Change of Antibiotic Susceptibility Testing Guidelines from CLSI to EUCAST: Influence on Cumulative Hospital Antibiograms

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Abstract

Objective: We studied whether the change in antibiotic susceptibility testing (AST) guidelines from CLSI to EUCAST influenced cumulative antibiograms in a tertiary care hospital in Switzerland.

Methods: Antibiotic susceptibilities of non-duplicate isolates collected within a one-year period before (period A) and after (period B) changing AST interpretation from CLSI 2009 to EUCAST 1.3 (2011) guidelines were analysed. In addition, period B isolates were reinterpreted according to the CLSI 2009, CLSI 2013 and EUCAST 3.1 (2013) guidelines.

Results: The majority of species/drug combinations showed no differences in susceptibility rates comparing periods A and B. However, in some gram-negative bacilli, decreased susceptibility rates were observed when comparing CLSI 2009 with EUCAST 1.3 within period B: *Escherichia coli* / cefepime, 95.8% (CLSI 2009) vs. 93.1% (EUCAST 1.3), P=0.005; *Enterobacter cloacae* / cefepime, 97.0 (CLSI 2009) vs. 90.5% (EUCAST 1.3), P=0.012; *Pseudomonas aeruginosa* / meropenem, 88.1% (CLSI 2009) vs. 78.3% (EUCAST 1.3), P=0.002. These differences were still evident when comparing susceptibility rates according to the CLSI 2013 guideline with EUCAST 3.1 guideline. For *P. aeruginosa* and imipenem, a trend towards a lower antibiotic susceptibility rate in ICUs compared to general wards turned into a significant difference after the change to EUCAST: 87.9% vs. 79.8%, *P*=0.08 (CLSI 2009) and 86.3% vs. 76.8%, *P*=0.048 (EUCAST 1.3).

Conclusions: The change of AST guidelines from CLSI to EUCAST led to a clinically relevant decrease of susceptibility rates in cumulative antibiograms for defined species/drug combinations, particularly in those with considerable differences in clinical susceptibility breakpoints between the two guidelines.

Introduction

The European Committee for Antimicrobial Susceptibility Testing (EUCAST) was initiated to harmonize minimum inhibitory concentration (MIC) breakpoints across Europe [1]. In line with many European clinical laboratories, the University of Zurich's Institute of Medical Microbiology, Switzerland, changed its antibiotic susceptibility testing (AST) system from the Clinical and Laboratory Standards Institute (CLSI) 2009 methodology to the EUCAST 1.3 AST guidelines on 1st July 2011 [2,3].

In general, EUCAST recommends lower resistance MIC breakpoints than CLSI, in particular for Gram-negative bacteria, and, in part, abandoned the intermediate susceptibility zone. These changes have been shown to result in different susceptibility rates, e.g. higher cefepime and meropenem resistance rates in *Pseudomonas aeruginosa* [4], higher ceftazidime and ceftriaxone resistance rates in *Escherichia coli* causing bacteremia [5], higher ceftazidime resistance in ESBL-producing *E. coli* and *Klebsiella pneumonia* [6], and higher cefepime and ceftazidime resistance in ESBL producing *E. coli* [7]. However, the actual effect of the guideline changes on cumulative hospital antibiograms is unknown, even though

local cumulative antibiograms are important for guiding empirical antibiotic therapy [8,9], and changes in cumulative resistance rates may influence the choice of empirical antimicrobial treatment [10].

This study was designed to determine whether and to which extent susceptibility rates in cumulative antibiograms of the five most prevalent bacterial species in our tertiary-care hospital would differ between two consecutive years before and after changing from CLSI 2009 to EUCAST 1.3 (2011) AST guidelines. Furthermore, we determined whether differences in cumulative antibiograms represented true changes in antimicrobial susceptibility, or if they were merely an effect of guideline changes.

In addition, as resistance rates of cumulative antibiograms from general ward specimens reportedly differ from those found on intensive care units (ICUs), we aimed to determine whether guideline dependent changes differed between ICUs and general wards [11,12].

Materials and Methods

Setting

The University Hospital Zurich, Zurich, Switzerland, is an 871 bed tertiary-care teaching hospital covering all medical specialties except paediatrics and orthopaedics. Six intensive care units (medical ICU, general, thoracic and transplant surgery ICU, trauma ICU, burn ICU, cardiac surgery ICU, neurosurgery ICU) with a total of 65 beds are assigned to different departments. Hematopoietic stem cell transplantations are performed in a specialized unit. All microbiologic samples are tested in the clinical microbiology laboratory of the Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland.

Data collection

Data were collected in two consecutive one-year periods just before (period A) and after (period B) the change of AST interpretation from CLSI 2009 to EUCAST 1.3 on 1 July 2011. All bacterial isolates from samples collected on general wards and intensive care units were taken into account. In addition, samples of the outpatients' clinic for respiratory medicine were included in a subgroup analysis for *P. aeruginosa*.

