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Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli

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Objectives: The aim of this study was to analyse the effects of clinical breakpoint changes in CLSI 2010 and 2011 guidelines and EUCAST 2011 guidelines on antibiotic susceptibility testing (AST) reports.

Methods: In total, 3713 non-duplicate clinical isolates of Enterobacteriaceae, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* were analysed. Inhibition zone diameters were determined for β-lactams, carbapenems, fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole. CLSI 2009–11 and EUCAST 2011 clinical breakpoints were applied.

Results: Changes in resistance as defined per the guidelines affected individual species and drug classes differently. The cefepime resistance rate in *Escherichia coli* and *Enterobacter cloacae* increased from 2.1% and 1.3% to 8.2% and 6.9%, respectively, applying CLSI 2009–11 versus EUCAST 2011 guidelines. Ertapenem resistance rates in *E. cloacae* increased from 2.6% with CLSI 2009 to 7.2% for CLSI 2010 and 2011, and to 10.1% when applying EUCAST 2011. Cefepime and meropenem resistance rates in *P. aeruginosa* increased from 12.2% and 20.6% to 19.8% and 27.7%, respectively, comparing CLSI 2009–11 with EUCAST 2011. Tobramycin and gentamicin resistance rates in *A. baumannii* increased from 15.9% and 25.4% to 34.9% and 44.4% applying CLSI 2009–11 versus EUCAST 2011.

Conclusions: Higher resistance rates reported due to breakpoint changes in CLSI and EUCAST guidelines will result in increasing numbers of Gram-negative bacilli reported as multidrug resistant. AST reports classifying amoxicillin/clavulanic acid, cefepime or carbapenem resistance will lead clinicians to use alternative agents. Upon implementation of the EUCAST guidelines, laboratories should be aware of the implications of modified drug susceptibility testing reports on antibiotic prescription policies.

Keywords: resistance rates, Enterobacteriaceae, non-fermenting Gram-negative bacilli

Introduction

The European Committee for Antimicrobial Susceptibility Testing (EUCAST) has published guidelines for performance and interpretation of antibiotic susceptibility testing (AST).[1] Laboratories in Europe are encouraged to change their AST system to facilitate comparability of AST results. The CLSI has updated its recommendations for interpretation of *in vitro* drug susceptibility testing results in the CLSI 2010 and 2011 guidelines; this update is based on clinical data, pharmacokinetic–pharmacodynamic (PK–PD) properties and MIC distributions, in part adopting EUCAST strategies.[2,3]

In particular for Gram-negative bacilli, significant changes in AST interpretation are notable. Inhibition zone diameter breakpoints for Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* defining susceptibility to third-generation cephalosporins, carbapenems and fluoroquinolones are significantly higher in the EUCAST 2011 version compared with CLSI 2009 breakpoints.[1,4] Most recently, CLSI recommended higher zone diameter susceptibility breakpoints for third-generation cephalosporins and carbapenems in its 2010 and 2011 updates, while breakpoints for fluoroquinolones were not changed.[2,3]

The main difference between EUCAST and CLSI is the elimination, or at least a reduction of, the intermediate AST category. EUCAST has, in part, removed the intermediate zone, because PKs/PDs and limited clinical data do not support an intermediate category. Consequently, AST reports are simplified by reporting an isolate as either susceptible or resistant. This strategy will change AST reports, mostly by reporting isolates as resistant that were formerly considered intermediate. Additionally, higher

diameter breakpoints will enhance increasing resistance rates reported for Gram-negative bacilli.[5,6] The definition of multidrug-resistant bacteria is commonly based on the presence of resistance to antibiotic agents from several different antibiotic classes.[7] A change in resistance rates of individual classes of antibiotic drugs will therefore inevitably influence the rate of multidrug-resistant isolates reported by the microbiological laboratory.

Adoption of new guidelines and breakpoints can have a significant effect on AST reports of diagnostic laboratories, with concomitant changes in antibiotic prescription by clinicians.[8] This study aimed at comparing the new standards with respect to changes in epidemiological parameters, i.e. reported rates of susceptibility and resistance of Gram-negative bacteria when applying CLSI 2009–11 and EUCAST 2011. The results of this study will support clinical microbiological laboratories in correct interpretation and antibiotic therapy recommendations to clinicians during the transition phase from the CLSI system to the EUCAST system. Close interaction with and information from clinicians is needed to avoid uncertainties in the interpretation of changes in AST reports.

Methods

Clinical isolates

In total, 3713 non-duplicate clinical isolates of Gram-negative bacilli isolated during a 17 month period from January 2010 to May 2011 in our clinical microbiological laboratory were included in the study. Our clinical laboratory primarily serves the 850 bed university hospital of Zurich. The 3713 isolates comprised 2834 Enterobacteriaceae (1360 *Escherichia coli*, 424 *Klebsiella pneumoniae*, 159 *Klebsiella oxytoca*, 306 *Enterobacter cloacae*, 75 *Enterobacter aerogenes*, 87 *Citrobacter freundii*, 68 *Citrobacter koseri*, 73 *Serratia marcescens*, 63 *Morganella morganii*, 47 *Proteus vulgaris*, 148 *Proteus mirabilis* and 24 *Hafnia alvei*), 656 *P. aeruginosa*, 160 *Stenotrophomonas maltophilia* and 63 *A. baumannii*.

Susceptibility testing

For susceptibility testing, the disc diffusion method according to Kirby–Bauer was used. Antibiotic discs were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). Susceptibility testing was done on Mueller–Hinton agar (bioMérieux, Marcy l'Étoile, France) using McFarland 0.5 from overnight cultures followed by incubation at 35°C for 16–18 h.

