-bp fragment was amplified by using the primers AB1(5'-GATCGTCTGCGCTGTG-3') and ampC2 (5'-GGGCAGCAAATG TGGAGCAA-3') (4). PCR amplicons were purified with a QIAquick PCR purification kit (Qiagen, Hombrechtikon, Switzerland), followed by cycle sequencing using a BigDye reagent kit (Applied Biosystems, Switzerland). Sequence analysis was performed on an ABI Prism 3100 DNA sequencer (Applied Biosystems) according to standard protocols. Sequences were analyzed and edited by using Lasergene 7 MegAlign software (DNASTAR, Inc.). The *ampC* promoter sequences were compared to the wild-type *ampC* sequence of *E. coli* strain ATCC 25922.

Detection of plasmid-mediated *ampC* beta-lactamase genes. For the detection of plasmid-mediated *ampC* beta-lactamase genes, a multiplex PCR was used (17), which detects the six plasmid-mediated *ampC* families. When necessary, PCR amplicons were sequenced with the amplification primers according to the protocol described above. Sequences were analyzed for homology by using the National Center for Biotechnology Information GenBank database (<http://www.ncbi.nlm.nih.gov/>).

Interpretation. Molecular methods were considered the gold standard for calculation of the performance parameters. The results of the CC-DDS and/or Etest AmpC analyses were considered inconclusive if visible zones of inhibition were lacking (i) with cefotetan or cefoxitin alone or (ii) with cefotetan-cloxacillin or cefoxitin-cloxacillin.

RESULTS

Analysis of *Enterobacteriaceae* isolates for AmpC production in clinical isolates. A total of 2,129 nonduplicate clinical strains of the *Enterobacteriaceae* family isolated in the diagnostic microbiological laboratory during a 7-month period were screened for AmpC production. Species with known chromosomally encoded AmpC beta-lactamases (9) were not included, except for *E. coli*. The majority of the isolates were identified as *E. coli* ($n = 1,435$) and *K. pneumoniae* ($n = 360$) (Table 1).

TABLE 1. Species distribution and numbers of AmpC-producing *Enterobacteriaceae* isolates^a

Species	No. (%) of isolates	No. (%) of <i>ampC</i> -positive isolates ^b
<i>Escherichia coli</i>	1,435 (67.4)	33 (2.3)
<i>Klebsiella pneumoniae</i>	360 (16.9)	2 (0.6)
<i>Klebsiella oxytoca</i>	99 (4.7)	0
<i>Salmonella enterica</i>	4 (0.2)	2 (50.0)
<i>Proteus vulgaris</i>	26 (1.2)	0
<i>Proteus mirabilis</i>	131 (6.2)	1 (0.8)
<i>Citrobacter koseri</i>	74 (3.5)	0
Total	2,129 (100)	38

^a Numbers are given for all *Enterobacteriaceae* species that were tested for antibiotic susceptibility.

^b A total of 19 *E. coli* strains harbored a plasmidic *ampC*, and 14 *E. coli* strains contained mutations in the chromosomal *ampC* promoter which resulted in overexpression. *E. coli* strains with both plasmidic *ampC* and chromosomal *ampC* promoter mutations present were not detected.

Of the 2,129 isolates, 211 were categorized as potential AmpC producers on the basis of (i) cefoxitin inhibition zone diameters of ≤18 mm, (ii) cefotetan inhibition zone diameters of ≤16 mm, and/or (iii) positive ESBL screening diameters according to CLSI guidelines. To further assess the sensitivity and specificity of the screening procedure, 94 of 1,922 isolates with (i) cefoxitin inhibition zones of >18 mm, (ii) cefotetan inhibition zone diameters of >16 mm, and (iii) negative ESBL screening diameters according to the CLSI were included in the analysis. In all, 305 isolates (211 potential AmpC producers and 94 determined to be negative by the AmpC screening procedure) were characterized by phenotypic methods, multiplex PCR and, in part, DNA sequence analysis (gold standard, see Fig. 2).