The species analysed comprised *E. coli*, *K. pneumoniae, Enterobacter cloacae*, *P. aeruginosa*, and *Staphylococcus aureus*. According to the guidelines for analysis and presentation of cumulative antibiograms, we excluded all repeated isolates, i.e. only the first isolate of a certain bacterium per patient and year was analysed, regardless of the material, the donor site, or the resistance profile [13].

In addition, isolates collected on the general wards and on the ICUs were analysed separately. The "ICU section" consisted of the six ICUs and the hematopoietic stem cell transplantation unit. The "ward-section" consisted of all general wards.

Susceptibility testing

For susceptibility testing, the disc diffusion method according to Kirby-Bauer was used [14]. Antibiotic discs were obtained from i2a (Montpellier, France). Susceptibility testing was done on Mueller-Hinton agar (Becton-Dickinson, Franklin Lakes, NJ, USA) using MacFarland 0.5 from overnight cultures followed by incubation at 35°C for 16-18h. Inhibition zone diameters were determined and recorded in the automated Sirweb/Sirscan system (i2a) and interpreted according to CLSI 2009 and EUCAST 1.3 guidelines [2,3].

Comparison of CLSI 2009 and EUCAST 1.3 (2011)

For certain drugs, e.g. ceftazidime, cefotaxime, and piperacillin/tazobactam, EUCAST guidelines contain other antibiotic disc loads than CLSI guidelines, preventing a direct comparison of disk diffusion AST results [2,3]. Thus, we only included drugs into analysis that have identical antibiotic disc loads in both CLSI and EUCAST guidelines, i.e., amoxicillin/ clavulanic acid, cefuroxime, cefoxitin, ceftriaxone, cefepime, imipenem, meropenem, tobramycin, sulfomethoxazole/ trimethoprim, ciprofloxacin, gentamicin, erythromycin, clindamycin, rifampin, and teicoplanin.

The first one-year period (period A) was interpreted according to CLSI 2009 guidelines only. Period B was originally interpreted according to EUCAST 1.3 guidelines. In order to analyze whether or not changes in susceptibility rates over time were due to the guideline change alone, results of period B were reinterpreted according to CLSI 2009 guidelines. Additionally, period B was reinterpreted according to CLSI 2013 and EUCAST 3.1.

Isolates of intermediate susceptibility were classified together with resistant isolates to the "non-susceptible"-group.

Statistical analyses

Differences in group proportions were assessed using chi-square or Fisher's exact tests, as appropriate. We used Stata (Version 12.1, StataCorp, College Station, Texas) for statistical analyses. *P*-values <0.05 were considered statistically significant.

Results

Cumulative hospital antibiogram

Between July 2010 and June 2011 (period A), 2540 isolates (1085 *E. coli*, 277 *K. pneumoniae*, 186 *E. cloacae*, 271 *P. aeruginosa*, 721 *S. aureus*) were included. Between July 2011 and June 2012 (period B), 2688 isolates (1177 *E. coli*, 310 *K. pneumoniae*, 200 *E. cloacae*, 282 *P. aeruginosa*, 719 *S. aureus*) were included.

Four different "patterns" of effects on susceptibility rates were found when comparing the cumulative antibiograms of period A and period B by either interpreting the antibiograms according to CLSI 2009 or according to EUCAST 1.3 AST guidelines (Figure 1).

