Original Article

Antimicrobial resistance to cefotaxime and ertapenem in *Enterobacteriaceae*: the effects of altering clinical breakpoints

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Abstract

Introduction: The Clinical and Laboratory Standards Institute (CLSI) updated its antimicrobial susceptibility testing interpretation criteria for *Enterobacteriaceae*. This study assessed the effects of clinical breakpoint changes in the CLSI 2009 to 2012 guidelines on antibiotic susceptibility testing reports.

Methodology: In total, 2,076 non-duplicate clinical isolates of Enterobacteriaceae were analyzed. The disk diffusion method was used for susceptibility testing. The CLSI 2009-12 clinical breakpoints were applied to determine susceptibility of cefotaxime and ertapenem. Combined-disk testing was used for phenotypic confirmation of extended-spectrum beta–lactamase (ESBL) production.

Results: In total, *Enterobacteriaceae* resistance rates to cefotaxime increased from 13.1% using the CLSI 2009 guidelines to 23.6% with the CLSI 2010-12 guidelines, and the resistance rates to ertapenem were 0.4%, 1.0% and 0.8% with CLSI 2009, 2011 and 2012, respectively. Based on the 2010-12 CLSI criteria, all ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* were resistant to cefotaxime. Marked differences in susceptibility to ertapenem between the 2009 CLSI criteria and 2012-12 CLSI criteria were noted in ESBL-producing *K. pneumoniae*.

Conclusions: Breakpoints changes in the updated CLSI guidelines resulted in higher resistance rates to cefotaxime and ertapenem. In addition, the effects were different in individual *Enterobacteriaceae* species. As a result, clinicians may opt to use alternative antimicrobial agents. Upon implementation of the newer CLSI guidelines, laboratories should be aware of the possible consequences and closely monitor the effects.

Key words: Drug Resistance; *Enterobacteriaceae*; microbial sensitivity tests; cefotaxime; ertapenem

J Infect Dev Ctries 2014; 8(3):289-296. doi:10.3855/jidc.3335

(Received 24 January 2013 – Accepted 22 April 2013)

Introduction

Antimicrobial susceptibility testing is one of the most important tasks in clinical microbiology laboratories. It is not only a valuable guide for antibiotic therapy but also an important epidemiological tool to monitor resistant organisms. Hence, antimicrobial susceptibility testing should be performed according to generally accepted and clinically relevant guidelines.

Several laboratories, particularly in countries without a national antibiotic susceptibility testing system, have been using the Clinical and Laboratory

Standards Institute (CLSI) guidelines to interpret antibiotic-sensitivity testing. Recently, the CLSI updated and included its recommendations into the CLSI 2010, 2011 and 2012 guidelines, which include major changes in the antibiotic susceptibility testing for *Enterobacteriaceae*. Inhibition zone diameter breakpoints for *Enterobacteriaceae* defining susceptibility to third-generation cephalosporins and carbapenems are significantly higher in the CLSI 2010/11 version compared to the CLSI 2009 breakpoints [1-3]. Recently, the CLSI recommended slightly lower zone diameter susceptibility breakpoints for ertapenem in its 2012 updates, while breakpoints for third-generation cephalosporins remained unchanged [4]. In addition, with the increase of cephalosporin breakpoints in the CLSI 2010-12 guidelines, it is no longer necessary to detect extended-spectrum beta–lactamase (ESBL) producers and to adjust all cephalosporin sensitivity results to resistance, as previously recommended by CLSI 2009 guideline.

Nevertheless, a controversy arose with regard to the appropriateness of current recommendations. Some investigators continue to support the importance of knowing the ESBL status in an isolate to ensure proper therapy [5, 6]. Especially in ESBL-endemic areas, where therapeutic options are very limited due to associated co-resistance in other classes of antimicrobial agents [7, 8]**.** Moreover, in a survey on 144 infectious disease specialists, most respondents reported that they would not treat *Enterobacteriaceae* infections based on the current breakpoints alone without testing for ESBL [9]. Some experts even suggested that clinical laboratories should not use the most recent CLSI breakpoints [9, 10].