Inhibition zone diameters were determined and recorded in the automated Sirweb/Sirscan system (i2a, Montpellier, France). Inhibition zone diameters were interpreted according to EUCAST 2011 and CLSI 2009–11 guidelines, respectively (see Tables 1 and 2).

Comparison of CLSI 2009–11 and EUCAST 2011 breakpoints

Inhibition zone diameters were used to compare CLSI 2009–11 recommended diameter breakpoints with EUCAST 2011 clinical breakpoints for Enterobacteriaceae. For *P. aeruginosa*, *S. maltophilia* and *A. baumannii*, inhibition zone diameter breakpoints as recommended in the CLSI 2009–11 AST guidelines were compared with EUCAST 2011 clinical breakpoints, since CLSI did not change breakpoints for these species from 2009 to 2011. EUCAST eliminates, in part, the intermediate category for some antibiotics such as amoxicillin/clavulanic acid. For other drugs such as ceftriaxone, an intermediate (or indeterminate) zone is kept but not specifically mentioned in the EUCAST breakpoint tables. Tables 3 and 4 list CLSI

Table 1. Clinical breakpoint values of CLSI 2009–11 and EUCAST 2011 for AST of Enterobacteriaceae

	Clinical breakpoints (mm)											
	CLSI 2009			CLSI 2010[a]			CLSI 2011[a]			EUCAST 2011[b]		
Drug	S	I	R	S	I	R	S	I	R	S	I	R
ampicillin	≥17	14–16	≤13							≥14		<14
amoxicillin/clavulanic acid	≥18	14–17	≤13							≥17		<17
cefuroxime	≥18	15–17	≤14							≥18		<18
cefoxitin	≥18	15–17	≤14							≥19		<19
cefpodoxime	≥21	18–20	≤17							≥21		<21
ceftriaxone	≥21	14–20	≤13	≥23	20–22	≤19				≥23	20–22	<20
cefepime	≥18	15–17	≤14							≥24	21–23	<21
meropenem	≥16	14–15	≤13				≥23	20–22	≤19	≥22	16–21	<16
imipenem	≥16	14–15	≤13				≥23	20–22	≤19	≥21	15–20	<15
ertapenem	≥19	16–18	≤15				≥23	20–22	≤19	≥25	20–24	<20
tobramycin	≥15	13–14	≤12							≥16	14–15	<14
amikacin	≥17	15–16	≤14							≥16	14–15	<14
gentamicin	≥15	13–14	≤12							≥17	15–16	<15
ciprofloxacin	≥21	16–20	≤15							≥22	19–21	<19
levofloxacin	≥17	14–16	≤13							≥22	19–21	<19
norfloxacin	≥17	13–16	≤12							≥22	19–21	<19
trimethoprim/sulfamethoxazole	≥16	11–15	≤10							≥16	13–15	<13

S, susceptible; I, intermediate; R, resistant.
[a]CLSI categories without value: interpretation guidelines were not changed compared with the previous version.
[b]EUCAST categories without value: interpretative category does not exist (intermediate category only).

Table 2. Clinical breakpoint values of CLSI 2009–11 and EUCAST 2011 for AST of glucose non-fermenting Gram-negative bacilli

	Clinical breakpoints (mm)					
	CLSI 2009–11[a]			EUCAST 2011[b]		
Species/drug	S	I	R	S	I	R
S. maltophilia						
trimethoprim/sulfamethoxazole	≥16	11–15	≤10	≥16		<16
P. aeruginosa						
cefepime	≥18	15–17	≤14	≥18		<18
imipenem	≥16	14–15	≤13	≥20	18–19	<18
meropenem	≥16	14–15	≤13	≥24	18–23	<18
tobramycin	≥15	13–14	≤12	≥16		<16
amikacin	≥17	15–16	≤14	≥18	15–17	<15
gentamicin	≥15	13–14	≤12	≥15		<15
ciprofloxacin	≥21	16–20	≤15	≥25	20–24	<20
levofloxacin	≥17	14–16	≤13	≥20	17–19	<15
A. baumannii						
imipenem	≥16	14–15	≤13	≥23	18–22	<18
meropenem	≥16	14–15	≤13	≥21	16–20	<16
tobramycin	≥15	13–14	≤12	≥17		<17
amikacin	≥17	15–16	≤14	≥18	16–17	<16
gentamicin	≥15	13–14	≤12	≥17		<17
ciprofloxacin	≥21	16–20	≤15	≥21		<21
levofloxacin	≥17	14–16	≤13	≥21	19–20	<19

S, susceptible; I, intermediate; R, resistant.
[a]CLSI 2009–11 guidelines were not changed.
[b]EUCAST categories without value: interpretative category does not exist (intermediate category only).

2009–11 and EUCAST 2011 clinical breakpoints and note the EUCAST inferred intermediate zone. For certain drugs, e.g. ceftazidime, cefotaxime and piperacillin/tazobactam, the EUCAST guidelines contain other antibiotic disc loads than the CLSI guidelines.[1] Only drugs were included in this comparison for which the same antibiotic disc loads are recommended in the CLSI and EUCAST system, i.e. ampicillin, amoxicillin/clavulanic acid, cefuroxime, cefoxitin, cefpodoxime, ceftriaxone, cefepime, meropenem, imipenem, ertapenem, tobramycin, amikacin, gentamicin, ciprofloxacin, levofloxacin, norfloxacin and trimethoprim/sulfamethoxazole.

Results

Enterobacteriaceae species

In aggregate, resistance rates to cefepime, ceftriaxone and ertapenem increased from 1.6%, 9.5% and 1.0% (CLSI 2009) to 6.3%, 14.2% and 1.6%/2.2% (CLSI 2010/2011 and EUCAST 2011, respectively; see Table 3).

