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Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β-lactamase production in clinical Enterobacteriaceae isolates

Silke Polsfuss†, Guido V. Bloemberg†, Jacqueline Giger, Vera Meyer and Michael Hombach*

Institut für Medizinische Mikrobiologie, Universität Zürich, 8006 Zürich, Switzerland

*Corresponding author. Institut für Medizinische Mikrobiologie, Universität Zürich, Gloriastr. 30/32, 8006 Zürich, Switzerland. Tel: +41-44-634-27-00; Fax: +41-634-49-06; E-mail: mhombach@imm.uzh.ch
†These authors contributed equally.

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Objectives: To compare the performance of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI breakpoints following their revision in 2010, for the detection of extended-spectrum β-lactamase (ESBL) production in Enterobacteriaceae.

Methods: 236 well-characterized clinical isolates (including 118 ESBL producers) were investigated by antibiotic disc testing with cefpodoxime, ceftriaxone, cefepime, cefotaxime EUCAST (5 μg/disc), ceftazidime EUCAST (10 μg/disc), cefotaxime CLSI (30 μg/disc) and ceftazidime CLSI (30 μg/disc) with the Kirby–Bauer method. Additionally, synergy phenomena were recorded between amoxicillin/clavulanic acid discs (20/10 μg/disc) and cefepime (30 μg/disc), EUCAST cefotaxime (5 μg/disc), EUCAST ceftazidime (10 μg/disc), CLSI cefotaxime (30 μg/disc) and CLSI ceftazidime [30 μg/disc; disc approximation method (DAM)].

Results: Overall sensitivity of the cefotaxime EUCAST non-susceptible breakpoint equalled sensitivity of the cefotaxime CLSI ESBL screening breakpoint (99.2%). With the ceftazidime EUCAST non-susceptible breakpoint, 27/118 ESBL-producing isolates were not detected, whereas the ceftazidime CLSI ESBL screening breakpoint missed 41/118 ESBL-producing isolates. For cefpodoxime the resistant EUCAST breakpoint showed higher sensitivity for ESBL detection compared with the CLSI ESBL screening breakpoint/disc content (100% versus 98.3%, respectively). Sensitivities of ceftazidime and cefotaxime DAM with CLSI or EUCAST disc contents were comparable (sensitivities ranging from 84.7% to 89.8%). DAM with cefepime displayed the highest overall sensitivity (96.6%). In AmpC-producing isolates, synergy of amoxicillin/clavulanic acid with cefepime showed sensitivity and specificity for ESBL detection of 100% and 97.4%, respectively.

Conclusions: EUCAST non-susceptible breakpoints for ceftazidime and cefpodoxime detect more ESBL-producing Enterobacteriaceae isolates compared with corresponding CLSI ESBL screening breakpoints. Implementation of the cefepime DAM can facilitate ESBL screening, especially in strains producing an AmpC β-lactamase since the test shows high sensitivity and specificity.

Keywords: breakpoints, cut-offs, Gram-negative

Introduction

The prevalence of extended-spectrum β-lactamase (ESBL) production in strains of the Enterobacteriaceae family, such as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp., has been increasing continuously during the past decade in Europe and worldwide.[1–4] The production on ESBLs can lead to life-threatening infections with increased morbidity, mortality and healthcare-associated costs.[5–8]

The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently changed their recommendations concerning the interpretation and reporting of *in vitro* drug susceptibility testing (DST) results. These changes apply to penicillins,

cephalosporins and monobactams, and are based on limited clinical data, pharmacokinetics/pharmacodynamics (PK/PD) properties and MIC distributions.

