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Consequences of revised CLSI and EUCAST guidelines for antibiotic susceptibility patterns of ESBL- and AmpC β-lactamase-producing clinical Enterobacteriaceae isolates

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Objectives: This study aimed to: (i) analyse the antibiotic susceptibility testing (AST) profiles of extended spectrum β-lactamase (ESBL)- and AmpC β-lactamase-producing clinical Enterobacteriaceae isolates applying EUCAST 2013 AST guidelines; and (ii) evaluate discrepancies in AST profiles according to EUCAST 2010 guidelines, EUCAST 2013 guidelines, CLSI 2009 guidelines and CLSI 2013 guidelines.

Methods: The 195 ESBL- and/or AmpC β-lactamase-producing Enterobacteriaceae isolates used in this study were systematically characterized by disc diffusion AST interpreted according to the 2013 guidelines of EUCAST and CLSI, the EUCAST 2010 guidelines and the CLSI 2009 guidelines.

Results: Individual cephalosporin AST patterns according to EUCAST 2013 guidelines were described for individual ESBL and AmpC β-lactamase genotypes. Significant differences in the susceptibility rates of important cephalosporins such as cefepime, ceftazidime and cefotaxime applying EUCAST 2013 and CLSI 2013 AST guidelines were demonstrated for ESBL- and AmpC β-lactamase-producing isolates.

Conclusions: The confirmation of ESBL and/or AmpC β-lactamase production can support the selection of an adequate antibiotic drug therapy. Despite a harmonized CLSI and EUCAST 'report as found' strategy for cephalosporins and ESBL-producing isolates, AST interpretation according to the CLSI 2013 and EUCAST 2013 guidelines shows significant differences in susceptibility rates for mainstay cephalosporins such as cefepime, ceftazidime and cefotaxime. Thus, further harmonization of clinical breakpoints is warranted.

Keywords: cephalosporins, penicillins, carbapenems, AST

Introduction

Extended-spectrum β-lactamase (ESBL)- and AmpC β-lactamase (AmpC)-producing strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* are increasingly reported worldwide.[1,2] ESBL- and AmpC-producing isolates are able to cause life-threatening infections with a significant impact on morbidity, mortality and healthcare-associated costs.[3–6] The ESBL classes that are most frequently encountered in the clinical laboratory are types TEM, SHV and CTX-M.[7] Currently, over 100 SHV ESBLs, 150 TEM ESBL types and ~90 CTX-M variants have been described. CTX-M-producing *E. coli* has become the most prevalent ESBL type in Europe and North America.[8,9] In addition to ESBLs, Enterobacteriaceae can acquire plasmid-encoded *ampC* genes (pAmpC) as an important resistance mechanism against β-lactams.[10] *E. coli* possesses a chromosomal *ampC* gene, which is regulated differently from other Enterobacteriaceae. *ampC* expression in *E. coli* is constitutive at low levels due to a weak promoter and a strong attenuator.[11] Mutations in the promoter region leading to *ampC* overexpression have been described.[12]

EUCAST and CLSI recently published new antimicrobial susceptibility testing (AST) guidelines that constitute a paradigm change in the interpretation and reporting of AST for ESBL- and AmpC-producing isolates and penicillins, cephalosporins and monobactams.[13,14] CLSI also changed its AST guidelines from the 2009 to the 2013 version, but significant differences in terms of the AST categorization of Enterobacteriaceae remain in the EUCAST guidelines.[15,16] Until 2009, the two institutions recommended either reporting *in vitro* susceptible and intermediate AST results for penicillins, cephalosporins and monobactams in ESBL-producing isolates as resistant (CLSI) or modifying the interpretation from susceptible to intermediate and from intermediate to resistant (EUCAST).[15,17] Such editing of *in vitro* AST results is no longer recommended.[14,16] These changes

were accompanied by significantly higher cephalosporin zone diameter clinical breakpoints (CBPs) in EUCAST 2013 compared with CLSI 2009 and, in part, EUCAST 2010. Higher EUCAST CBPs were, in part, adopted by CLSI in its 2010 to 2013 guidelines.[13,15,16,18]

Data systematically describing the AST profiles of ESBL- and AmpC-producing Enterobacteriaceae isolates are of significant interest as antibiotic therapy for ESBL- and AmpC-producing isolates that are categorized as susceptible to cephalosporins, such as cefepime, cefotaxime, ceftazidime and ceftriaxone, is now allowed by EUCAST and CLSI. We determined here the AST patterns of a well-defined set of clinical isolates of ESBL- and AmpC-producing Enterobacteriaceae according to the former and revised versions of the EUCAST and CLSI guidelines.