Due to elimination of the intermediate zone for amoxicillin/clavulanic acid by EUCAST 2011, the resistance rate (corrected for natural resistance in several species, e.g. *Enterobacter* spp., *Citrobacter freundii*) increased from 6.0% to 20.6% comparing CLSI 2009–11 with EUCAST 2011.

The overall resistance rates to fluoroquinolones moderately increased, e.g. the resistance rate to levofloxacin increased from 14.7% to 18.3% comparing CLSI 2009–11 with EUCAST 2011 (see Table 3). Increased fluoroquinolone resistance was noted for all Enterobacteriaceae species except *E. coli*, *C. koseri* and *P. vulgaris*, e.g. in *E. coli* resistance rates to fluoroquinolones were similar comparing CLSI 2009–11 with EUCAST 2011 (Table 3). The most important species-specific changes are described below.

E. coli

As observed in the trends for Enterobacteriaceae spp., resistance rates to cefepime and ceftriaxone increased from 2.1% (CLSI 2009) to 8.2% (EUCAST 2011) and from 9.9% (CLSI 2009) to 13.7% (CLSI 2010/11 and EUCAST 2011), respectively. Due to elimination of the intermediate zone, the resistance rate for amoxicillin/clavulanic acid increased from 6.6% to 23.7% comparing CLSI 2009–11 with EUCAST 2011 (see Table 3).

K. pneumoniae

Resistance rates to fluoroquinolones increased from 12.0%, 10.1% and 13.2% to 17.5%, 15.8% and 19.8% for ciprofloxacin, levofloxacin and norfloxacin, respectively, comparing CLSI 2009–11 with EUCAST 2011.

K. oxytoca

The resistance rate to ceftriaxone markedly increased from 0.6% (CLSI 2009) to 10.1% (CLSI 2010/2011 and EUCAST 2011).

E. cloacae, E. aerogenes and C. freundii

The resistance rate to cefepime in *E. cloacae and E. aerogenes* increased from 1.3% and 0% to 6.9% and 2.6% comparing CLSI 2009–11 with EUCAST 2011.

The resistance rate to ertapenem in *E. cloacae*, *E. aerogenes* and *C. freundii* increased from 2.6%, 11.7% and 0% with CLSI 2009/2010 to 7.2%, 11.7% and 0% for CLSI 2011 and to 10.1%, 14.3% and 1.1% with EUCAST 2011, respectively. Concomitantly, the susceptibility rate to ertapenem in *E. cloacae* decreased from 94.8% (CLSI 2009/2010) to 78.4% (CLSI 2011 and EUCAST 2011).

Resistance rates to fluoroquinolones in *E. cloacae* increased from 2.6%, 2.3% and 0% to 6.0%, 6.5% and 1.8% for ciprofloxacin, levofloxacin and norfloxacin, respectively, comparing CLSI 2009–11 with EUCAST 2011. For *E. aerogenes* and *C. freundii* the same trends as in *E. cloacae* were noted.

S. marcescens

The resistance rate for ciprofloxacin remained at 0%. However, resistance rates to levofloxacin and norfloxacin increased from 0% to 1.4% and 5.3% when applying CLSI 2009–11 standards compared with EUCAST 2011 standards, respectively.

C. koseri, M. morganii, P. mirabilis, P. vulgaris and H. alvei

Only marginal changes in resistance and susceptibility rates were demonstrated for *C. koseri*, *M. morganii*, *P. mirabilis*, *P. vulgaris* and *H. alvei* (Table 3).

Table 3. Assignment of Enterobacteriaceae clinical isolates to interpretative category according to CLSI 2009–11 and EUCAST 2011[a]

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories											
	CLSI 2009			CLSI 2010			CLSI 2011			EUCAST 2011		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
E. coli												
ampicillin	38.7	8.5	56.8							43.2		56.8
amoxicillin/clavulanic acid	76.3	15.5	6.6							76.3		23.7
cefuroxime	82.8	2.5	14.7							82.8		17.2
cefoxitin	94.9	2.1	3.4							94.2		5.5
cefpodoxime	83.7	0.4	15.9							83.7		16.3
ceftriaxone	85.4	4.3	9.9	84.8	1.2	13.7				84.8	1.2	13.7
cefepime	94.0	3.3	2.1							88.8	2.5	8.2
meropenem	99.8	0.0	0.0				99.4	0.4	0.0	99.5	0.3	0.0
imipenem	99.9	0.0	0.0				99.5	0.4	0.1	99.8	0.2	0.0
ertapenem	99.1	0.1	0.1				98.8	0.2	0.3	98.7	0.2	0.4
tobramycin	84.0	2.8	13.2							83.0	2.2	14.8
amikacin	97.1	1.8	1.1							98.1	1.0	1.0
gentamicin	86.6	0.4	13.0							86.2	0.5	13.4
ciprofloxacin	70.8	1.6	27.5							70.3	1.4	28.3
levofloxacin	72.2	2.4	25.2							68.7	2.4	28.8
norfloxacin	75.5	0.9	23.4							74.2	0.7	24.9
trimethoprim/sulfamethoxazole	63.5	0.8	35.6							63.5	0.3	36.2
K. pneumoniae												
amoxicillin/clavulanic acid	80.4	9.8	5.3							80.4		19.6
cefuroxime	78.9	4.1	17.0							78.9		21.1
cefoxitin	89.5	7.9	4.5							87.4		12.4
cefpodoxime	83.7	1.0	15.3							83.7		16.3
ceftriaxone	85.8	3.8	10.1	84.4	1.7	13.7				84.4	1.7	13.7
cefepime	94.3	2.6	2.9							87.3	3.1	9.3
meropenem	97.6	0.7	1.4				97.6	0.0	2.1	97.6	0.0	2.1
imipenem	97.6	0.5	1.7				97.1	0.5	2.2	97.6	0.5	1.7
ertapenem	97.6	0.0	2.1				97.4	0.2	2.1	95.5	1.9	2.4
tobramycin	85.1	0.7	14.2							84.7	0.7	14.6
amikacin	96.5	0.9	2.6							97.4	0.9	1.7
gentamicin	91.4	0.5	8.1							90.4	1.0	8.6
ciprofloxacin	79.1	8.9	12.0							77.6	4.8	17.5
levofloxacin	87.7	2.1	10.1							74.8	9.4	15.8
norfloxacin	81.0	5.8	13.2							74.4	5.8	19.8
trimethoprim/sulfamethoxazole	74.3	1.7	24.0							74.3	1.7	24.0
K. oxytoca												
amoxicillin/clavulanic acid	82.9	6.3	3.8							82.9		17.1
cefuroxime	80.4	1.9	17.7							80.4		19.6
cefoxitin	98.7	1.3	0.6							98.1		1.9
cefpodoxime	90.5	1.9	7.6							90.5		9.5
ceftriaxone	88.7	10.7	0.6	86.8	3.1	10.1				86.8	3.1	10.1
cefepime	99.4	0.6	0.0							97.5	1.9	0.6
meropenem	99.4	0.0	0.0				99.4	0.0	0.0	99.4	0.0	0.0
imipenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
ertapenem	99.4	0.0	0.0				99.4	0.0	0.0	99.4	0.0	0.0
tobramycin	96.2	0.6	3.1							96.2	0.6	3.1
amikacin	99.4	0.0	0.6							99.4	0.6	0.0
gentamicin	96.2	0.6	3.2							94.9	1.3	3.8