If the production of an ESBL was confirmed, both institutions until 2009 recommended to edit all *in vitro* susceptible and intermediate DST results for penicillins, cephalosporins and monobactams to 'resistant' (CLSI),[9] or to change interpretative categories 'susceptible' and 'intermediate' to 'intermediate' and 'resistant', respectively (EUCAST).[10] In 2010 EUCAST published inhibition-zone diameter susceptibility breakpoints for cephalosporins in Enterobacteriaceae that were significantly higher than CLSI breakpoints up to 2009.[11] In parallel, CLSI increased zone diameter susceptibility breakpoints as well.[12] Currently, editing of *in vitro* susceptibility test results for β-lactams in ESBL-producing isolates is no longer recommended.[13,14] However, for epidemiological and infection control purposes, screening for ESBL production in Enterobacteriaceae is still useful (CLSI)[13] or even mandatory (EUCAST).[14]

In isolates producing an AmpC-type β-lactamase, phenotypic detection of ESBL production is often hampered by the interference of AmpC with ESBL screening and confirmatory tests leading to false reports to clinicians and, thus, to inadequate therapy.[15,16] In addition, unnecessary time, effort and cost are generated in the laboratories to further study false-positive ESBL screening tests resulting from the low specificity of ESBL screening methods in AmpC-positive isolates.[16] As a tool to counter this problem, cloxacillin-containing Muller–Hinton agar, which inhibits AmpC activity, has been successfully evaluated.[17] Furthermore, cefepime may be the most suitable cephalosporin for ESBL detection in AmpC-positive isolates since it is less affected by AmpC than other third-generation cephalosporins, such as ceftazidime, cefotaxime, cefpodoxime and ceftriaxone.[15]

The rapid advance of molecular methods for the detection of ESBL has raised the question of using these techniques as routine screening methods.[18–20] However, implementation in routine clinical diagnostic laboratories is complex and needs personal resources with specialist qualifications. Moreover, the costs of molecular screening methods for multidrug-resistant isolates are still significantly higher than those for phenotypic methods.[21]

In this study the performance of CLSI screening breakpoints for ESBL detection in clinical isolates were compared with EUCAST breakpoints for a set of phenotypically and genotypically well-characterized Enterobacteriaceae isolates. Many clinical laboratories in Europe are currently adopting the EUCAST system, although a direct comparison of the performance of CLSI and EUCAST standards has not yet been reported. EUCAST does not provide specific screening breakpoints for ESBL; therefore, EUCAST inhibition zone diameter clinical breakpoints for third-generation cephalosporins were applied as determinants for ESBL production.

Methods

Clinical isolates

The 236 Enterobacteriaceae clinical isolates used in this study have previously been systematically characterized for the production of ESBL and/or AmpC-type β-lactamases, using phenotypic and molecular methods (for ESBL, S. Polsfuss, G. V. Bloemberg, J. Giger, V. Meyer, E. C. Bottger and M. Hombach, unpublished results).[22] All isolates had initially been screened for potential ESBL production on the basis of: (i) positive CLSI screening breakpoint values for ESBL for at least one third-generation cephalosporin (cefpodoxime and/or ceftazidime and/or ceftriaxone and/or cefotaxime); and (ii) observation of a synergy zone between amoxicillin/clavulanic acid and cefpodoxime and/or ceftazidime and/or ceftriaxone and/or cefotaxime. For 118/236 isolates ESBL production was confirmed by molecular methods, while another 118/236 isolates were ESBL-negative (see Table 1).

Susceptibility testing

For susceptibility testing the disc diffusion method according to Kirby–Bauer was used. Antibiotic discs (Becton Dickinson, Franklin Lakes, NJ, USA) were selected, and results were interpreted according to the 2011 guidelines of EUCAST and CLSI.[13,14] Screening breakpoint values are shown in Tables 2–4.

Susceptibility testing was performed on Mueller–Hinton agar (bioMérieux, Marcy L'Etoile, France) using McFarland 0.5 with overnight cultures and incubated at 35°C for 16–18 h.