Methods

Clinical isolates

This study comprised 195 ESBL- and/or AmpC-producing non-duplicate Enterobacteriaceae clinical isolates (i.e. one isolate per patient and species) originating from the University Hospital of Zurich from 2009 until 2012 (Table 1). No clonal outbreak strains were detected by PFGE (data not shown). Isolates had been characterized for the production of an ESBL and/or an AmpC in previous studies.[19,20]

Susceptibility testing

Disc diffusion AST was carried out on Mueller–Hinton agar (Becton Dickinson, Franklin Lakes, NJ, USA) using overnight cultures with a turbidity equivalent to that of a 0.5 McFarland standard followed by incubation at 35°C for 16–18 h. Antibiotic discs (i2a, Montpellier, France) were used. Results were interpreted according to the EUCAST 2010, EUCAST 2013, CLSI 2009 and CLSI 2013 guidelines.[13,15,16,18] The CBPs for Enterobacteriaceae are summarized in Table S1 (available as Supplementary data at *JAC* Online).

Genotypic ESBL characterization

DNA was extracted using the InstaGene Matrix (Bio-Rad, Reinach, Switzerland) from colonies grown on sheep blood agar according to the manufacturer's instructions. Detection of TEM and SHV ESBLs was carried out as described previously.[21] Wild-type *E. coli* AF427133.1 TEM-1 and *E. coli* AF148850 SHV-1 according to the database at http://www.lahey.org/studies were used as references to compare TEM and SHV β-lactamase sequences. CTX-M β-lactamase genes were detected by a multiplex PCR described by Pitout *et al.*[8] This multiplex PCR discriminates CTX-M group 1, CTX-M group 2, CTX-M group 8 and CTX-M group 9 genes.

Genotypic ampC characterization

DNA extraction from colonies grown on sheep blood agar medium using the InstaGene Matrix (Bio-Rad) was carried out following the manufacturer's instructions. Plasmid-mediated *ampC* genes were detected by a multiplex PCR described by Perez-Perez and Hanson.[22] This PCR detects six plasmid-mediated *ampC* families. PCR amplicons were sequenced when necessary, using the amplification primers following the protocol described above. Sequences were analysed for homology using the National Center for Biotechnology Information's GenBank (http://www.ncbi.nlm.nih.gov/).

For the analysis of *ampC* promoter mutations, a 271 bp fragment was amplified using primers AB1 (5′-GATCGTTCTGCCGCTGTG-3′) and ampC2 (5′-GGGCAGCAAATGTGGAGCAA-3′).[23] PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hombrechtikon, Switzerland) followed by cycle sequencing using the BigDye Reagent Kit (Applied Biosystems, Switzerland). Sequence analysis was performed using an ABI Prisma 3100 DNA Sequencer (Applied Biosystems, Switzerland) following standard protocols. Sequences were analysed and edited using Lasergene 7 MegAlign software (DNASTAR Inc., USA). The *ampC* promoter sequences were compared with the wild-type *ampC* sequence of *E. coli* strain ATCC 25922.

Software

All calculations were done using IBM SPSS version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). To test for the statistical significance of categorization differences, Wilcoxon's signed-rank test and Friedman's χ^2 test were used.

