Influence of Clinical Breakpoint Changes from CLSI 2009 to EUCAST 2011 Antimicrobial Susceptibility Testing Guidelines on Multidrug Resistance Rates of Gram-Negative Rods

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Multidrug resistance (MDR) rates of Gram-negative rods were analyzed comparing CLSI 2009 and EUCAST 2011 antibiotic susceptibility testing guidelines. After EUCAST 2011 was applied, the MDR rates increased for *Klebsiella pneumoniae* (2.2%), *Enterobacter cloacae* (1.1%), *Pseudomonas aeruginosa* (0.7%), and *Escherichia coli* (0.4%). A total of 24% of *Enterobacteriaceae* MDR isolates and 12% of *P. aeruginosa* MDR isolates were categorized as MDR due to breakpoint changes.

European countries without national antibiotic susceptibility testing (AST) systems used Clinical and Laboratory Standards Institute (CLSI) guidelines for many years until the European Committee on Antimicrobial Susceptibility Testing (EUCAST) published AST guidelines in 2010 (1). EUCAST AST guidelines contained significantly higher clinical susceptibility breakpoints (CBPs) for disk diffusion compared to those of the CLSI until 2009, in particular for Gram-negative rods (GNR) (2, 3). The CLSI concomitantly increased GNR CBPs for several antibiotic drug classes, particularly newer cephalosporins and carbapenems, in part adopting EUCAST CBPs (4, 5). Changed CLSI guidelines or the use of EUCAST guidelines will lead to more isolates considered cephalosporin and carbapenem nonsusceptible (i.e., intermediate or resistant), and higher GNR MDR rates can be anticipated, since most GNR MDR definitions are based on nonsusceptibility to different antibiotic classes (6–8). Hospital isolation policies and related costs for health care systems will be influenced. The extent to which CBP changes actually influence MDR rates remained unknown.

We analyzed the effect of CBP changes on GNR MDR classification comparing GNR-MDR rates in the University Hospital Zurich (UHZ) in two 17-month periods in which either CLSI 2009 or EUCAST 2011 AST guidelines were applied (CBP values, see Table 1). Analyzed GNR numbers are listed in Table 2.

Disk diffusion AST was performed on Mueller-Hinton agar (Becton Dickinson, Franklin Lakes, NJ) according to CLSI and EUCAST guidelines (2, 3). Antibiotic disks were obtained from i2a (Montpellier, France).

UHZ MDR definitions are based on clinical reasoning, local resistance rates, antibiotic use policies, and international expert proposals (7, 9). *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are classified MDR by nonsusceptibility to at least one agent in three or more out of five drug classes. Drug class criteria (DCC) are nonsusceptibility to (i) $\geq 2/3$ aminoglycosides (amikacin, gentamicin, and tobramycin), (ii) $3/3$ cephalosporins (ceftazidime, cefepime, and ceftriaxone, with the last only for *Enterobacteriaceae*), (iii) $2/2$ fluoroquinolones (ciprofloxacin and levofloxacin), (iv) $2/2$ carbapenems in *P. aeruginosa* and *A. baumannii* (imipenem and meropenem), or $2/3$ carbapenems

(imipenem, meropenem, and ertapenem) in *Enterobacteriaceae*, and (v) nonsusceptibility to piperacillin-tazobactam.

In the EUCAST period, 22/90 *Enterobacteriaceae* MDR isolates (24%) were classified MDR due to CBP changes from CLSI 2009 to EUCAST 2011 (Table 2). CBP related MDR rate increases differed for individual species: 5/9 (56%) *Enterobacter cloacae*, 12/58 (21%) *Escherichia coli*, 5/23 (22%) *Klebsiella pneumoniae*, and 7/59 (12%) *P. aeruginosa* were classified MDR due to higher CBPs (Table 2). No additional *A. baumannii* MDR classifications due to CBP changes were found. For the complete study population, the average increase of *Enterobacteriaceae* MDR rates in the EUCAST 2011 period caused by CBP changes was 0.5%. The increase in MDR rates due to CBP changes in the EUCAST 2011 period was most prominent in *K. pneumoniae* (2.2%), followed by *E. cloacae* (1.1%), *P. aeruginosa* (0.7%), and *E. coli* (0.4%, Table 2).