Pattern 1 - Artificial changes in susceptibility rates. A decrease in the cefepime susceptibility rates of *E. coli* and *E. cloacae* from period A to period B was observed (Table 1,
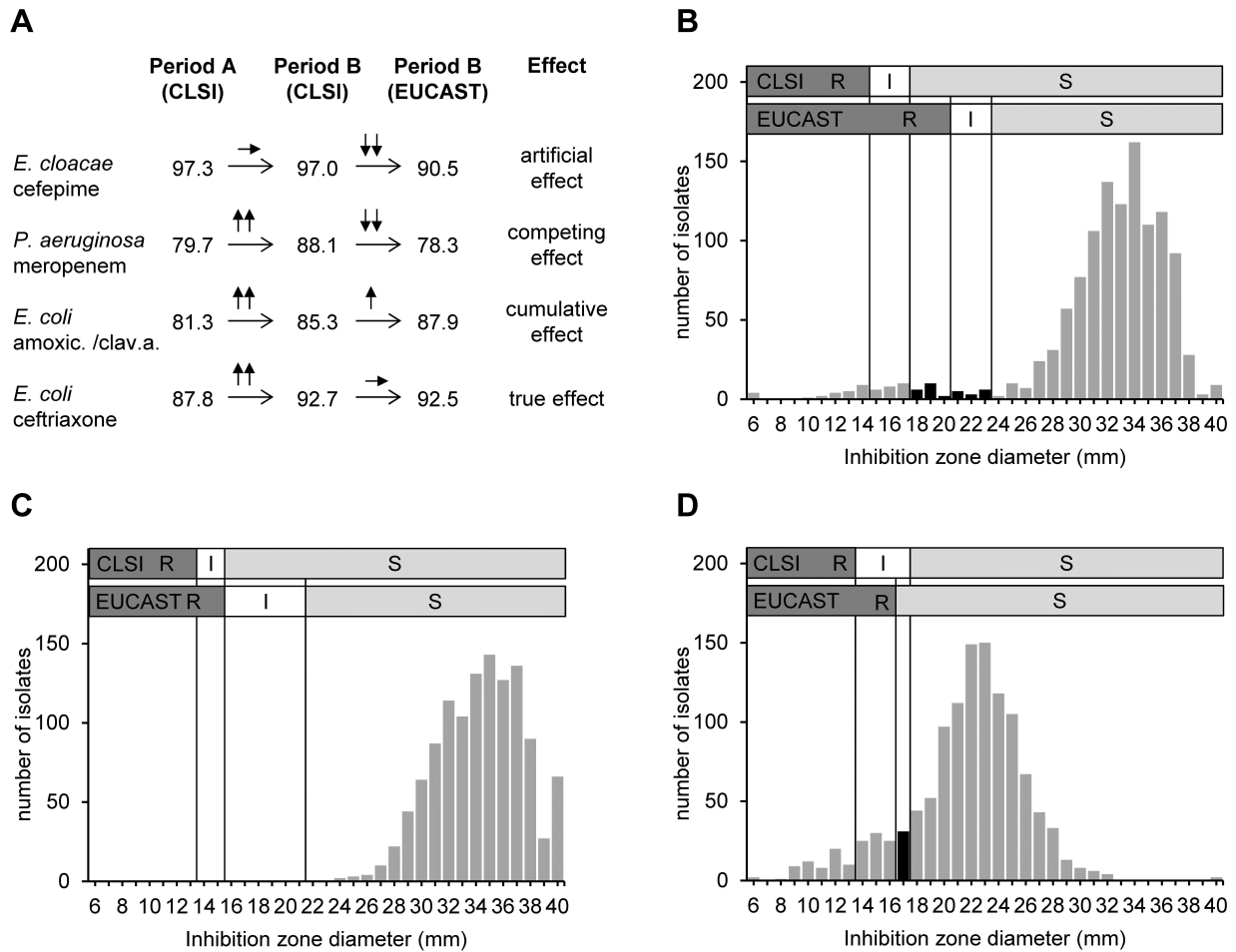
Figure 1. Different patterns of effects and distributions of inhibition zone diameters. Part A: Different "patterns of effects" when analysing period A according CLSI 2009 guidelines and period B according CLSI 2009 and EUCAST 1.3 guidelines; numbers are percent susceptible. Part B: Distribution of inhibition zone diameters of *E.coli* and cefepime: isolates of columns in black are not classified as "susceptible" any more when reported according to EUCAST 1.3 guidelines. Part C: Distribution of inhibition zone diameters of *E. coli* and meropenem: no change of susceptibility rate when EUCAST 1.3 guidelines are applied. Part D: Distribution of inhibition zone diameters of *E. coli* and amoxicillin/clavulanic acid: overlap of wild-type and resistent bacteria, leading to a change in classification from "intermediate" to "susceptible" (black column) of a near significant number of isolates when EUCAST 1.3 guidelines are applied.

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comparison 1). These differences disappeared when periods A and B were both interpreted according to CLSI 2009 AST guidelines (Table 1, comparison 2).

Pattern 2 - Competing effects. When comparing *P. aeruginosa* / meropenem susceptibility rates defined according to CLSI 2009 guidelines in both periods, more specimens were reported susceptible in period B than in period A (Table 1, comparison 2), reflecting a true epidemiological change. When period A was analysed according to CLSI 2009 and period B according to EUCAST 1.3 AST guidelines, the reported susceptibility rates did not differ (Table 1, comparison 1). A

similar effect was shown for *P. aeruginosa* / imipenem and ciprofloxacin susceptibility rates.

Pattern 3 - Cumulative effects. Amoxicillin/clavulanic acid susceptibility rates of *E. coli* and *K. pneumonia* increased between period A and B when both periods were interpreted according to CLSI 2009 AST guidelines (Table 1, comparison 2). This increase was amplified by interpreting period B according to EUCAST 1.3 AST guidelines (Table 1, comparison 3).

Pattern 4 - True changes in susceptibility rates. *E. coli* ceftriaxone susceptibility and *K. pneumoniae* tobramycin susceptibility rates increased from period A to period B, while

Table 1. Comparison of cumulative antibiograms of two adjacent one-year periods by either applying CLSI or EUCAST guidelines.