Of the 211 potential AmpC producers, 37 were confirmed as AmpC-producing isolates by phenotypic and molecular methods (see Fig. 2). Of 211 isolates with a CIT type plasmid-encoded AmpC beta-lactamase, 1 was detected by molecular methods exclusively. AmpC production in this isolate was not detected by either the cefoxitin or the cefotetan disk diffusion test (which showed inhibition zone diameters of 21 or 27 mm, respectively), nor was it detected by the cefoxitin-cloxacillin double-disk synergy test. The cefoxitin and cefotetan MICs for this isolate were 4 and 0.75 mg/liter, respectively. Both values are in the susceptible range of the CLSI 2009 guidelines. The majority of plasmid-encoded AmpCs belonged to the CIT type (22 of 24 isolates), and two plasmid-encoded AmpCs were identified as the DHA type.

The prevalence of AmpC production among all tested isolates was 1.8%. Most frequently, AmpC production was observed in *E. coli* (33 of 38 potential AmpC-producing isolates); 19 of these isolates were plasmid encoded, and 14 were due to mutations in the *ampC* promoter region (Table 1). The majority of the isolates with AmpC production were isolated from urine (52.6%), respiratory tract (18.4%), rectogenital (7.9%), and wound (7.9%) specimens. A total of 13.9% of the specimens represent swabs without localization indicated that were originally sent to the laboratory for ESBL screening.

Comparison of primary screening markers for AmpC production. Cefoxitin and cefotetan were compared as primary screening markers. For cefoxitin, an 18-mm inhibition zone

TABLE 2. Performance of screening tests, confirmation tests, and the proposed AmpC detection algorithm

Examination method	No. of isolates examined ^a						%	
	Total	TP	FP	TN	FN	IR	Sensitivity	Specificity
Screening tests								
Cefoxitin screening	305	37	57	210	1	0	97.4	78.7
Cefotetan screening	305	20	2	265	18	0	52.6	99.3
Confirmation tests								
Etest AmpC	305	24	0	264	7	10	77.4	100.0
Cefoxitin ± cloxacillin	305	35	0	267	1	2	97.2	100.0
AmpC algorithm	305	37	0	267	1	0	97.4	100.0

^a TP, true positive; TN, true negative; FP, false positive; FN, false negative; IR, inconclusive result. Results were rated inconclusive if the MICs exceeded the scale of the Etest for cefotetan alone and/or cefotetan in combination with cloxacillin (Etest AmpC) or when no inhibition zone could be detected for both cefoxitin alone or in combination with cloxacillin (CC-DDS). Inconclusive results were excluded from the calculation of performance parameters.

diameter was chosen as the cutoff, and 16-mm diameter was used for cefotetan (i.e., the CLSI 2009 susceptibility breakpoints). Performance parameters were calculated considering molecular methods as the gold standard (multiplex PCR for the detection of plasmid-mediated *ampC* beta-lactamase genes and *E. coli* chromosomal *ampC* promoter sequence analysis). The sensitivities of cefoxitin and cefotetan for the detection of AmpC production were 97.4 and 52.6%, respectively, and the specificities were 78.7 and 99.3%, respectively. The absolute numbers of isolates and calculated performance parameters are summarized in Table 2.

Figure 1 shows the zone diameter distributions for cefoxitin and cefotetan in all isolates with a genotypically confirmed *ampC*. An AmpC screening cutoff for cefoxitin of ≤ 18 mm (CLSI susceptibility breakpoint) missed only one isolate with a genotypically detected CIT type AmpC that produced a diameter of 21 mm. Plasmid-encoded AmpCs clustered at a cefoxitin inhibition zone of 6 mm (19 of 24 isolates), which corresponds to the absence of a visible inhibition zone since the disc diameter itself is 6 mm. For isolates with plasmid-encoded AmpCs, the largest cefoxitin inhibition zone observed was 13 mm, in an isolate that was identified as *Salmonella enterica* serovar Typhimurium with a CIT-type AmpC. In contrast, the majority of *E. coli* isolates with promoter mutations produced inhibition zone diameters of ≥ 13 mm (10 of 14 isolates). Cefotetan, in general, showed higher variation in zone diameters than cefoxitin. An AmpC screening cutoff of ≤ 16 mm (CLSI susceptibility breakpoint) missed all 14 *E. coli* isolates with AmpC promoter mutations and 5 of 24 isolates with a plasmid-encoded AmpC. All isolates with promoter mutations showed cefotetan zone diameters of ≥ 18 mm and the majority of isolates with plasmid-encoded AmpCs showed cefotetan zone diameters of ≤ 17 mm (20 of 24 isolates).