The majority of the 0.5% ertapenem nonsusceptible *E. coli* isolates in the EUCAST period (16/17, i.e., 94.1%) were classified nonsusceptible due to higher CBPs. However, none of these classifications resulted in a positive carbapenem DCC since meropenem and imipenem remained susceptible. *K. pneumoniae* and *E. cloacae* ertapenem nonsusceptibility rates increased by 1.4 and 12.9% due to increased CBP values. Nonsusceptibility rates to imipenem and meropenem were found at 1.0 and 1.0% for *K. pneumoniae* and at 0.7 and 1.1% for *E. cloacae*, respectively, resulting in a total of three MDR classifications that were directly related to increased carbapenem CBPs.

Increased CBPs for newer cephalosporins (ceftriaxone and cefepime) strongly contributed to additional MDR classifications. This was observed for *E. coli* (9 of 12 additional MDR classifications), *K. pneumoniae* (1 of 5 additional MDR classifications), and *E. cloacae* (2 of 5 additional MDR classifications). One reason for this may be the prevalence of plasmid-encoded extended-spectrum β -lactamases (ESBL) and/or AmpC β -lactamases in MDR

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TABLE 1 Clinical breakpoint values for inhibition zone diameters of CLSI 2009 and EUCAST 2011 guidelines for antibiotic susceptibility testing using disk diffusion

Species or drug ^a	Clinical breakpoint (zone diam [mm]) ^b					
	CLSI 2009			EUCAST 2011		
	S	I	R	S	I	R
<i>Enterobacteriaceae</i>						
Ceftazidime*	≥18	15–17	≤14	≥22	19–21	<19
Ceftriaxone	≥21	14–20	≤13	≥23	20–22	<20
Cefepime	≥18	15–17	≤14	≥24	21–23	<21
Meropenem	≥16	14–15	≤13	≥22	16–21	<16
Imipenem	≥16	14–15	≤13	≥21	15–20	<15
Ertapenem	≥19	16–18	≤15	≥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
Piperacillin-tazobactam*	≥21	18–20	≤17	≥18	15–17	<15
<i>P. aeruginosa</i>						
Piperacillin-tazobactam*	≥18		≤17	≥19		<19
Ceftazidime*	≥18	15–17	≤14	≥16		<16
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
<i>A. baumannii</i> group						
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

^a *, Some EUCAST diameter values cannot directly be compared to CLSI values due to different disk contents (ceftazidime at 10 µg/disk versus 30 µg/disk and piperacillin-tazobactam at 30/6 µg/disk versus 100/10 µg/disk for EUCAST and CLSI, respectively).

^b EUCAST categories without values indicate that an intermediate category does not exist. S, susceptible; I, intermediate; R, resistant.

Enterobacteriaceae or the overexpression of a chromosomal AmpC in *E. cloacae*, e.g., 43 of 58 *E. coli* MDR isolates (74%), 14 of 23 *K. pneumoniae* MDR isolates (61%), and 4 of 9 *E. cloacae* MDR isolates (44%) in the present study were ESBL producers.

Increased fluoroquinolone nonsusceptibility due to CBP changes contributed to additional MDR definitions for all species save *A. baumannii*. The contribution of the fluoroquinolone DCC to additional MDR classifications was higher for *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa* (2 of 5 additional MDR for each *E. cloacae* and *K. pneumoniae* and 3 of 7 additional MDR for *P. aeruginosa*) compared to *E. coli* (1 of 12 additional MDR). The aminoglycoside DCC contributed little to additional MDR classifications (2 of 12 additional *E. coli* MDR, both caused by increased tobramycin nonsusceptibility).