Species	Drug	Comparison 1 EUCAST CLSI 2009 1.3 (2011)			Comparison 2 CLSI 2009 CLSI 2009			Comparison 3 EUCAST CLSI 2009 1.3 (2011)			Comparison 4 EUCAST CLSI 2013 3.1 (2013)		
		Period A[a]	Period B[b]	P-value	Period A[a]	Period B[b]	P-value	Period B[b]	Period B[b]	P-value	Period B[b]	Period B[b]	P-value
E.coli	Amoxicillin/clavulanic acid	81.3	87.9	<0.001	81.3	85.3	0.011	85.3	87.9	0.07	85.3	87.9	0.07
	Cefuroxime	89.6	90.9	0.32	89.6	90.9	0.32	90.9	90.9	1.00	90.9	90.9	1.00
	Ceftriaxone	87.8	92.5	0.001	87.8	92.7	<0.001	92.7	92.5	0.94	92.5	92.5	1.00
	Cefepime	97.1	93.1	<0.001	97.1	95.8	0.11	95.8	93.1	0.005	95.8	93.1	0.005
	Imipenem	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.
	Meropenem	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.
	Tobramycin	88.1	88.8	0.70	88.1	89.7	0.34	89.7	88.8	0.51	89.7	88.6	0.43
	Sulfomethoxazole/trimethoprim	68.6	65.2	0.08	68.6	65.2	0.08	65.2	65.2	1.00	65.2	65.2	1.00
	Ciprofloxacin	80.0	81.3	0.46	80.0	81.2	0.49	81.2	81.3	0.96	81.2	81.3	0.96
K. pneumoniae	Amoxicillin/clavulanic acid	85.6	92.3	0.011	85.6	89.7	0.13	89.7	92.3	0.33	89.7	92.3	0.33
	Cefuroxime	87.0	89.6	0.37	87.0	89.6	0.37	89.6	89.6	n.a.	89.6	89.6	1.00
	Ceftriaxone	88.6	91.6	0.28	88.6	91.6	0.28	91.6	91.6	1.00	91.6	91.6	1.00
	Cefepime	96.0	92.6	0.08	96.0	95.5	0.84	95.5	92.6	0.17	95.5	92.6	0.17
	Imipenem	98.9	99.4	0.67	98.9	99.4	0.67	99.4	99.4	1.00	98.4	99.0	0.72
	Meropenem	98.0	99.0	0.44	98.0	99.4	0.22	99.4	99.0	1.00	98.7	99.0	1.00
	Tobramycin	87.1	93.9	0.010	87.1	94.2	0.006	94.2	93.9	1.00	94.2	93.2	0.74
	Sulfomethoxazole/trimethoprim	81.2	81.3	1.00	81.2	81.3	1	81.3	81.3	1.00	81.3	81.3	1.00
	Ciprofloxacin	88.8	93.5	0.06	88.8	93.9	0.037	93.9	93.5	1.00	93.9	93.5	1.00
E. cloacae	Amoxicillin/clavulanic acid	4.8	9.0	0.12	4.8	7.5	0.30	7.5	9.0	0.72	7.5	9.0	0.72
	Cefuroxime	66.5	70.5	0.44	66.5	70.5	0.44	70.5	70.5	1.00	70.5	70.5	1.00
	Ceftriaxone	73.8	74.5	0.90	73.8	76.0	0.63	76.0	74.5	0.82	74.5	74.5	1.00
	Cefepime	97.3	90.5	0.006	97.3	97.0	1.00	97.0	90.5	0.012	97.0	90.5	0.012
	Imipenem	100.0	99.5	1.00	100.0	100.0	n.a.	100.0	99.5	1.00	95.0	98.5	0.09
	Meropenem	100.0	97.5	0.07	100.0	100.0	n.a.	100.0	97.5	0.06	97.0	97.5	0.77
	Tobramycin	95.7	96.0	1.00	95.7	96.0	1.00	96.0	96.0	1.00	96.0	96.0	1.00
	Sulfomethoxazole/trimethoprim	93.0	91.0	0.57	93.0	91.0	0.57	91.0	91.0	1.00	91.0	91.0	1.00
	Ciprofloxacin	95.1	96.0	0.81	95.1	96.5	0.61	96.5	96.0	1.00	96.5	96.0	1.00
P. aeruginosa	Cefepime	85.6	88.7	0.31	85.6	88.7	0.31	88.7	88.7	1.00	88.7	88.7	1.00
	Imipenem	76.7	82.9	0.07	76.7	85.1	0.013	85.1	82.9	0.57	83.3	82.9	1.00
	Meropenem	79.7	78.3	0.74	79.7	88.1	0.013	88.1	78.3	0.002	86.3	78.3	0.015
	Tobramycin	91.6	94.6	0.21	91.6	94.6	0.21	94.6	94.6	1.00	94.6	94.6	1.00
	Ciprofloxacin	84.1	90.7	0.021	84.1	93.9	<0.001	93.9	90.7	0.20	93.9	90.7	0.20
S. aureus	Cefoxitin	96.7	94.4	0.041	96.7	94.4	0.041	94.4	94.4	1.00	94.4	94.4	1.00
	Gentamicin	98.2	98.7	0.52	98.2	98.9	0.38	98.9	98.7	1.00	98.9	98.7	1.00
	Sulfomethoxazole/trimethoprim	99.2	99.3	1.00	99.2	99.3	1.00	99.3	99.3	1.00	99.3	99.3	1.00
	Erythromycin	87.2	87.8	0.74	87.2	86.9	0.87	86.9	87.8	0.63	86.9	87.8	0.63
	Clindamycin	97.6	98.4	0.33	97.6	98.4	0.33	98.4	98.4	1.00	98.4	98.4	1.00
	Rifampicin	99.4	98.6	0.18	99.4	98.7	0.27	98.7	98.6	1.00	98.7	98.6	1.00
	Teicoplanin	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.	100.0	100.0	n.a.

cefoxitin susceptibility rates of *S. aureus* decreased (Table 1, comparison 1). These changes in susceptibility rates remained when CLSI 2009 guidelines were applied to period B (Table 1, comparison 2).