Table 1. Enterobacteriaceae clinical isolates included in the study

	All isolates (%)	ESBL producers	AmpC producers	ESBL and AmpC producers	Non-ESBL, non-AmpC	CTX-M types			SHV ESBL type	TEM ESBL type
						Group I	Group III	Group IV		
All species	236 (100.0)	105	78	13	40					
Escherichia coli	131 (55.6)	86	30	2	13	62	1	16	8	1
Klebsiella pneumoniae	31 (13.1)	16	2	1	12	14		2	1	
Klebsiella oxytoca	17 (7.2)	2	0	0	15			2[a]	1[a]	
Enterobacter cloacae	33 (14.0)	0	24	9	0	6		3		
Citrobacter sp.	1 (0.4)	0	0	1	0				1	
Proteus mirabilis	2 (0.8)	1	1	0	0					1
Others[b]	21 (8.9)	0	21	0	0					

[a]One isolate co-produced both SHV and CTX-M IV.
[b]Others comprised *Enterobacter aerogenes* (8 isolates), *Citrobacter freundii* (7 isolates), *Morganella morganii* (2 isolates), *Serratia marcescens* (2 isolates) and *Salmonella enterica* (2 isolates).

Table 2. Performance parameters of critical diameters and DAM for the detection of ESBL production in 236 Enterobacteriaceae clinical isolates

Method	Breakpoint (mm)	Interpretation/category	Isolates (*N*)	True		False		Sensitivity (%)	Specificity (%)
				positive (*N*)	negative (*N*)	positive (*N*)	negative (*N*)		
Critical diameters									
CTX CLSI (30 μg/disc)	≤27	Screening breakpoint CLSI	236	117	48	70	1	99.2	40.7
CTX EUCAST (5 μg/disc)	<18	EUCAST=R	236	112	63	55	6	94.9	53.4
	<21	EUCAST=I+R	236	117	53	65	1	99.2	44.9
CAZ CLSI (30 μg/disc)	≤22	Screening breakpoint CLSI	236	77	66	52	41	65.3	55.9
CAZ EUCAST (10 μg/disc)	<19	EUCAST=R	236	77	67	51	41	65.3	56.8
	<22	EUCAST=I+R	236	91	52	66	27	77.1	44.1
CRO (30 μg/disc)	≤25	Screening breakpoint CLSI	236	117	49	69	1	99.2	41.5
	<20	EUCAST=R	236	100	68	50	18	84.7	57.6
	<23	EUCAST=I+R	236	113	57	61	5	95.8	48.3
CPD (10 μg/disc)	≤17	Screening breakpoint CLSI	236	116	53	65	2	98.3	44.9
	<21	EUCAST=R (no I category)	236	118	44	74	0	100.0	37.3
FEP (30 μg/disc)	<21	EUCAST=R	236	77	109	9	41	65.3	92.4
	<24	EUCAST=I+R	236	91	93	25	27	77.1	78.8
	≤14	CLSI=R	236	14	117	1	104	11.9	99.2
	≤17	CLSI=I	236	48	114	4	70	40.7	96.6
DAM									
CTX CLSI+AMC			236	106	106	12	12	89.8	89.8
CTX EUCAST+AMC			236	103	110	8	15	87.3	93.2
CAZ CLSI+AMC			236	100	116	2	18	84.7	98.3
CAZ EUCAST+AMC			236	102	116	2	16	86.4	98.3
FEP AMC			236	114	106	12	4	96.6	89.8

AMC, amoxicillin/clavulanic acid; CAZ, ceftazidime; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; DAM, disc approximation method; I, intermediate category; R, resistant category.

Table 3. Performance parameters of critical diameters and DAM for the detection of ESBL production in 91 AmpC-producing Enterobacteriaceae isolates[a]