The contribution of ceftazidime and piperacillin-tazobactam CBP changes to increased MDR rates could not be assessed since EUCAST and the CLSI use different disk contents (ceftazidime at 10 µg/disk versus 30 µg/disk, and piperacillin-tazobactam at 30/6

µg/disk versus 100/10 µg/disk for EUCAST and CLSI, respectively). Only EUCAST disk contents were used in the EUCAST period, and reinterpretation using CLSI CBPs was not possible. Thus, the actual increase in MDR rates may have been underestimated.

Regarding the whole study population, the effect of increased CBPs on absolute numbers of MDR classifications was comparably low (22 additional MDR classifications in 4,537 *Enterobacteriaceae* isolates and 7 additional MDR classifications in 977 *P. aeruginosa* isolates [Table 2]). The relative MDR increase of 24% for *Enterobacteriaceae* in a low-prevalence area such as Switzerland may still have a considerable impact on patients and health care system. The consequences of increased MDR reports include, e.g., more patients in contact isolation prone to inadequacies of procedures and adverse events, including reduced patient well-being (10–12). In addition, infection control measures for MDR are associated with substantial costs (13). When introducing new CBPs, MDR definitions may have to be adapted to avoid dispro-

TABLE 2 Change in MDR rates in selected species of Gram-negative rods applying CLSI 2009 and EUCAST 2011 AST guidelines in two adjacent 17-month periods

Species	Total isolates		MDR isolates ^a				
	CLSI 2009 period (n)	EUCAST 2011 period (n)	CLSI 2009 period (n, %)	EUCAST 2011 period (n, %)	Difference EUCAST/ CLSI (%)	Cases due to EUCAST 2011 increased CBP (n, %) ^b	Difference without EUCAST increased CBP (%)
<i>E. coli</i>	3,303	3,295	43 (1.3)	58 (1.8)	0.5	12 (0.4, 21)	0.1
<i>K. pneumoniae</i>	767	830	25 (3.3)	23 (2.8)	−0.5	5 (2.2, 22)	−2.7
<i>E. cloacae</i>	420	448	6 (1.4)	9 (2.0)	0.6	5 (1.1, 56)	−0.5
Total	4,490	4,573	74 (1.6)	90 (2.0)	0.3	22 (0.5, 24)	−0.2
<i>P. aeruginosa</i>	783	977	57 (7.3)	59 (6.0)	−1.3	7 (0.7, 12)	−2.0
<i>A. baumannii</i> group	84	137	13 (15.5)	16 (11.7)	−3.8	0 (0, 0)	−3.8
Total	901	1,114	87 (9.7)	75 (6.7)	−2.9	7 (0.6, 9)	−3.5

^a n, Number of isolates. Where applicable, the percentage(s) is indicated in parentheses.

^b Not including the piperacillin-tazobactam DCC and the extended-spectrum cephalosporin DCC since EUCAST 2011 uses different disk contents than CLSI 2009 for piperacillin-tazobactam and ceftazidime. The increase in MDR rate may therefore be underestimated, in particular for *P. aeruginosa*, since ceftazidime and cefepime alone define the extended-spectrum cephalosporin DCC, and therefore ceftazidime has a higher impact on MDR reporting rates compared to the *Enterobacteriaceae*. The first number in parentheses gives the absolute increase in the percentage of the total number of isolates in the EUCAST period; the second number gives the relative increase in the percentage of all MDR classified isolates in the EUCAST period.

portionate MDR rates and related isolation measures. A limitation of this study is a missing generally accepted MDR definition. However, the MDR definition used was based on current expert recommendations (7). Our results may thus be extrapolated to other institutions.

Our study illustrates the effects of changing from CLSI 2009 to EUCAST 2011 guidelines on GNR MDR rates. Increased-diameter CBPs had a significant effect on MDR rates affecting the number of patients requiring intensified infection control precautions. Furthermore, our results can be of value for institutions that changed from CLSI 2009 to CLSI 2010-2013 guidelines since CLSI, in part, adopted EUCAST CBPs.

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