Table 1 (continued).

Table 1 shows that, besides changing AST patterns described above, there was no difference in susceptibility rates between the two periods in the majority of isolates, neither when comparing CLSI 2009 AST guidelines applied to period A and EUCAST 1.3 guidelines to period B (comparison 1), nor when periods A and B were both interpreted according to CLSI 2009 AST guidelines (comparison 2). The results of comparison 3 were unchanged when inhibition zone diameters of period B interpreted according to CLSI 2103 and EUCAST 3.1 were compared (comparison 4).

Comparison of cumulative antibiograms of intensive care units, general wards and other units

Regardless of the methodology applied, susceptibility rates of *E. coli* to cefuroxime, ceftriaxone and cefepime, and susceptibility rates of *S. aureus* to clindamycin were lower in cumulative antibiograms of intensive care units as compared to those of general wards (Table 2). Similarly, a trend to lower susceptibility rates in ICUs could be detected in some other species/drug combinations.

For *P. aeruginosa* and imipenem, a trend towards a lower antibiotic susceptibility rate in ICUs compared to general wards turned into a significant difference after the change to EUCAST 1.3 (Table 2). Moreover, in *P. aeruginosa* isolates collected in the outpatients' clinic for respiratory medicine, which include specimens of numerous patients with cystic fibrosis and lung transplant recipients, a decrease in ciprofloxacin susceptibility rate was notable when applying EUCAST 1.3, but not CLSI 2009 guidelines (data not shown).

Discussion

This observational study was designed to analyse cumulative hospital antibiograms in two adjacent one-year periods before and after the clinical laboratory changed antibiotic susceptibility test interpretation from CLSI 2009 to EUCAST 1.3 (2011) guidelines, and to analyse whether possible differences are due to true epidemiologic changes or result only from CLSI / EUCAST guideline differences. Differences resulting from guideline changes alone may misdirect physicians in the interpretation of antibiotic susceptibility trends in that truly increasing resistance rates may be missed or unchanging resistance rates may be reported as increasing or decreasing, resulting in a change of antibiotic use patterns and thus patient management and quality of care.

Clinical breakpoint (CBP) setting is a multi-step process comprising the determination of epidemiological cut off (ECOFF) values from MIC distributions, correlating these ECOFFs to zone diameters distributions, comparing putative CBPs to available PK/PD data and, finally, clinical validation of putative CBPs in clinical outcome studies [15].

CLSI and EUCAST use different methods for the determination of disc diffusion CBPs: CLSI uses a variant of the error-rate-bounded method, sometimes incorporating an intermediate zone [16], and EUCAST first defines MIC breakpoints on the basis of epidemiological MIC cut-offs (ECOFFs) and pharmacokinetic/pharmacodynamic (PK/PD) parameters, and correlates those MIC CBPs to zone diameter values using the "MIC-coloured zone diameter histogram technique" [17,18]. The EUCAST policy of CBP setting promises more transparency in this still complex, rather consensus based process as all documents on diameter/MIC distributions, and ECOFF data are publicly available [19].

Such different CBP determination methods inevitably lead to different CBPs in CLSI and EUCAST AST guidelines for many species/drug combinations. EUCAST 1.3 (generally unchanged in EUCAST 2.0 and 3.1) disk diffusion CBPs are frequently higher as compared to CLSI 2009, and in many cases even higher as compared to revised CLSI guideline versions 2010 to 2013 [2,3,20-24]. Several studies have shown a significant impact of guideline changes on the reporting of AST results [4-7].

Instead of a uniform trend towards lower reported susceptibility rates for all species/drug combinations after implementation of EUCAST guidelines, this study showed four distinct patterns of effects of guidelines changes on susceptibility rates: i) a clearly artificial change of susceptibility rates due to changes in AST guidelines (e.g. *E. coli* / cefepime and *E. cloacae* / cefepime; Table 1, comparison 3); ii) competing effects of artificial changes in susceptibility rates and true epidemiologic variations (e.g. *P. aeruginosa* / meropenem; Table 1, comparisons 2 and 3); iii) cumulative effects resulting from artificial changes enhancing a true epidemiologic variation (e.g. *E. coli*/amoxicillin and clavulanic acid; Table 1, comparisons 2 and 3) and iv) a true change of susceptibility rates due to epidemiologic variation (e.g. *E. coli* / ceftriaxone; Table 1, comparison 2).