Results

β-Lactam AST profiles according to EUCAST 2013 guidelines

Resistance rates to amoxicillin/clavulanic acid were significantly higher in AmpC-producing isolates compared with ESBL-producing isolates (e.g. resistance rates of 94.1% and 78.6%, $P=0.000$, for pAmpCs and *ampC* promoter mutations in *E. coli*, respectively;

Table 1. ESBL and/or AmpC genotypes of the Enterobacteriaceae isolates included in the study

		ESBL genotypes ($n=150$)			AmpC genotypes ($n=32$)		ESBL[a] combined with AmpC ($n=13$)		
Species	Total isolates (n)	CTX-M	SHV	TEM	pAmpC	promoter mutation[b]	pAmpC	promoter mutation[b]	chromosomal AmpC
E. coli	159	117	9	1	17	14	1	—	—
K. pneumoniae	25	21	2	0	1	—	1	—	—
E. cloacae	11	0	0	0	—	—	—	—	11
Total	195	138	11	1	18	14	2	—	11

[a]ESBL and AmpC genotypes comprised nine *E. cloacae* CTX-M (combined with the natural chromosomal AmpC of *E. cloacae*), two *E. cloacae* SHV (combined with the natural chromosomal AmpC of *E. cloacae*), one *E. coli* SHV-ESBL combined with DHA-type pAmpC, and one *K. pneumoniae* CTX-M combined with DHA-type pAmpC.
[b]Applies to *E. coli* isolates only. Promoter mutation of the chromosomal *ampC* in *E. coli*, which results in overexpression of AmpC.

Table 2. Resistance profiles of *E. cloacae*, *E. coli* and *K. pneumoniae* isolates applying EUCAST 2013 AST guidelines to ESBL- and AmpC-producing isolates

			E. coli				
Drug/interpretation (%)		*E. cloacae* CTX-M ($n=9$)	CTX-M ($n=117$)	SHV ($n=9$)	pAmpC ($n=17$)	*ampC* promoter mutation[a] ($n=14$)	*K. pneumoniae* CTX-M ($n=21$)
Ampicillin	R		100	100	100	100	
	I	NR	—	—	—	—	NR
	S		0	0	0	0	
Amoxicillin/clavulanic acid	R		43.6	11.1	94.1	78.6	61.9
	I	NR	—	—	—	—	—
	S		56.4	88.9	5.9	21.4	38.1
Piperacillin/tazobactam	R	36.3	13.7	11.1	29.4	7.1	28.6
	I	27.3	13.7	0	29.4	14.3	9.5
	S	36.4	72.6	88.9	41.2	78.6	61.9
Cefuroxime	R	100	97.4	33.3	82.4	35.7	100
	I	—	0	0	0	0	—
	S	0	2.6	66.7	17.6	64.3	0
Cefoxitin	R		6.8	11.1	94.1	85.7	33.3
	I	NR	—	—	—	—	—
	S		93.2	88.9	5.9	14.3	66.7
Cefpodoxime	R	100	97.4	88.9	100	92.9	100
	I	—	—	—	—	—	—
	S	0	2.6	11.1	0	7.1	0
Cefotaxime	R	100	93.2	22.2	88.2	21.4	100
	I	0	3.4	44.4	0	14.3	0
	S	0	3.4	33.3	11.8	64.3	0
Ceftazidime	R	81.8	61.5	77.8	94.1	42.9	90.5
	I	0	6.0	0	0	14.3	0
	S	18.2	32.5	22.2	5.9	42.9	9.5
Ceftriaxone	R	100	94.0	33.3	94.1	7.1	100
	I	0	3.4	33.3	0	14.3	0
	S	0	2.6	33.3	5.9	78.6	0
Cefepime	R	63.6	69.2	0	11.8	0	81.0
	I	0	10.3	11.1	0	0	9.5
	S	36.4	20.5	88.9	88.2	100	9.5
Ertapenem	R	18.1	0	0	0	0	4.8
	I	36.4	0.9	0	5.9	0	9.5
	S	45.5	99.1	100	94.1	100	85.7
Imipenem	R	0	0	0	0	0	0
	I	0	0	0	0	0	0
	S	100	100	100	100	100	100
Meropenem	R	0	0	0	0	0	0
	I	0	0	0	0	0	0
	S	100	100	100	100	100	100

R, resistant; I, intermediate; S, susceptible; NR, natural resistance.
[a]Promoter regions of the chromosomal *ampC* were analysed for mutations resulting in overexpression only when a pAmpC was not detected.