Continued

Table 3. *Continued*

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories											
	CLSI 2009			CLSI 2010			CLSI 2011			EUCAST 2011		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
ciprofloxacin	96.8	3.2	0.0							94.9	3.2	1.9
levofloxacin	98.7	0.6	0.6							94.3	3.8	1.9
norfloxacin	100	0.0	0.0							100	0.0	0.0
trimethoprim/sulfamethoxazole	91.8	0.0	8.2							91.8	0.0	8.2
E. cloacae												
cefuroxime	64.0	2.3	33.7							64.0		36.0
cefpodoxime	55.8	10.2	34.0							55.8		44.2
ceftriaxone	70.2	11.1	18.7	68.5	3.6	27.9				68.5	3.6	27.9
cefepime	95.7	3.0	1.3							84.2	8.9	6.9
meropenem	98.0	0.7	1.3				97.1	0.0	2.9	97.1	1.0	2.0
imipenem	98.7	0.3	1.0				93.1	5.0	2.0	97.7	1.0	1.3
ertapenem	94.8	2.6	2.6				85.6	7.2	7.2	78.4	11.4	10.1
tobramycin	90.2	0.3	9.5							89.5	0.7	9.8
amikacin	98.0	1.3	0.7							98.4	1.3	0.3
gentamicin	92.4	4.3	3.3							90.8	1.6	7.6
ciprofloxacin	91.1	6.3	2.6							90.1	4.0	6.0
levofloxacin	95.8	2.0	2.3							90.6	2.9	6.5
norfloxacin	98.2	1.8	0.0							98.2	0.0	1.8
trimethoprim/sulfamethoxazole	87.5	2.3	10.2							87.5	1.0	11.6
E. aerogenes												
cefuroxime	59.7	1.3	39.0							59.7		40.3
cefpodoxime	59.7	0.0	40.3							59.7		40.3
ceftriaxone	68.8	18.2	13.0	63.6	6.5	29.9				63.6	6.5	29.9
cefepime	97.4	2.6	0.0							92.2	5.2	2.6
meropenem	97.4	2.6	0.0				88.3	0.0	11.7	88.3	9.1	2.6
imipenem	96.1	3.9	0.0				81.8	9.1	9.1	88.3	10.4	1.3
ertapenem	88.3	0.0	11.7				84.4	3.9	11.7	76.6	9.1	14.3
tobramycin	98.7	0.0	1.3							98.7	0.0	1.3
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	98.7	0.0	1.3							96.0	2.7	1.3
ciprofloxacin	92.0	4.0	4.0							90.7	2.7	6.7
levofloxacin	96.0	1.3	2.7							90.7	4.0	5.3
norfloxacin	100	0.0	0.0							100	0.0	0.0
trimethoprim/sulfamethoxazole	96.1	2.6	1.3							96.1	2.6	1.3
C. freundii												
cefuroxime	69.0	4.6	26.4							69.0		31.0
cefpodoxime	49.4	13.8	36.8							49.4		50.6
ceftriaxone	74.7	6.9	18.4	71.3	4.6	24.1				71.3	4.6	24.1
cefepime	100	0.0	0.0							95.4	4.6	0.0
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				93.1	6.9	0.0	100	0.0	0.0
ertapenem	97.7	0.0	0.0				95.4	2.3	0.0	88.5	8.0	1.1
tobramycin	98.9	0.0	1.1							97.7	1.1	1.1
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	100	0.0	0.0							97.7	2.3	0.0
ciprofloxacin	93.1	2.3	4.6							93.1	0.0	6.9
levofloxacin	94.3	1.1	4.6							90.8	2.3	6.9
norfloxacin	94.1	5.9	0.0							94.1	0.0	5.9