Method	Breakpoint (mm)	Interpretation/category	Isolates (*N*)	True		False		Sensitivity (%)	Specificity (%)
				positive (*N*)	negative (*N*)	positive (*N*)	negative (*N*)		
Critical diameters									
CTX CLSI (30 μg/disc)	≤27	Screening breakpoint CLSI	91	13	27	51	0	100.0	34.6
CTX EUCAST (5 μg/disc)	<18	EUCAST=R	91	13	30	48	0	100.0	38.5
	<21	EUCAST=I+R	91	13	27	51	0	100.0	34.6
CAZ CLSI (30 μg/disc)	≤22	Screening breakpoint CLSI	91	11	31	47	2	84.6	39.7
CAZ EUCAST (10 μg/disc)	<19	EUCAST=R	91	11	31	47	2	84.6	39.7
	<22	EUCAST=I+R	91	13	27	51	0	100.0	34.6
CRO (30 μg/disc)	≤25	Screening breakpoint CLSI	91	13	32	46	0	100.0	41.0
	<20	EUCAST=R	91	13	36	42	0	100.0	46.2
	<23	EUCAST=I+R	91	13	33	45	0	100.0	42.3
CPD (10 μg/disc)	≤17	Screening breakpoint CLSI	91	13	25	53	0	100.0	32.1
	<21	EUCAST=R (no I category)	91	13	21	57	0	100.0	26.9
FEP (30 μg/disc)	<21	EUCAST=R	91	10	75	3	3	76.9	96.2
	<24	EUCAST=I+R	91	12	64	14	1	92.3	82.1
	≤14	CLSI=R	91	1	78	0	12	7.7	100.0
	≤17	CLSI=I	91	5	78	0	8	38.5	100.0
DAM									
CTX CLSI+AMC			91	10	77	1	3	76.9	98.7
CTX EUCAST+AMC			91	9	78	0	4	69.2	100.0
CAZ CLSI+AMC			91	7	78	0	6	53.8	100.0
CAZ EUCAST+AMC			91	7	78	0	6	53.8	100.0
FEP AMC			91	13	76	2	0	100.0	97.4

AMC, amoxicillin/clavulanic acid; CAZ, ceftazidime; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; DAM, disc approximation method; I, intermediate category; R, resistant category.

[a]Detailed numbers are listed in Table 1.

Table 4. Performance parameters of critical diameters and DAM for the detection of ESBL production in 145 Enterobacteriaceae isolates without AmpC production[a]

Method	Breakpoint (mm)	Interpretation/category	Isolates (*N*)	True		False		Sensitivity (%)	Specificity (%)
				positive (*N*)	negative (*N*)	positive (*N*)	negative (*N*)		
Critical diameters									
CTX CLSI (30 μg/disc)	≤27	Screening breakpoint CLSI	145	104	21	19	1	99.0	52.5
CTX EUCAST (5 μg/disc)	<18	EUCAST=R	145	99	33	7	6	94.3	82.5
	<21	EUCAST=I+R	145	104	26	14	1	99.0	65.0
CAZ CLSI (30 μg/disc)	≤22	Screening breakpoint CLSI	145	66	35	5	39	62.9	87.5
CAZ EUCAST (10 μg/disc)	<19	EUCAST=R	145	66	36	4	39	62.9	90.0
	<22	EUCAST=I+R	145	78	25	15	27	74.3	62.5
CRO (30 μg/disc)	≤25	Screening breakpoint CLSI	145	104	17	23	1	99.0	42.5
	<20	EUCAST=R	145	87	32	8	18	82.9	80.0
	<23	EUCAST=I+R	145	100	24	16	5	95.2	60.0
CPD (10 μg/disc)	≤17	Screening breakpoint CLSI	145	103	28	12	2	98.1	70.0
	<21	EUCAST=R (no I category)	145	105	23	17	0	100.0	57.5
FEP (30 μg/disc)	<21	EUCAST=R	145	67	34	6	38	63.8	85.0
	<24	EUCAST=I+R	145	79	29	11	26	75.2	72.5
	≤14	CLSI=R	145	13	39	1	92	12.4	97.5
	≤17	CLSI=I	145	43	36	4	62	41.0	90.0
DAM									
CTX CLSI+AMC			145	96	29	11	9	91.4	72.5
CTX EUCAST+AMC			145	94	32	8	11	89.5	80.0
CAZ CLSI+AMC			145	93	38	2	12	88.6	95.0
CAZ EUCAST+AMC			145	95	38	2	10	90.5	95.0
FEP AMC			145	101	30	10	4	96.2	75.0

AMC, amoxicillin/clavulanic acid; CAZ, ceftazidime; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; DAM, disc approximation method; I, intermediate category; R, resistant category.

[a]Detailed numbers are listed in Table 1.