Three species/drug combinations (*E. coli* / cefepime, *E. cloacae* / cefepime and *P. aeruginosa* / meropenem) showed a statistically significant, yet artificial, decrease in susceptibility rates when period B was analysed according to EUCAST 1.3 (2011) instead of CLSI 2009 guidelines. For these three species/drug combinations, susceptible clinical inhibition zone diameter breakpoints differ substantially between EUCAST 1.3 and CLSI 2009 guidelines, i.e., 6 mm difference for cefepime susceptible CBP and *Enterobacteriaceae* (susceptible CPB CLSI 2009 18 mm; EUCAST 1.3 24 mm), and 8 mm difference in meropenem CBP for *P. aeruginosa* (susceptible CPB CLSI 2009 16 mm; EUCAST 1.3 24 mm).

Table 2. Comparison of cumulative antibiograms of wards vs. ICUs by either applying CLSI 2009 or EUCAST 1.3 (2011) guidelines to period B[a].

Species	Drug	CLSI 2009			EUCAST 1.3 (2011)		
		Wards	ICUs	P-value	Wards	ICUs	P-value
E. coli	Amoxicillin/ clavulanic acid	85.9	82.4	0.20	88.4	85.7	0.29
	Cefuroxime	92.1	85.1	0.003	92.1	85.1	0.003
	Ceftriaxone	93.5	89.0	0.039	93.3	89.0	0.043
	Cefepime	96.6	92.4	0.012	94.2	88.2	0.004
	Imipenem	100	100	n.a.	100	100	n.a.
	Meropenem	100	100	n.a.	100	100	n.a.
	Tobramycin	90.5	86.2	0.08	89.5	85.2	0.07
	Sulfomethoxazole/ trimethoprim	65.0	65.9	0.87	65.0	65.9	0.87
	Ciprofloxacin	81.6	79.1	0.44	81.8	79.1	0.38
K. pneumoniae	Amoxicillin/ clavulanic acid	89.9	89.3	0.84	92.5	91.7	0.81
	Cefuroxime	89.3	90.5	0.84	89.3	90.5	0.84
	Ceftriaxone	91.2	92.9	0.82	91.2	92.9	0.82
	Cefepime	95.1	96.4	0.77	92.5	92.9	1.00
	Imipenem	99.6	98.8	0.47	99.6	98.8	0.47
	Meropenem	99.6	98.8	0.47	99.1	98.8	1.00
	Tobramycin	94.7	92.9	0.59	94.2	92.9	0.60
	Sulfomethoxazole/ trimethoprim	79.6	85.7	0.25	79.6	85.7	0.25
	Ciprofloxacin	93.4	95.2	0.79	93.4	94.0	1.00
E. cloacae	Amoxicillin/ clavulanic acid	7.6	7.4	1.00	9.8	7.4	0.79
	Cefuroxime	72.0	67.6	0.62	72.0	67.6	0.62
	Ceftriaxone	77.3	73.5	0.60	75.8	72.1	0.61
	Cefepime	96.2	98.5	0.67	90.9	89.7	0.80
	Imipenem	100	100	n.a.	99.2	100	1.00
	Meropenem	100	100	n.a.	97.0	98.5	0.66
	Tobramycin	94.7	98.5	0.27	94.7	98.5	0.27
	Sulfomethoxazole/ trimethoprim	88.6	95.6	0.12	88.6	95.6	0.12
	Ciprofloxacin	96.2	97.1	1.00	95.4	97.1	0.72
P. aeruginosa	Cefepime	88.0	89.9	0.70	88.0	89.9	0.70
	Imipenem	87.9	79.8	0.08	86.3	76.8	0.048
	Meropenem	90.5	83.8	0.12	80.8	73.7	0.18
	Tobramycin	95.0	93.9	0.78	95.0	93.9	0.78
	Ciprofloxacin	93.3	94.9	0.79	90.6	90.9	1.00
S. aureus	Cefoxitin	94.1	95.2	0.72	94.1	95.2	0.72
	Gentamicin	98.8	99.0	1.00	98.6	99.0	1.00
	Sulfomethoxazole/ trimethoprim	99.0	100	0.33	99.0	100	0.33
	Erythromycin	86.4	87.9	0.63	87.5	88.4	0.80
	Clindamycin	99.2	96.6	0.02	99.2	96.6	0.02
	Rifampicin	98.6	99.0	1.00	98.4	99.0	0.73
	Teicoplanin	100	100	n.a.	100	100	n.a.

[a]1 July 2011 to 30 June 2012, numbers are percent susceptible

n.a. not applicable.

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The present study showed significant changes in susceptibility rates for individual species/drug combinations with substantial differences in susceptible CBPs between both former and current CLSI and EUCAST guidelines. The probability and extent of changes, however, both depend on the inhibition zone diameters distributions of individual species/ drug combinations and, thus, depend on the epidemiological situation present. This is seen in *E. coli* and meropenem, where a 6 mm difference between CBPs does not lead to changes in susceptibility rates (Figure 1b and c).