Continued

Table 3. *Continued*

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories											
	CLSI 2009			CLSI 2010			CLSI 2011			EUCAST 2011		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
trimethoprim/sulfamethoxazole	90.8	0.0	9.2							90.8	0.0	9.2
C. koseri												
amoxicillin/clavulanic acid	95.6	0.0	4.4							95.6		4.4
cefuroxime	88.4	7.2	4.3							88.4		11.6
cefoxitin	97.1	2.9	2.9							94.1		5.9
cefpodoxime	98.5	0.0	1.5							98.5		1.5
ceftriaxone	100	0.0	0.0	100	0.0	0.0				100	0.0	0.0
cefepime	100	0.0	0.0							100	0.0	0.0
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				98.5	1.5	0.0	100	0.0	0.0
ertapenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
tobramycin	100	0.0	0.0							100	0.0	0.0
amikacin	98.5	1.5	0.0							100	0.0	0.0
gentamicin	100	0.0	0.0							100	0.0	0.0
ciprofloxacin	100	0.0	0.0							98.5	1.5	0.0
levofloxacin	100	0.0	0.0							100	0.0	0.0
norfloxacin	100	0.0	0.0							100	0.0	0.0
trimethoprim/sulfamethoxazole	98.5	0.0	1.5							98.5	0.0	1.5
S. marcescens												
cefpodoxime	87.7	4.1	8.2							87.7		12.3
ceftriaxone	94.5	4.1	1.4	93.2	2.7	4.1				93.2	2.7	4.1
cefepime	100	0.0	0.0							98.6	1.4	0.0
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				95.9	2.7	1.4	98.6	1.4	0.0
ertapenem	97.3	1.4	0.0				97.3	0.0	1.4	97.3	0.0	1.4
tobramycin	95.9	2.7	1.4							91.8	5.5	2.7
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	100	0.0	0.0							100	0.0	0.0
ciprofloxacin	94.5	5.5	0.0							90.4	9.6	0.0
levofloxacin	98.6	1.4	0.0							90.4	8.2	1.4
norfloxacin	100	0.0	0.0							89.5	5.3	5.3
trimethoprim/sulfamethoxazole	94.5	1.4	4.1							94.5	1.4	4.1
M. morganii												
cefpodoxime	87.3	1.6	11.1							87.3		12.7
ceftriaxone	98.4	0.0	1.6	98.4	0.0	1.6				98.4	0.0	1.6
cefepime	100	0.0	0.0							98.4	1.6	0.0
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				74.6	23.8	1.6	95.2	4.8	0.0
ertapenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
tobramycin	95.2	3.2	1.6							93.7	4.8	1.6
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	88.9	0.0	11.1							87.3	1.6	11.1
ciprofloxacin	87.3	3.2	9.5							87.3	0.0	12.7
levofloxacin	90.5	0.0	9.5							84.1	3.2	12.7
norfloxacin	94.7	0.0	5.3							89.5	5.3	5.3
trimethoprim/sulfamethoxazole	79.4	1.6	19.0							79.4	1.6	19.0

Continued

Table 3. *Continued*

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories											
	CLSI 2009			CLSI 2010			CLSI 2011			EUCAST 2011		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
P. vulgaris												
amoxicillin/clavulanic acid	93.6	2.1	4.3							93.6		6.4
cefoxitin	100	4.3	0.0							95.7		4.3
cefpodoxime	95.7	0.0	4.3							95.7		4.3
ceftriaxone	97.9	0.0	2.1	97.9	0.0	2.1				97.9	0.0	2.1
cefepime	97.9	2.1	0.0							97.9	0.0	2.1
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				87.2	10.6	2.1	97.9	2.1	0.0
ertapenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
tobramycin	100	0.0	0.0							100	0.0	0.0
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	100	0.0	0.0							100	0.0	0.0
ciprofloxacin	100	0.0	0.0							100	0.0	0.0
levofloxacin	100	0.0	0.0							100	0.0	0.0
norfloxacin	100	0.0	0.0							100	0.0	0.0
trimethoprim/sulfamethoxazole	91.5	0.0	8.5							91.5	0.0	8.5
P. mirabilis												
ampicillin	63.5	4.5	35.1							64.9		35.1
amoxicillin/clavulanic acid	92.6	2.7	6.1							92.6		7.4
cefuroxime	94.6	1.4	4.1							94.6		5.4
cefoxitin	97.3	1.4	1.4							97.3		2.7
cefpodoxime	95.3	0.0	4.7							95.3		4.7
ceftriaxone	94.6	1.4	0.7	94.6	0.0	2.0				94.6	0.0	2.0
cefepime	99.3	0.0	0.7							98.6	0.0	1.4
meropenem	100	0.0	0.0				99.3	0.0	0.7	99.3	0.7	0.0
imipenem	99.3	0.0	0.7				94.5	4.8	0.7	98.6	0.7	0.7
ertapenem	98.0	0.0	0.7				98.0	0.0	0.7	97.3	0.7	0.7
tobramycin	92.6	0.7	6.8							89.9	3.4	6.8
amikacin	97.3	1.4	1.4							97.3	2.0	0.7
gentamicin	85.8	1.4	12.8							84.5	1.4	14.2
ciprofloxacin	85.1	5.4	8.8							83.8	2.7	12.8
levofloxacin	90.5	2.7	6.8							84.5	4.1	11.5
norfloxacin	86.1	8.3	2.8							80.6	5.6	11.1
trimethoprim/sulfamethoxazole	75.0	1.4	23.6							75.0	0.7	24.3
H. alvei												
cefuroxime	62.5	4.2	33.3							62.5		37.5
cefpodoxime	70.8	8.3	20.8							70.8		29.2
ceftriaxone	87.5	0.0	12.5	79.2	8.3	12.5				79.2	8.3	12.5
cefepime	100	0.0	0.0							100	0.0	0.0
meropenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
imipenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
ertapenem	100	0.0	0.0				100	0.0	0.0	100	0.0	0.0
tobramycin	100	0.0	0.0							100	0.0	0.0
amikacin	100	0.0	0.0							100	0.0	0.0
gentamicin	100	0.0	0.0							100	0.0	0.0
ciprofloxacin	100	0.0	0.0							100	0.0	0.0
levofloxacin	100	0.0	0.0							100	0.0	0.0