Comparison of confirmation assays for AmpC production. The Etest AmpC and the cefoxitin-cloxacillin CC-DDS method were compared as phenotypic confirmation tests. In 10 of the 305 isolates, the results of the Etest AmpC analysis were inconclusive since MICs exceeded the scale of the test for cefotetan alone and/or cefotetan in combination with cloxacillin (Fig. 2). Therefore, the calculation of a ratio was not possible. With the CC-DDS, two inconclusive results were observed. In these two isolates no inhibition zone was present for cefoxitin alone or in combination with cloxacillin. Isolates with

inconclusive results were not included in the calculation of performance parameters. The sensitivities for Etest AmpC and CC-DDS were 77.4 and 97.2%, respectively, and the specificity was 100% when both methods were combined (Table 2).

Development of an algorithm for AmpC detection in *Enterobacteriaceae*. Combining the most sensitive screening method with the most accurate confirmation assay for AmpC production, we developed a comprehensive diagnostic flow chart (Fig. 3), which consists of (i) cefoxitin as a screening marker for AmpC production and (ii) CC-DDS as phenotypic confirmation, along with (iii) molecular methods in the case of inconclusive results. For AmpC detection in the isolates of the present study, this diagnostic approach would have displayed a calculated sensitivity and specificity of 97.4 and 100%, respectively (Table 2), with molecular analysis for inconclusive results only necessary for two isolates (1% of all isolates positive in the AmpC screening procedure).

DISCUSSION

Detection of AmpC production in pathogens might be important for ensuring effective antibiotic therapy (20) since the presence of an AmpC beta-lactamase frequently seems to result in therapeutic failure when broad-spectrum cephalosporins are used (14, 24). However, further studies are required to assess whether AmpC production is an independent risk factor for clinical outcome. Several methods have been evaluated for phenotypic screening and confirmation of AmpC beta-lactamase production (9, 25). However, a comprehensive diagnostic algorithm integrating both screening and confirmation has not been established. In the present study we evaluated individual screening and confirmation methods for AmpC production. Subsequently, we developed a diagnostic algorithm that (i) combines the most efficient and accurate methods, (ii) is simple, and (iii) can be implemented in the diagnostic laboratory (Fig. 2).

When cefoxitin and cefotetan (both cephamycins) were compared as the primary screening marker, cefoxitin was clearly superior to cefotetan regarding sensitivity (see Table 2). Our results for cefoxitin are in agreement with those of other authors (20, 25). However, the specificity in the present study was significantly lower, e.g., 78.7% versus the 95% reported by Tan et al. (25). In contrast to MIC determination by automated systems, the determination of drug susceptibility by disc diffu-