Table 2). Amoxicillin/clavulanic acid resistance rates in ESBL-producing *E. coli* isolates were significantly higher for CTX-M ESBLs (43.6%, $P=0.016$) compared with SHV ESBLs (11.1%). The amoxicillin/clavulanic acid resistance rate of CTX-M-containing isolates was higher for *K. pneumoniae* than for *E. coli* (61.9% versus 43.6%, $P=0.121$; Table 2). Piperacillin/tazobactam showed a comparable susceptibility pattern to amoxicillin/clavulanic acid with one exception: the elevated production of AmpC mediated by promoter mutation(s) in *E. coli* resulted in a relatively low resistance rate to piperacillin/tazobactam of 7.1% (Table 2).

Resistance to cefotaxime, ceftriaxone and cefepime was significantly more common in CTX-M- and pAmpC-producing isolates compared with *E. coli ampC* promoter mutations ($P\leq0.05$) and SHV ESBLs ($P\leq0.05$). Resistance to ceftazidime was comparably more common in SHV ESBLs and *E. coli ampC* mutations (77.8% and 42.9%, respectively; Table 2). CTX-M producing *K. pneumoniae* isolates displayed significantly higher resistance rates for ceftazidime and cefoxitin compared with CTX-M-producing *E. coli* (90.5% versus 61.5%, $P=0.034$, and 33.3% versus 6.8%, $P=0.000$; Table 2).

Carbapenems remained active against ESBL- and AmpC-producing isolates with two exceptions: susceptibility rates to ertapenem were significantly lower in *K. pneumoniae* and *E. cloacae* producing CTX-M (85.7%, $P=0.002$, and 45.5%, $P=0.000$) compared with CTX-M-producing *E. coli* (99.1%; Table 2). In addition, ertapenem susceptibility was slightly decreased in pAmpC-producing *E. coli* isolates (94.1%; Table 2).

Comparison of β-lactam AST profiles of ESBL- and AmpC-producing isolates according to CLSI 2009, CLSI 2013, EUCAST 2010 and EUCAST 2013 guidelines

The amoxicillin/clavulanic acid susceptibility rates of ESBL-producing isolates were similar when applying the EUCAST 2013, CLSI 2009 and CLSI 2013 guidelines (Table 3). In contrast, the EUCAST 2010 susceptibility rate was significantly higher (90.7%, $P=0.000$). Piperacillin/tazobactam susceptibility rates according to EUCAST 2010 and 2013 were slightly higher for ESBL-producing isolates compared with the CLSI 2009/2013 guidelines (78.0% and 72.0% versus 64.0%, $P=0.165$), but lower for AmpC-producing isolates and EUCAST 2013 as compared with EUCAST 2010 and CLSI 2009/2013 (56.2% versus 78.1% and 71.9%, respectively, $P\leq0.052$; Table 3).

Various patterns of susceptibility to cephalosporins were detected applying the EUCAST 2010, EUCAST 2013, CLSI 2009 and CLSI 2013 guidelines: similar rates of susceptibility were found for cefuroxime and cefpodoxime for both ESBL- and AmpC-producing isolates comparing all guideline versions (Table 3). EUCAST 2010 and 2013 did not significantly differ in terms of cephalosporin categorization except for the cefepime resistance rates of ESBL producers, which were significantly higher applying the 2013 version (40.7% versus 66.0%, $P=0.021$). Significantly lower susceptibility rates were found when applying EUCAST 2010 and 2013 compared with CLSI 2009 and CLSI 2013 for AmpC-producing isolates and cefotaxime and ceftazidime ($P\leq0.001$), and for ESBL-producing isolates and cefepime ($P=0.000$, Table 3). ESBL-producing isolates had significantly different susceptibility rates to ceftazidime when comparing EUCAST 2010 and EUCAST 2013 with CLSI 2009 and CLSI 2013 (32.0%, 28.0%, 54.0% and 38.7%, respectively, $P=0.000$). The ceftriaxone diameter CBPs are equal in EUCAST 2010/2013 and CLSI 2013 compared with CLSI 2009, resulting in significantly lower ceftriaxone susceptibility rates for ESBL-positive, AmpC-positive and both ESBL- and AmpC-positive isolates [CLSI 2009 versus EUCAST 2010/2013 and CLSI 2013: 52.0% versus 4.0% ($P=0.000$), 46.9% versus 37.5% ($P=0.004$) and 23.1% versus 0% ($P=0.038$) for ESBL-positive, AmpC-positive and both ESBL- and AmpC-positive isolates, respectively; Table 3].