Continued

Table 3. *Continued*

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories											
	CLSI 2009			CLSI 2010			CLSI 2011			EUCAST 2011		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
norfloxacin	100	0.0	0.0							100	0.0	0.0
trimethoprim/sulfamethoxazole	100	0.0	0.0							100	0.0	0.0
All Enterobacteriaceae species[b]												
ampicillin	41.2	4.2	54.6							45.4		54.6
amoxicillin/clavulanic acid	79.4	12.3	6.0							79.4		20.6
cefuroxime	77.2	3.8	19.1							77.2		22.8
cefoxitin	94.7	3.2	3.2							93.6		6.4
cefpodoxime	80.6	2.2	17.2							80.6		19.4
ceftriaxone	85.2	5.3	9.5	84.0	1.8	14.2				84.0	1.8	14.2
cefepime	95.9	2.4	1.6							90.6	3.1	6.3
meropenem	99.4	0.2	0.4				98.8	0.2	1.0	98.9	0.5	0.6
imipenem	99.4	0.2	0.4				96.7	2.3	1.0	98.8	0.8	0.5
ertapenem	98.6	0.4	1.0				97.3	1.1	1.6	95.7	2.2	2.2
tobramycin	88.2	1.7	10.1							87.2	1.7	11.0
amikacin	97.6	1.3	1.1							98.3	0.9	0.8
gentamicin	90.3	0.8	8.9							89.4	0.9	9.7
ciprofloxacin	80.3	3.6	16.1							79.3	2.5	18.2
levofloxacin	83.3	2.0	14.7							78.0	3.7	18.3
norfloxacin	82.4	2.0	15.6							80.0	1.8	18.3
trimethoprim/sulfamethoxazole	74.5	1.1	24.4							74.5	0.7	24.8

S, susceptible; I, intermediate; R, resistant.
[a]Drugs to which species are naturally resistant are not listed. CLSI categories without value: interpretation guidelines were not changed compared with the previous version. EUCAST categories without value: interpretative category does not exist (applies to intermediate category only).
[b]Values were corrected for natural resistance.

P. aeruginosa

Resistance rates to cefepime, imipenem and meropenem in *P. aeruginosa* increased from 12.2%, 25.5% and 20.6% to 19.8%, 30.4% and 27.7% comparing CLSI 2009–11 standards with EUCAST 2011 standards, respectively (see Table 4).

Resistance rates to gentamicin increased from 18.6% to 25.2% with CLSI 2009–11 standards compared with EUCAST 2011 standards due to elimination of the intermediate category by EUCAST.

Resistance rates to ciprofloxacin and levofloxacin increased from 15.9% and 21.3% to 29.7% and 30.8% with CLSI 2009–11 standards compared with EUCAST 2011 standards, respectively.

S. maltophilia

Trimethoprim/sulfamethoxazole is the only substance for which EUCAST 2011 provides AST breakpoints. No significant differences in the CLSI 2011 system were found except the elimination of the intermediate category by EUCAST and a resulting slight increase in the resistant category (9.4% and 10.6% resistance rate comparing CLSI 2009–11 with EUCAST 2011; see Table 4).

A. baumannii complex

Resistance rates to tobramycin and gentamicin increased from 15.9% and 25.4% to 34.9% and 44.4% comparing CLSI 2009–11 standards with EUCAST 2011 standards, while the resistance rate to amikacin remained unchanged (34.9%). In the case of tobramycin and gentamicin, the increased resistance rate results from the elimination of the intermediate zone in the EUCAST system. The gentamicin susceptibility rate was comparable in both the CLSI 2009–11 and the EUCAST 2011 system (58.7% and 55.6%, respectively). In the case of tobramycin, however, the increased resistance rate was accompanied by a decrease in the susceptibility rate (84.1% with the CLSI 2009–11 versus 77.8% with the EUCAST 2011 system; see Table 4).

Discussion

Many European laboratories are currently preparing to implement the new EUCAST guidelines for AST.[1] Besides the national AST systems (e.g. in Germany, France, the UK and Sweden), many laboratories, in particular in countries without a national AST system, have been using CLSI guidelines for many years.[2–4] Prior to implementing new guidelines in the diagnostic microbiology laboratory, the consequences of changed AST reports need to be considered to prevent misunderstandings in interpretation. The introduction of new guidelines should be accompanied by communicating the scientific rationale and the practical implications of changes in AST reporting.[9] In this study the interpretation of AST for

Table 4. Assignment of glucose non-fermenting Gram-negative bacilli clinical isolates to interpretative categories according to CLSI 2009–11 and EUCAST 2011 guidelines

	Assignment of clinical isolates (%) to antibiotic susceptibility interpretative categories					
	CLSI 2009–11			EUCAST 2011[a]		
Species/drug	S	I	R	S	I	R
P. aeruginosa						
cefepime	80.2	7.6	12.2	80.2		19.8
imipenem	71.7	2.4	25.5	67.0	2.3	30.4
meropenem	73.5	3.5	20.6	63.0	8.8	27.7
tobramycin	87.5	2.3	10.2	86.3		13.7
amikacin	80.3	4.0	15.7	76.6	7.6	15.7
gentamicin	74.8	6.6	18.6	74.8		25.2
ciprofloxacin	72.9	10.7	15.9	63.0	6.9	29.7
levofloxacin	69.2	9.5	21.3	62.8	6.4	30.8
S. maltophilia						
trimethoprim/sulfamethoxazole	89.4	1.3	9.4	89.4		10.6
A. baumannii						
imipenem	65.1	1.6	33.3	65.1	0.0	34.9
meropenem	66.7	0.0	33.3	61.9	4.8	33.3
tobramycin	84.1	0.0	15.9	77.8		34.9
amikacin	65.1	0.0	34.9	65.1	0.0	34.9
gentamicin	58.7	15.9	25.4	55.6		44.4
ciprofloxacin	51.6	3.2	45.2	51.6		48.4
levofloxacin	58.1	1.6	40.3	54.8	1.6	43.5

S, susceptible; I, intermediate; R, resistant.
[a]EUCAST categories without value: interpretative category does not exist (applies to intermediate category only).