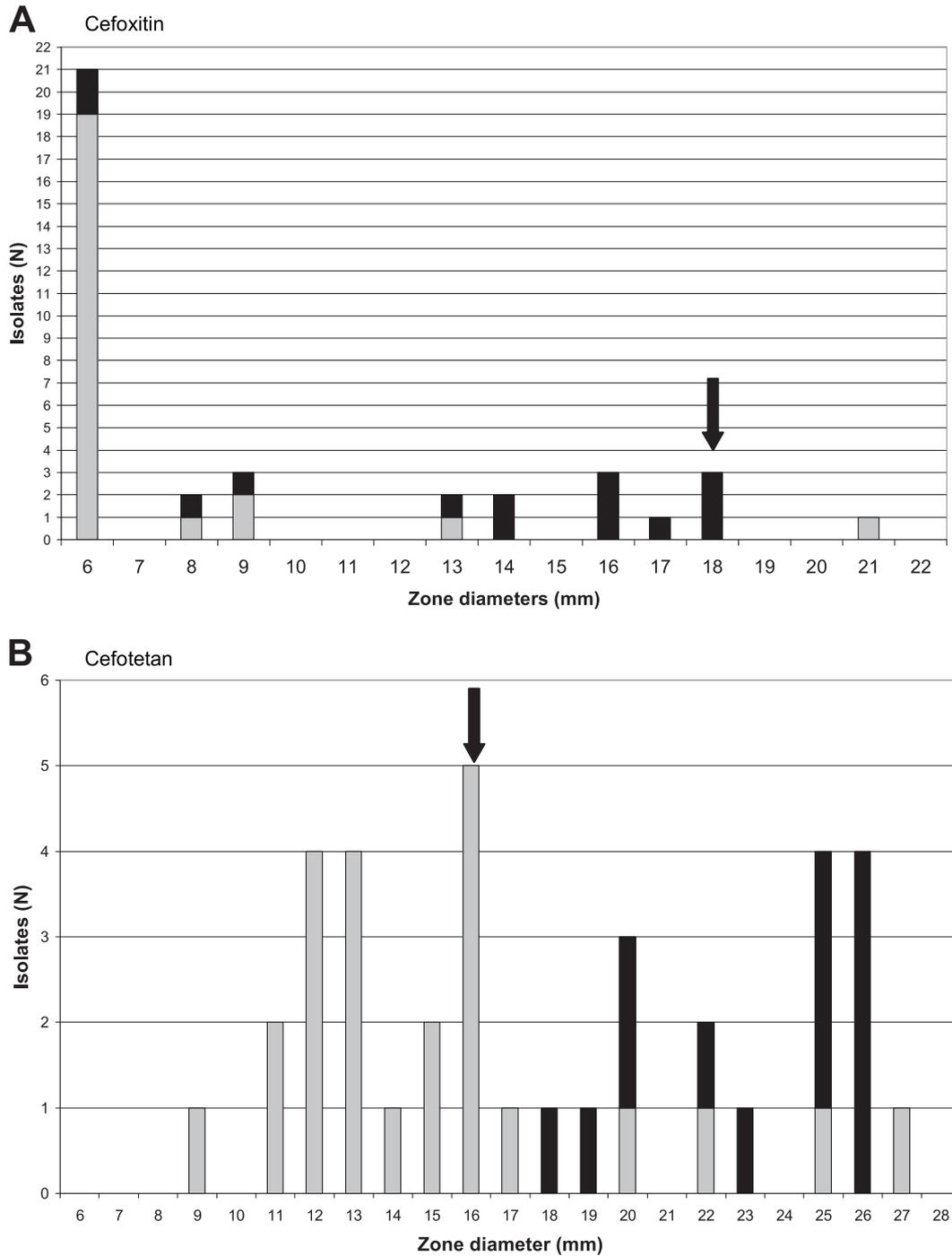


FIG. 1. Inhibition zone diameter distributions in AmpC beta-lactamase-producing isolates. The inhibition zone diameters of cefoxitin (A) and cefotetan (B) are presented. The numbers of *Enterobacteriaceae* isolates with plasmidic *ampC* (□) and chromosomal *ampC* promoter mutations (■, *E. coli* only) are given; the CLSI 2009 susceptibility breakpoints are indicated by black arrows.

sion may further enhance sensitivity since synergy and antagonism phenomena are readily observed, e.g., when placing a cefoxitin disc near a expanded-spectrum cephalosporin disc. For example, the presence of DHA type enzymes will lead to flattening of inhibition zones (antagonism phenomena) of expanded-spectrum cephalosporins toward inducers such as cefoxitin, carbapenems, or clavulanic acid. Otherwise, ACC-

type enzymes are characteristically inhibited by cefoxitin visible as enhancement of the inhibition zones (synergy phenomena) of expanded-spectrum cephalosporins and cefoxitin. With this strategy, the detection of ACC-type AmpC enzymes is possible, although ACC enzymes appear to be cefoxitin susceptible (1, 22). In contrast, cefoxitin screening by MIC alone would miss ACC types. Other authors recommend additional screening