Most important, some species/drug combinations, for which wild-type and resistant population zone diameters are not clearly separated, may be affected by only minor CBP changes, e.g. *E. coli* and amoxicillin/clavulanic acid (Figure 1d), for which a susceptible CBP difference of 1 mm (susceptible CBP CLSI 2009 18 mm; EUCAST 1.3 17 mm) led to an almost significant increase in susceptibility rates from 85.3% to 87.9% (Table 1, Comparison 3).

Empirical antibiotic treatment is not only guided by individual patient characteristics, but also by epidemiological data such as local susceptibility rates [9]. Usually, empirical antibiotic treatment of specific pathogens or specific infections is recommended only if a certain level of resistance is not exceeded. Examples are the empirical treatment of cystitis with a threshold of 80% susceptible isolates for any antibiotic agent [25], or community acquired pneumonia and macrolide-therapy with a threshold of 75% susceptible isolates [26]. Consequently, changes in reported susceptibility rates will influence empirical antibiotic therapy. Meropenem susceptibility of *P. aeruginosa* dropped from 88.1% to 78.3% when analyzed according to CLSI 2009 and EUCAST 1.3 guidelines. This significant decrease in the meropenem susceptibility rate by EUCAST interpretation may, therefore, have a practical impact on the choice of the empirical antibiotic therapy in patients with life-threatening infections, and could result in more toxic (e.g. aminoglycosides, colistin) or less effective antibiotic regimens.

The results of this study are in concordance with those of other authors demonstrating that susceptibility rates differ between ICU's and general wards [11,27]. Corresponding differences were observed for cephalosporin susceptibility rates of *E. coli*, and clindamycin susceptibility rates of *S. aureus* when applying both CLSI and EUCAST guidelines. However, for imipenem susceptibility rates of *P. aeruginosa*, a significant difference between ICU's and general wards was found only if applying EUCAST 2011, but not with CLSI 2009 (Table 2). Moreover, ciprofloxacin susceptibility rates of *P.aeruginosa* isolates collected in the outpatient clinic for respiratory medicine decreased significantly when EUCAST 2011 guidelines were applied. The most likely reason is a different inhibition zone diameter distribution (i.e. a shift towards lower inhibition zone diameters) of isolates originating from this patient population, resulting in a comparably stronger effect of guideline changes on susceptibility rates. These two examples show that guideline changes can affect different hospital wards to a different extent.

Thus, effects of AST guidelines are not easily predictable and are dependent on the epidemiological situation. Both clinicians and microbiologists should, therefore, know their

local epidemiology to be able to foresee effects of AST guideline changes on clinical practice. In addition, switching guidelines should be accompanied by thorough analyses during one time period where both guidelines are applied in order to detect bacteria/drug combinations belonging to one of the patterns described here. This might be particularly important in settings with higher resistance rates.

Despite a broad general set of data, the smaller sample size for the general ward/ICU distinction may have obscured significant differences in this study. In addition, this study is limited to the epidemiological situation in one single institution in north-eastern Switzerland, which is a low prevalence region for antibiotic resistance, hampering generalizability to other geographic settings. Effects of CBP changes as shown in this work may, thus, differ from the situation in high-prevalence regions.

In conclusion, this study demonstrates that changes in AST guidelines, e.g. from CLSI 2009 to EUCAST 1.3 (2011) guidelines, can influence a hospital's cumulative antibiogram in various ways. These changes cannot be easily predicted and

may differ between hospital units. Even cautious interpretation can only serve as an approximation of the real epidemiological changes. Nevertheless, these issues have to be taken into account when interpreting cumulative antibiograms in a period after significant AST guideline changes. Further studies are needed to assess the effect of the guideline changes on different local epidemiological situations.