Gram-negative bacilli using the CLSI guidelines of 2009–11 and the EUCAST 2011 system was investigated.

Implementation of EUCAST 2011 will lead to significantly more isolates of Gram-negative species being reported resistant to extended-spectrum cephalosporins (ceftriaxone, cefepime), carbapenems and fluoroquinolones (Tables 1 and 2). The same applies to the CLSI 2011 standards. Since usage volume and resistance rate for an individual drug are generally linked, higher numbers of Gram-negative bacilli reported resistant to third-generation cephalosporins, carbapenems and fluoroquinolones will most likely lead to an increased therapeutic usage volume and thus increased selection pressure on other antimicrobial classes or drugs such as aminoglycosides, trimethoprim/sulfamethoxazole, fosfomycin or nitrofurantoin.[10–12]

The effects of a change from CLSI 2009 to CLSI 2010/2011 or from CLSI 2009 to EUCAST 2011 differ between Enterobacteriaceae species: for example, while the resistance rate for fluoroquinolones in *E. coli* remains almost unchanged, *E. cloacae* and *K. pneumoniae* are more frequently reported as resistant to these antibiotics using EUCAST 2011 (Table 3). This finding is most probably related to the different natural diameter distributions of individual Enterobacteriaceae species as compared with the breakpoints, which are defined for the whole Enterobacteriaceae family. In the EUCAST diameter distribution tables (available at http://www.eucast.org/zone_diameter_distributions/), the putative wild-type population of *E. cloacae*, for example, shows a diameter range of 20–38 mm and 19–33 mm for ciprofloxacin and levofloxacin, respectively, while the putative wild-type population of *E. coli* shows a diameter range of 25–42 mm and 26–39 mm for ciprofloxacin and levofloxacin, respectively. Considering the uniform resistant breakpoint definitions for both drugs and species, the lower average diameters of *E. cloacae* readings explain the greater increase in fluoroquinolone resistance rates compared with *E. coli*. We suggest that species-adapted breakpoints for Enterobacteriaceae species would eliminate these artefacts and improve interpretation of AST.

Reportedly the number of extended-spectrum β-lactamase (ESBL)-producing strains isolated in the clinical laboratory is increasing, and ESBL-producing isolates are frequently treated with carbapenems, thereby selecting for carbapenemase-producing strains.[13–15] The EUCAST 2011 and CLSI 2011 guidelines have led to a paradigm change in AST reporting of ESBL-producing isolates. Until 2009 the CLSI guidelines recommended reporting *in vitro* intermediate and susceptible AST results for third- and fourth-generation cephalosporins as resistant in confirmed ESBL producers. In its 2008 expert rules EUCAST recommended changing the interpretation of AST results of third- and fourth-generation cephalosporins from 'susceptible' to 'intermediate' and from 'intermediate' to 'resistant' for confirmed ESBL producers.[16] These recommendations have been abandoned. Classification as susceptible, intermediate or resistant is now based on the reading of inhibition zone diameters alone, and not on interpretative reading, i.e. considering the underlying resistance mechanism.[17,18] Based on the new EUCAST and CLSI recommendations, a significant fraction of ESBL-producing strains will be categorized as susceptible to third- and fourth-generation cephalosporins.[19] In a recent study we showed ceftazidime to be more frequently categorized as susceptible (22.9%) in ESBL producers, whereas the susceptibility rate to cefotaxime was low (0.8%). This finding is probably due to the high prevalence of CTX-M ESBL types in our study population, which resembles the epidemiological situation in Europe.[20] One effect of implementing the new EUCAST and CLSI guidelines is treatment of ESBLs with third- and fourth-generation cephalosporins, and thus less frequent use of carbapenems, reducing selection pressure on this increasingly used drug family.[21] In contrast, the increased diameter breakpoints for third- and fourth-generation cephalosporins in EUCAST will result in higher resistance rates reported for those drugs. Carbapenems will most likely be chosen to serve as alternative substances due to their currently low resistance rates, broad activity and low side effects. It is difficult to predict which effect will prevail: less frequent use of carbapenems due to using third- and fourth-generation cephalosporins for ESBL-producing isolates, or more frequent use of carbapenems due to higher resistance rates reported for third- and fourth-generation cephalosporins resulting from increased diameter breakpoints. One limitation of this study is the exclusion of ceftazidime, cefotaxime and piperacillin/tazobactam. Analyses were done in a routine microbiological laboratory using CLSI disc contents. EUCAST uses lower disc loads than CLSI (10 μg/disc, 5 μg/disc and 30/6 μg/disc EUCAST versus 30 μg/disc, 30 μg/disc and 100/10 μg/disc CLSI for ceftazidime, cefotaxime and piperacillin/tazobactam, respectively), making interpretation of diameters

with both systems impossible. Taking into account the importance of piperacillin/tazobactam in clinical practice, more studies are needed to analyse further the impact of the new AST guidelines.

The number of useful antimicrobial treatment options for glucose non-fermenting Gram-negative bacilli like *P. aeruginosa* and *A. baumannii* will probably decrease after implementation of the EUCAST guidelines due to higher resistance rates. Especially for *P. aeruginosa*, resistance rates of cefepime, carbapenems and fluoroquinolones will increase (Table 4). Resistance rates of aminoglycosides in *P. aeruginosa* are hardly affected by changing from CLSI to EUCAST guidelines (Table 4). These classes of drugs may therefore become a frequently chosen alternative, but will hardly be used as monotherapy.