Enterobacteriaceae isolates N = 2129																							
Cefoxitin zone diameter ≤ 18 mm and/or cefotetan diameter ≤ 16 mm. Critical ESBL diameters (CLSI) positive for CDP and/or CAZ and/or CTX and/or CRO																							
AmpC screening negative N = 1922												Potential AmpC producers N = 211											
AmpC screening negative control group N = 94																							
CC-DDS N = 94			Etest AmpC N = 94			CC-DDS N = 211			Etest AmpC N = 211														
negative: N = 94	positive: N = 0	inconclusive N = 0	negative N = 93	positive N = 0	inconclusive N = 1	negative N = 174	positive N = 35	inconclusive N = 2	negative N = 178	positive N = 24	inconclusive N = 9												
ampC Multiplex PCR (all isolates) and ampC promoter sequence characterization (<i>E. coli</i> only) N = 305																							
pos N = 0	neg N = 94	pos N = 0	neg N = 0	pos N = 0	neg N = 0	pos N = 0	neg N = 93	pos N = 0	neg N = 0	pos N = 0	neg N = 1	pos N = 1 [#]	neg N = 173	pos N = 35	neg N = 0	pos N = 2	neg N = 0	pos N = 7	neg N = 171	pos N = 24	neg N = 0	pos N = 7	neg N = 2

FIG. 2. Study layout and numbers of isolates. CPD, cefpodoxime; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; CC-DDS, double-disk synergy test. #, AmpC missed by the algorithm.

criteria for ACC enzymes such as critical inhibition zone diameters for amoxicillin-clavulanic acid or expanded-spectrum cephalosporins (26). To date, the ACC types seem to be the only known enzymes that can be missed by cefoxitin screening. The isolation numbers of ACC enzymes are still significantly lower than those of CIT (CMY), FOX, and DHA types (10, 14, 19, 25, 26). No ACC-type AmpC was detected in the present study.

The AmpC flow chart (Fig. 2) can be combined with a flow chart for ESBL detection (unpublished data). If cefoxitin is not routinely tested, an alternative branch may be chosen that substitutes the cefoxitin screening criteria by CLSI screening criteria for ESBL (Fig. 3). With a combined ESBL/AmpC screening strategy, ACC enzymes will readily be detected. ACC confers high resistance to expanded-spectrum cephalosporins, which serve as primary screening markers for ESBL detection (19, 21). Thus, corresponding isolates will be assigned to a combined ESBL/AmpC confirmation test via the CLSI screening criteria for ESBL (5, 6).

The single false-negative result for the cefoxitin screening test in the present study (Fig. 2) resulted from the presence of a CIT-type *ampC* detected by multiplex PCR. MICs of this isolate for cefoxitin and cefotetan were well within the susceptible range, and both phenotypic confirmation tests were clearly negative (Etest AmpC ratio of 1.0; CC-DDS, no difference). Sequence analysis of the CIT *ampC* gene did not reveal any mutation affecting the structure and/or function of the enzyme. However, mutations in the regulatory regions may result in very low expression or no expression of the structural gene (8). If the CIT type enzyme in this isolate were nonfunctional, the sensitivity of the cefoxitin screening procedure would be close to 100%.

We compared the performance of the Etest AmpC and the cefoxitin-cloxacillin CC-DDS as a phenotypic confirmation test (25). Boronic acid can be used as alternative to cloxacillin as AmpC inhibitor, since it was found to be almost as sensitive and specific as cloxacillin by Tan et al. (25). However, boronic acid may produce false-positive results in isolates carrying class A carbapenemases, whereas cloxacillin does not (15, 16).

Therefore, we chose cloxacillin as AmpC inhibitor in our algorithm. Regarding sensitivity, the CC-DDS was clearly superior to the Etest AmpC (97.2% versus 77.4%, respectively, see Table 2). This result may be explained by the use of cefotetan in the Etest AmpC. Cefotetan has a lower sensitivity than cefoxitin concerning the detection of AmpC production. This is also apparent when cefotetan disc diffusion was used as a screening test (see Table 2). Ten inconclusive results were obtained with the Etest AmpC, due to MICs exceeding the Etest scale of cefotetan with or without cloxacillin (Table 2). In routine use, this may hamper the sensitivity and practicability of this method. In contrast, with CC-DDS only two inconclusive results were obtained. For both isolates with an inconclusive result, no inhibition zone for cefoxitin was observed both with or without cloxacillin. Eventually, AmpC enzymes of the CIT type were found in both strains. The results for the CC-DDS are in agreement with other studies that reported a high sensitivity and specificity for this test (25).