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Author Contributions

References

1. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW et al. (2003) European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52: 145-148. doi:10.1093/jac/dkg312. PubMed: 12837738.
2. Clinical and Laboratory Standards Insitute (2009) Performance Standards for Antimicrobial Susceptebility Testing: Nineteenth Informational Supplement M100-S19. Wayne, PA: CLSI..
3. European Commitee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Versions 1.3 and 2.0. Available: http://www.eucast.org/antimicrobial_susceptibility_testing/previous_versions_of_tables/. Accessed 23 May 2013.
4. Hombach M, Bloemberg GV, Böttger EC (2012) Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. J Antimicrob Chemother England, 67: 622-632. PubMed: 22167240.
5. van der Bij AK, van Dijk K, Muilwijk J, Thijsen SF, Notermans DW et al. (2012) Clinical breakpoint changes and their impact on surveillance of antimicrobial resistance in Escherichia coli causing bacteraemia. Clin Microbiol Infect 18: E466-E472. doi:10.1111/j.1469-0691.2012.03996.x. PubMed: 22925456.
6. Hawser SP, Badal RE, Bouchillon SK, Hoban DJ, Hsueh PR (2010) Comparison of CLSI 2009, CLSI 2010 and EUCAST cephalosporin clinical breakpoints in recent clinical isolates of Escherichia coli, Klebsiella pneumoniae and Klebsiella oxytoca from the SMART Global Surveillance Study. Int J Antimicrob Agents Neth, 36: 293-294. PubMed: 20598511.
7. Rodríguez-Baño J, Picón E, Navarro MD, López-Cerero L, Pascual A (2012) Impact of changes in CLSI and EUCAST breakpoints for susceptibility in bloodstream infections due to extended-spectrum beta-lactamase-producing Escherichia coli. Clin Microbiol Infect 18: 894-900. doi:10.1111/j.1469-0691.2011.03673.x. PubMed: 21985560.
8. Bax R, Bywater R, Cornaglia G, Goossens H, Hunter P et al. (2001) Surveillance of antimicrobial resistance--what, how and whither? Clin Microbiol Infect 7: 316-325. doi:10.1046/j.1198-743x.2001.00239.x. PubMed: 11442565.
9. Pakyz AL (2007) The utility of hospital antibiograms as tools for guiding empiric therapy and tracking resistance. Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy 27: 1306-1312. doi:10.1592/phco.27.9.1306. PubMed: 17723084.
10. Marchese A, Esposito S, Barbieri R, Bassetti M, Debbia E (2012) Does the adoption of EUCAST susceptibility breakpoints affect the selection of antimicrobials to treat acute community-acquired respiratory tract infections? BMC Infect Dis England, 12: 181. PubMed: 22866984.
11. Kuster SP, Ruef C, Zbinden R, Gottschalk J, Ledergerber B et al. (2008) Stratification of cumulative antibiograms in hospitals for hospital

unit, specimen type, isolate sequence and duration of hospital stay. J Antimicrob Chemother England, 62: 1451-1461. PubMed: 18776189.
12. Rhomberg PR, Fritsche TR, Sader HS, Jones RN (2006) Antimicrobial susceptibility pattern comparisons among intensive care unit and. Diagn Microbiol Infect Dis 56: 57-62. doi:10.1016/j.diagmicrobio.2005.12.009. PubMed: 16631338.
13. Hindler JF, Stelling J (2007) Analysis and presentation of cumulative antibiograms: a new consensus guideline from the Clinical and Laboratory Standards Institute. Clin Infect Dis 44: 867-873. doi:10.1086/511864. PubMed: 17304462.
14. European Committee on Antimicrobial Susceptibility Testing EUCAST Disk diffusion manual 2013. http://www.eucast.org/eucast_susceptibility_testing/disk_diffusion_methodology. Accessed 7 August 2013
15. Hombach M, Böttger EC, Roos M (2013) The critical influence of the intermediate category on interpretation errors in revised EUCAST and CLSI antimicrobial susceptibility testing guidelines. Clin Microbiol Infect 19: E59-E71. doi:10.1111/1469-0691.12090. PubMed: 23210931.
16. Clinical and Laboratory Standards Institute (2008). Developement of in vitro susceptibility testing criteria and quality control parameters: approved guideline, 3rd edn. Wayne, PA: CLSI. CLSI Document M23-A3
17. Kronvall G, Giske CG, Kahlmeter G (2011) Setting interpretive breakpoints for antimicrobial susceptibility testing using disk diffusion. Int J Antimicrob Agents 38: 281-290. doi:10.1016/j.ijantimicag.2011.04.006. PubMed: 21640560.
18. European Committee on Antimicrobial Susceptibility Testing (2010) Setting breakpoints for new agents, EUCAST SOP. I.0. Available: http://www.eucast.org/documents/sops/. Accessed 23 May 2013.
19. European Committee on Antimicrobial Susceptibility Testing EUCAST rationale documents. Available: http://www.eucast.org/documents/rd/. Accessed 7 August 2013
20. CLSI, editor (2010), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20. CLSI, Wayne, PA, USA.
21. CLSI, editor (2011), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21. CLSI, Wayne, PA, USA.
22. CLSI, editor (2012), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptebility Testing: Twenty-second Informational Supplement M100-S22. CLSI, Wayne, PA, USA.
23. CLSI, editor (2013), Clinical and Laboratory Standards Insitute. Performance Standards for Antimicrobial Susceptebility Testing: Twenty-third Informational Supplement M100-S23. CLSI, Wayne, PA, USA.

24. European Commitee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 3.0. Available: http://www.eucast.org/clinical_breakpoints/. Accessed 23 May 2013

25. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R et al. (2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society

for Microbiology and Infectious Diseases. Clin Infect Dis 52: e103-e120. doi:10.1093/cid/ciq257. PubMed: 21292654.

26. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD et al. (2007) Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44: S27-S72. doi: 10.1086/511159. PubMed: 17278083.

27. Binkley S, Fishman NO, LaRosa LA, Marr AM, Nachamkin I et al. (2006) Comparison of unit-specific and hospital-wide antibiograms: potential. Infect Control Hosp Epidemiol 27: 682-687. doi: 10.1086/505921. PubMed: 16807842.