Similar rates of susceptibility were demonstrated for the carbapenems with both ESBL- and AmpC-producing isolates. For ESBL- and AmpC-co-producing isolates, ertapenem susceptibility rates applying the EUCAST 2013 guidelines (46.2%) were significantly lower than for the CLSI 2009 (92.3%, $P=0.014$) and CLSI 2013 (76.9%, $P=0.014$) categorizations.

Discussion

Since implementation of their 2010 AST guidelines EUCAST and CLSI recommend reporting AST results for ESBL-producing Enterobacteriaceae to penicillins and cephalosporins 'as found' *in vitro*, i.e. results are no longer edited to intermediate and/or resistant, if an ESBL is present.[13,16] The treatment of ESBL-producing isolates with cephalosporins is allowed depending on the AST categorization. EUCAST 2013 and CLSI 2013 aim to assure the correct treatment recommendations by higher zone diameter breakpoints compared with CLSI 2009 and, partly, EUCAST 2010, classifying more isolates as resistant. This particularly accounts for newer cephalosporins, such as cefpodoxime, cefotaxime, ceftazidime, ceftriaxone (EUCAST and CLSI) and cefepime (EUCAST).[13,15,16,18] Using cephalosporins for ESBL treatment could result in a lower selection pressure on reserve drugs such as the carbapenems. However, few data are available showing antibiotic susceptibility patterns for defined populations of ESBL-producing isolates according to revised EUCAST and CLSI guidelines.[24] The same accounts for the increasing number of AmpC-producing isolates.

Testing for the presence of an ESBL is considered useful for epidemiological purposes by CLSI and EUCAST.[13,16] Nevertheless, it remains controversial whether the exclusive presence of a certain resistance mechanism, e.g. ESBL or AmpC, should be considered in the selection of a calculated antibiotic drug therapy (interpretative reading).[25,26]

This study describes various resistance patterns for individual ESBL and AmpC genotype/species combinations if EUCAST 2013 CBPs are applied (Table 2). Low rates of susceptibility to cefpodoxime, cefotaxime and ceftriaxone were found for CTX-M-type ESBL-producing isolates (0%–3.4%; Table 2), whereas ceftazidime and cefepime were categorized as susceptible in 9.5%–32.5% and 9.5%–36.4% of CTX-M-positive isolates, respectively, which is in concordance with other studies.[27] For pAmpC-positive *E. coli* isolates, susceptibility rates varied from 0% in the case of cefpodoxime up to 88.2% for cefepime, while *E. coli* isolates with *ampC* promoter mutations generally displayed higher levels of susceptibility to newer cephalosporins except for cefpodoxime (42.9% to 100%; Table 2). Our results underline that different β-lactamase genotypes produce distinct phenotypic AST patterns. ESBL and AmpC co-expression can even lead to non-susceptibility to carbapenems, particularly in the case of ertapenem (see, for

Table 3. Comparison of antibiotic susceptibility profiles of ESBL- and AmpC-producing Enterobacteriaceae isolates applying the CLSI 2009, CLSI 2013, EUCAST 2010 and EUCAST 2013 AST guidelines