Combining the high sensitivity of cefoxitin screening with the high specificity of the cefoxitin-cloxacillin CC-DDS confirmation test, we propose a flow chart for the phenotypic detection and characterization of AmpC beta-lactamases (Fig. 3). In the case of (rarely occurring) inconclusive results, molecular methods are used for resolution. The proposed flow chart would have a calculated sensitivity and specificity of 97.4 and 100%, respectively, with respect to the isolates in the present study. Phenotypic AmpC screening and confirmation tests are inexpensive but nevertheless highly sensitive and specific. Therefore, it can be performed in all types of clinical laboratories, whereas the implementation of molecular methods is often complex, requires specially trained personnel, and is associated with higher costs.

In conclusion, the proposed flow chart for detection of AmpC is simple to use and easy to implement in a diagnostic laboratory. If molecular methods are not available, the few inconclusive isolates can be submitted to a reference laboratory for further investigations. In parallel, we have developed a flow chart for ESBL detection (unpublished), which in combi-

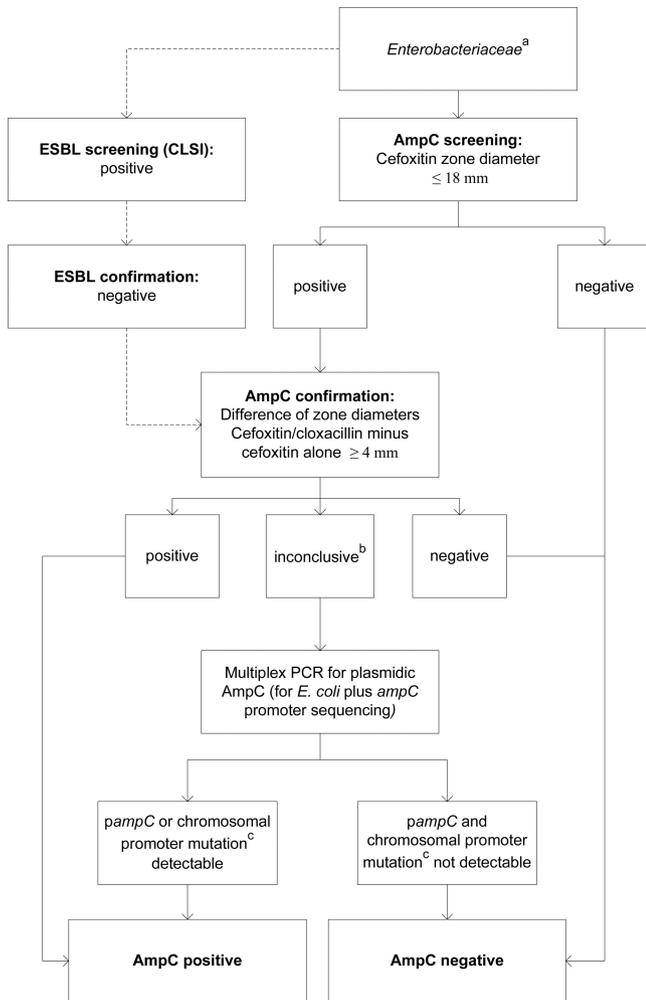


FIG. 3. Flow chart for detection of AmpC beta-lactamase production in *Enterobacteriaceae*. Superscript letters: a, this category includes *Enterobacteriaceae* spp. with no known chromosomal *ampC* production plus *E. coli*; b, differences of zone diameters indicated as “inconclusive” means there were no visible inhibition zones around both cefoxitin discs with or without cloxacillin (*pampC*, plasmidic AmpC beta-lactamase); c, this category refers to mutations in the *ampC* promoter region of *E. coli* that result in the overexpression of *ampC*.

nation with the AmpC detection flow chart, covers a broad spectrum of beta-lactamases, facilitating therapeutic decisions and epidemiological surveillance.

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