No standard definitions exist to define Gram-negative bacilli as multidrug resistant, but most systems are based on the detection of non-susceptibility to several antibiotic classes.[7] Following implementation of the CLSI 2011 and EUCAST 2011 guidelines more Gram-negative bacilli will be reported as multidrug resistant, resulting in higher rates of patients in isolation and, concomitantly, higher costs.[22] A higher rate of multidrug-resistant Gram-negative bacilli will not only result in higher costs for hospitals and hospital hygiene measures, but will also result in more confirmatory testing in the laboratory.

AST reports influence prescription policy and antibiotic use.[8] Implementation of EUCAST guidelines will affect antibiotic prescription, in part because of the partial elimination of the intermediate category. Defining isolates as resistant that were formerly considered intermediate will most likely lead clinicians to use other antimicrobial classes.

This study was limited to the epidemiological situation in the Zurich region with low to moderate levels of resistance. The increase in resistance rates reported due to changes in guidelines may be more prominent in populations with higher levels of resistance, e.g. the Mediterranean region, since diameter distributions are shifted to lower mean values. In contrast, a lesser increase of reported resistance rates may be seen in regions with low resistance levels, such as Scandinavia.

Implementation of the EUCAST standards for AST makes results in Europe more comparable, incorporating PK–PD studies and clinical data, e.g. the possible use of extended-spectrum cephalosporins versus carbapenems for ESBL-producing isolates.[1] However, evidence-based clinical studies should further accompany and validate proposed changes in guidelines. During implementation of the EUCAST system, laboratories should be aware of the implications of modified AST reports on antibiotic prescription policy. Information from clinicians on changes in guidelines resulting in apparently increased resistance rates may help to prevent excessive use of reserve antibiotic drugs. Antibiotic stewardship has been proven to effectively control antibiotic prescription, resulting in lower resistance rates.[23] Considering a changed description of antimicrobial drug susceptibility in Gram-negative bacilli with higher resistance rates following implementation of the CLSI 2011 and EUCAST 2011 standards, the need for antibiotic stewardship must once more be emphasized.

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Transparency declarations

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References

1 European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 1.3, 2011*. http://www.eucast.org/clinical_breakpoints (7 November 2011, date last accessed).

2 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

3 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21*. CLSI, Wayne, PA, USA, 2011.

4 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement M100-S19*. CLSI, Wayne, PA, USA, 2009.

5 Chastre J. Evolving problems with resistant pathogens. *Clin Microbiol Infect* 2008; **14** Suppl 3: 3–14.

6 Livermore DM. The threat from the pink corner. *Ann Med* 2003; **35**: 226–34.

7 Kallen AJ, Hidron AI, Patel J *et al*. Multidrug resistance among Gram-negative pathogens that caused healthcare-associated infections reported to the National Healthcare Safety Network, 2006–2008. *Infect Control Hosp Epidemiol* 2010; **31**: 528–31.

8 MacKenzie FM, Gould IM, Bruce J *et al*. The role of microbiology and pharmacy departments in the stewardship of antibiotic prescribing in European hospitals. *J Hosp Infect* 2007; **65** Suppl 2: 73–81.

9 Jenkins SG, Jerris RC. Critical assessment of issues applicable to development of antimicrobial susceptibility testing breakpoints. *J Clin Microbiol* 2011; **49** Suppl: S5–10.

10 Harada K, Asai T. Role of antimicrobial selective pressure and secondary factors on antimicrobial resistance prevalence in *Escherichia coli* from food-producing animals in Japan. *J Biomed Biotechnol* 2010; **2010**: 180682.

11 Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009; **64** Suppl 1: i3–10.

12 Lai CC, Wang CY, Chu CC *et al*. Correlation between antibiotic consumption and resistance of Gram-negative bacteria causing healthcare-associated infections at a university hospital in Taiwan from 2000 to 2009. *J Antimicrob Chemother* 2011; **66**: 1374–82.

13 Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008; **13**: pii=19044.

14 Walsh TR. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents* 2010; **36** Suppl 3: S8–14.

15 Pakyz AL, Oinonen M, Polk RE. Relationship of carbapenem restriction in 22 university teaching hospitals to carbapenem use and carbapenem-resistant *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009; **53**: 1983–6.

16 European Committee on Antimicrobial Susceptibility Testing. *Expert Rules in Antimicrobial Susceptibility Testing, Version 1, 2008*. http://www.eucast.org/expert_rules (7 November 2011, date last accessed).

17 Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *J Antimicrob Chemother* 2001; **48** Suppl S1: 87–102.

18 Winstanley T, Courvalin P. Expert systems in clinical microbiology. *Clin Microbiol Rev* 2011; **24**: 515–56.

19 Wang P, Hu F, Xiong Z *et al*. Susceptibility of extended-spectrum-β-lactamase-producing Enterobacteriaceae according to the new CLSI breakpoints. *J Clin Microbiol* 2011; **49**: 3127–31.

20 Polsfuss S, Bloemberg GV, Giger J *et al*. 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. *J Antimicrob Chemother* 2011; **67**: 159–66.

21 Masterton RG. The new treatment paradigm and the role of carbapenems. *Int J Antimicrob Agents* 2009; **33**: 105–10.

22 Lee NY, Lee HC, Ko NY *et al*. Clinical and economic impact of multidrug resistance in nosocomial *Acinetobacter baumannii* bacteremia. *Infect Control Hosp Epidemiol* 2007; **28**: 713–9.

23 Kaki R, Elligsen M, Walker S *et al*. Impact of antimicrobial stewardship in critical care: a systematic review. *J Antimicrob Chemother* 2011; **66**: 1223–30.