		ESBL-producing isolates[c] (n=150)				AmpC-producing isolates (n=32)				ESBL- and AmpC-producing isolates[a,c] (n=13)			
Drug/interpretation (%)		CLSI 2009	CLSI 2013	EUCAST 2010	EUCAST 2013	CLSI 2009	CLSI 2013	EUCAST 2010	EUCAST 2013	CLSI 2009	CLSI 2013	EUCAST 2010	EUCAST 2013
Ampicillin	R	100	100	100	100	100	100	100	100	100	100	100	100
	I	0	0	—	—	0	0	—	—	0	0	—	—
	S	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin/clavulanic acid	R	26.0	26.0	9.3	43.3	84.4	84.4	81.2	87.5	100	100	92.3	100
	I	23.3	23.3	—	—	12.5	12.5	—	—	0	0	—	—
	S	50.7	50.7	90.7	56.7	3.1	3.1	18.8	12.5	0	0	7.7	0
Piperacillin/tazobactam[b]	R	20	20	9.3	15.3	15.6	15.6	15.6	21.9	46.2	46.2	46.2	46.2
	I	16.0	16.0	12.7	12.7	12.5	12.5	6.3	21.9	15.4	15.4	15.4	23.0
	S	64.0	64.0	78.0	72.0	71.9	71.9	78.1	56.2	38.4	38.4	38.4	30.8
Cefuroxime	R	91.3	91.3	93.3	93.3	43.8	43.8	62.5	62.5	100	100	100	100
	I	2.0	2.0	—	—	18.8	18.8	—	—	0	0	—	—
	S	6.7	6.7	6.7	6.7	37.5	37.5	37.5	37.5	0	0	0	0
Cefoxitin	R	2.7	2.7	—	12.0	75.0	75.0	—	90.6	100	100	—	100
	I	8.0	8.0	—	—	9.4	9.4	—	—	0	0	—	—
	S	89.3	89.3	—	88.0	15.6	15.6	—	9.4	0	0	—	0
Cefpodoxime	R	96.7	96.7	97.3	97.3	96.9	96.9	96.9	96.9	100	100	100	100
	I	0.7	0.7	—	—	0	0	—	—	0	0	—	—
	S	2.7	2.7	2.7	2.7	3.1	3.1	3.1	3.1	0	0	0	0
Cefotaxime[b]	R	66.7	91.3	89.3	89.3	9.4	40.6	59.4	59.4	76.9	100	100	100
	I	24.7	1.3	3.4	5.3	31.2	3.1	6.2	6.2	23.1	0	0	0
	S	8.7	7.3	7.3	5.3	59.4	56.2	34.4	34.4	0	0	0	0
Ceftazidime[b]	R	18.7	46.0	56.0	67.3	15.6	31.2	53.1	71.9	46.2	69.2	76.9	84.6
	I	27.3	15.3	12.0	4.7	15.6	3.1	15.6	6.2	23.1	15.4	7.7	0
	S	54.0	38.7	32.0	28.0	68.8	65.6	31.3	21.9	30.8	15.4	15.4	15.4
Ceftriaxone	R	44.7	91.3	91.3	91.3	34.4	56.2	56.2	56.2	61.5	100	100	100
	I	3.3	4.7	4.7	4.7	18.8	6.2	6.2	6.2	15.4	0	0	0
	S	52.0	4.0	4.0	4.0	46.9	37.5	37.5	37.5	23.1	0	0	0
Cefepime	R	15.3	15.3	40.7	66.0	0	0	0	6.2	7.7	7.7	15.4	69.2
	I	26.0	26.0	35.3	10.0	0	0	6.2	0	7.7	7.7	53.8	0
	S	58.7	58.7	24.0	24.0	100	100	93.1	93.1	84.6	84.6	30.8	30.8
Ertapenem	R	0.7	0.7	0.7	0.7	0	0	0	0	0	7.7	23.1	23.1
	I	0	0	2.0	2.0	0	0	3.1	3.1	7.7	15.4	30.8	30.8
	S	99.3	99.3	97.3	97.3	100	100	96.9	96.9	92.3	76.9	46.2	46.2
Imipenem	R	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	S	100	100	100	100	100	100	100	100	100	100	100	100
Meropenem	R	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0
	S	100	100	100	100	100	100	100	100	100	100	100	100

R, resistant; I, intermediate; S, susceptible.