Comparison of EUCAST and CLSI ESBL screening breakpoints

CLSI-recommended inhibition zone diameter breakpoints for third-generation cephalosporins for ESBL screening were compared with EUCAST clinical breakpoints for their ability to detect ESBL-producing clinical isolates.[13,14] EUCAST eliminates the intermediate category for some antibiotics, such as cefpodoxime. The resulting single breakpoint was used as the ESBL-screening breakpoint. However, for other third-generation cephalosporins, like ceftazidime and cefotaxime, an intermediate (or indeterminate) zone is retained, but not specifically mentioned in the EUCAST breakpoint tables. In such cases EUCAST provides different breakpoints for clinical resistance and susceptibility. For example, with ceftazidime all isolates showing an inhibition zone ≥22 mm are considered clinically susceptible, and all isolates presenting an inhibition zone <19 mm are considered clinically resistant. Isolates showing an inhibition zone of 19–21 mm are not specifically categorized in the EUCAST guidelines, and the intermediate zone is only implied. A non-susceptible breakpoint was deduced from the EUCAST susceptible breakpoint, e.g. if EUCAST defines a ceftazidime inhibition zone ≥22 mm as susceptible, a breakpoint of <22 mm was referred to in this publication as the corresponding non-susceptible breakpoint. Non-susceptible isolates in this definition include, therefore, all intermediate and resistant isolates (see Tables 2–4).

Disc approximation method (DAM)

DAM with amoxicillin/clavulanic acid was conducted as described.[23] Synergy phenomena were recorded between amoxicillin/clavulanic acid discs (20/10 μg/disc) and/or cefepime (30 μg/disc), and/or EUCAST cefotaxime (5 μg/disc), and/or EUCAST ceftazidime (10 μg/disc), and/or CLSI cefotaxime (30 μg/disc) and/or CLSI ceftazidime (30 μg/disc) discs. Antibiotic discs were placed 30 mm apart (centre to centre). β-Lactam inhibitor-mediated enhancement of a third-generation cephalosporin inhibition zone was interpreted as synergy positive. Molecular methods were considered the gold standard for the calculation of performance parameters.

Results

Comparison of CLSI and EUCAST inhibition zone breakpoints for third-generation cephalosporins

For cefotaxime, overall sensitivity of EUCAST non-susceptible breakpoints for ESBL with corresponding EUCAST loaded discs equalled those of CLSI ESBL screening breakpoints/loads (sensitivity 99.2%, see also Table 2). If the EUCAST resistant breakpoint was applied, sensitivity decreased from 99.2% (1/118 ESBL-producing isolates not detected) to 94.9% (6/118). For ceftazidime, sensitivity of the EUCAST non-susceptible breakpoint for ESBL with corresponding EUCAST disc content was higher than that for the CLSI ESBL screening breakpoint/load (77.1% and 65.3% for EUCAST and CLSI, respectively). If the EUCAST resistant breakpoint was applied, sensitivity equalled that of the CLSI breakpoint/disc content. When the EUCAST non-susceptible breakpoint for ceftriaxone was used, sensitivity for ESBL detection compared with the CLSI ESBL screening breakpoint/disc content was lower (95.8% versus 99.2%, respectively). For cefpodoxime the non-susceptible EUCAST breakpoint showed higher sensitivity for ESBL detection compared with the CLSI ESBL screening breakpoint/load (100% versus 98.3%, respectively).

In AmpC-producing isolates ($n=91$), of which 13 were ESBL positive (see Table 1), all diameter breakpoints showed low specificities for ESBL detection, except the EUCAST breakpoints for cefepime, which displayed a specificity of 82.1% and 96.2% for the non-susceptible and resistant breakpoints, respectively (Table 3).