[a]ESBL- and AmpC-positive isolates comprised nine *E. cloacae* CTX-M (combined with the natural chromosomal AmpC of *E. cloacae*), two *E. cloacae* SHV (combined with the natural chromosomal AmpC of *E. cloacae*), one *E. coli* SHV-ESBL combined with DHA-type pAmpC, and one *K. pneumoniae* CTX-M combined with DHA-type pAmpC.

[b]For these drugs, CLSI and EUCAST use different disc contents: ceftazidime (CLSI 30 μg/disc, EUCAST 10 μg/disc), cefotaxime (CLSI 30 μg/disc, EUCAST 5 μg/disc) and piperacillin/tazobactam (CLSI 100/10 μg/disc, EUCAST 30/6 μg/disc). All isolates were tested with both disc contents in parallel and interpretation was carried out accordingly.

[c]Until 2009, CLSI recommended editing susceptible and intermediate *in vitro* AST results for all penicillins and cephalosporins to 'resistant' for clinical reports if the presence of an ESBL was confirmed.

example, the decreased ertapenem susceptibility rates for CTX-M- and pAmpC-producing isolates; Table 2). Carbapenem non-susceptibility may be caused by ESBL and/or AmpC production combined with a loss of outer membrane porins.[28–30] Confirming the presence of a certain resistance mechanism (ESBL and/or AmpC for instance) may, therefore, still be of value in selecting an adequate calculated antibiotic therapy, as previously suggested.[25,26] Systematic prospective clinical studies analysing whether the exclusive presence of an ESBL influences clinical outcome are largely lacking.

Equal (but low) susceptibility rates were demonstrated for ceftriaxone due to CLSI and EUCAST harmonized CBPs (Table 3). The most prominent difference in susceptibility rates of ESBL-producing isolates applying EUCAST 2010/2013 and CLSI 2009/2013 was found for cefepime (58.7% versus 24.0%, $P=0.000$; Table 3). This difference resulted from CLSI retaining low cefepime diameter CBPs in its 2013 guidelines, whereas zone diameter CBPs for ceftazidime and cefotaxime were significantly increased.[15,16] Furthermore, the AST interpretation in EUCAST 2010, EUCAST 2013 and CLSI 2009/2013 differed for amoxicillin/clavulanic acid, piperacillin/tazobactam and ertapenem depending on the presence of an ESBL, an AmpC or the co-expression of both types of β-lactamases (Table 3). Considering the harmonized 'report as found' strategy of the current EUCAST and CLSI AST guidelines, such discrepancies in AST interpretation by CLSI and EUCAST warrant a further validation of CBPs. For some drugs, EUCAST 2013 and CLSI 2009/2013 recommend different diameter CBPs, but equal MIC CBPs, e.g. for ampicillin, amoxicillin/clavulanic acid and ertapenem. However, resulting differences in disc diffusion susceptibility rates were statistically significant only for ertapenem and ESBL and AmpC co-producers (46.2% versus 76.9% for EUCAST 2013 and CLSI 2013, respectively, $P=0.014$). MIC/zone correlation data to verify whether CLSI or EUCAST disc diffusion breakpoints correspond better to MIC breakpoints would be of interest, but exceeded the capacity of this study.

A limitation of this study was the local origin of the clinical strains. However, the predominance of CTX-M-type ESBLs as in this study is found worldwide.[9,31] The number of SHV-ESBL and TEM-ESBL types in the present work, however, was relatively low. Thus, further studies are needed to characterize SHV-ESBL and TEM-ESBL AST profiles according to the new CLSI and EUCAST guidelines.

To conclude, the CLSI 2013 and EUCAST 2013 AST guidelines displayed significant differences in disc diffusion susceptibility rates for important drugs such as cefepime, ceftazidime or cefotaxime despite harmonized reporting strategies for ESBLs (and AmpC). Thus, further adjustment of CBPs and correlation of CBPs with MIC data and clinical outcome studies seems warranted.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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