In non-AmpC-producing isolates ($n=145$) the EUCAST non-susceptible breakpoint for cefpodoxime was the most sensitive single marker for ESBL production (sensitivity 100%, see Table 4). In comparison, the corresponding CLSI breakpoint showed a sensitivity of 98.1% (2 out of 105 ESBL-producing isolates not detected). Sensitivities of EUCAST and CLSI breakpoints for cefotaxime were equal (99.0%, 1 out of 105 ESBL positive isolates not detected). For third-generation cephalosporins, only the EUCAST non-susceptible breakpoint for ceftriaxone showed lower sensitivity in non-AmpC-producing isolates than the CLSI ESBL screening breakpoint (sensitivities of 95.2% versus 99.0%, respectively).

Comparison of DAM with CLSI and EUCAST disc contents

Sensitivities of DAM with amoxicillin/clavulanic acid (clavulanic acid serving as the ESBL inhibitor) and ceftazidime or cefotaxime discs with CLSI or EUCAST disc contents, respectively, were similar (ranging from 84.7% to 89.8%, see Table 2). Considering all isolates independent of the production of an AmpC-type β-lactamase, DAM with amoxicillin/clavulanic acid and cefepime displayed the highest sensitivity (96.6%). The other combinations had sensitivities less than 90%.

In AmpC-producing isolates, synergy of amoxicillin/clavulanic-acid with cefepime showed a sensitivity and specificity of 100% and 97.9%, respectively. The other DAMs in AmpC-producing isolates showed low sensitivity (Table 3).

Discussion

In 2010 EUCAST and CLSI changed their guidelines concerning ESBL detection and interpretation.[11,12] Reporting of penicillins and cephalosporins as resistant, independent of *in vitro* results, is no longer recommended. However, detection of ESBL is still considered useful (CLSI, 2011)[13] or even mandatory (EUCAST, 2011)[14] for epidemiological purposes. Additionally, it remains controversial as to whether the presence of ESBL-producing bacterial strains alone is an independent risk factor that may influence the selection of an adequate therapy.[24–28] CLSI inhibition zone screening breakpoints for ESBL have been evaluated in several studies, as it has for DAM.[26,29] The current adoption of the new EUCAST guidelines in Europe raises the question of how sensitive and specific EUCAST clinical breakpoints are for third-generation cephalosporins, in comparison with CLSI values, for the detection of ESBL.

Overall, this study shows that EUCAST non-susceptible breakpoints for cefotaxime and ceftazidime with corresponding EUCAST disc contents may be used without loss of performance compared with CLSI ESBL screening breakpoints. Using the EUCAST non-susceptible breakpoints for ceftriaxone will slightly decrease sensitivity compared with the CLSI ESBL screening breakpoint. However, using the EUCAST non-susceptible breakpoint for cefpodoxime will result in a sensitivity of 100% with specificity marginally decreased compared with the CLSI ESBL screening breakpoint.

Furthermore, EUCAST recommends lower antibiotic disc contents for ceftazidime and cefotaxime compared with CLSI. Evaluation of the influence of the new disc contents on the performance of the commonly applied DAM for ESBL detection and confirmation was another aim of this study. The sensitivities of DAM with ceftazidime and cefotaxime were found to be independent of disc contents of CLSI and EUCAST, respectively; however, these are dispensable for routine use, since other markers showed a higher sensitivity. Cefepime DAM and the EUCAST resistant breakpoint for cefpodoxime proved to be the most sensitive markers for screening of potential ESBL producers. Notably, cefepime synergy showed a sensitivity of 100% in isolates producing chromosomally encoded or plasmid-encoded AmpC β-lactamases. Our results are in agreement with other studies that found cefepime to be the most suitable substance for screening and confirmation of ESBL-producing isolates that also produce AmpC.[30,31] Thus, for AmpC-positive isolates such as *Enterobacter* spp., but also for isolates with plasmid-encoded AmpC, cefepime DAM may be used as a sole screening marker for ESBL. Taking into account the high specificity (97.9%) of the cefepime DAM, positive isolates may even be reported as ESBL-positive without further confirmation.

In conclusion, changing from CLSI to EUCAST breakpoints for ESBL detection will retain or even enhance sensitivity for ESBL detection. The fear that large proportions of ESBL-producing organisms will be reported susceptible to third- and fourth-generation cephalosporins could not be substantiated.

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Transparency declarations

None to declare.

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