Adoption of new guidelines and breakpoints can have significant effects on antibiotic susceptibility testing reports, with concomitant changes in antibiotic prescriptions by clinicians. Prior to the implementation of new guidelines in diagnostic microbiology laboratories, the impact of changes in antibiotic susceptibility testing reports needs to be evaluated.

This study compared the new standards with respect to changes in cefotaxime and ertapenem among *Enterobacteriaceae* isolates.

Methodology

Clinical isolates

Between March 2011 and June 2011, the study collected consecutive non-duplicate clinical isolates of *Enterobacteriaceae* in our clinical microbiological laboratory. Our clinical laboratory primarily serves a 1500-bed veterans hospital in central Taiwan. Identification to the species level was performed by routine laboratory methods including the Vitek 2 system (bioMérieux, Marcy l'Etoile, France).

Susceptibility testing

The Kirby-Bauer disk diffusion test was used for susceptibility testing. Cefotaxime and ertapenem were tested during the study period. Antibiotics discs were obtained from Becton Dickinson (BD Diagnostic Systems, Cockeysville, USA). Susceptibility testing was done on Mueller-Hinton agar (BD Diagnostic Systems, Cockeysville, USA) using McFarland 0.5 from overnight cultures followed by incubation at 35℃ for 16-18 hours. Inhibition zone diameters were determined and recorded by two experienced microbiologists.

ESBL testing

In keeping with CLSI guideline, phenotypic identification of ESBL production in *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis* isolates was determined using double disk diffusion method [4, 11]. Briefly, ESBL combined-disk testing was performed with commercial disks (BD Diagnostic Systems, Cockeysville, USA), according to the manufacturer's instructions, using Mueller-Hinton II (BD Diagnostic Systems, Cockeysville, USA) agar and an inoculation turbidity of 0.5 McFarland. The disks used were cefotaxime 30 μg, cefotaxime-clavulanic acid 30/10 μg, ceftazidime 30 μg and ceftazidime-clavulanic acid 30/10 μg.

Comparison of CLSI 2009-2012 breakpoints

Inhibition zone diameters were interpreted according to the CLSI 2009-12 recommended zone diameter breakpoints for *Enterobacteriaceae* (Table 1). The CLSI 2009 susceptibility breakpoint for cefotaxime was ≥ 23 mm which increased to ≥ 26 mm based on the CLSI 2010-12 guidelines. The CLSI 2009 susceptibility breakpoint for ertapenem was ≥ 19 mm using CLSI 2009, which increased to ≥ 23 mm using CLSI 2011 and reduced to ≥ 22 mm with CLSI 2012. Rates of susceptibility to cefotaxime and ertapenem measured using 2009 CLSI breakpoints were compared with those obtained using 2010-12 CLSI breakpoints [1, 3, 4].

Table 1. CLSI 2009-12 susceptibility testing breakpoints of *Enterobacteriaceae*

	Clinical breakpoints (mm)											
	CLSI 2009			CLSI 2010			CLSI 2011			CLSI 2012		
Drug	S	I	R	S	I	R	S	I	R	S	I	R
cefotaxime	≥23	15-22	≤14	≥26	23-25	≤22						
ertapenem	≥19	16-18	≤15				≥23	20-22	≤19	≥22	19-21	≤18

Categories without value: interpretation guidelines were not changed compared to the previous version.
S, susceptible; I, intermediate; R, resistant.

Table 2. Susceptibility of *Enterobacteriaceae* clinical isolates according to CLSI 2009-12

	Assignment of *Enterobacteriaceae* clinical isolates (%) to interpretative category											
	CLSI 2009			CLSI 2010			CLSI 2011			CLSI 2012		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
E.coli												
cefotaxime	663	120	125	646	17	245						
%	73.0	13.2	13.8	71.1	1.9	27.0						
ertapenem	907	1	0				898	10	0	904	4	0
%	99.9	0.1	0.0				98.9	1.1	0.0	99.6	0.4	0.0
Klebsiella pneumoniae												
cefotaxime	403	32	55	388	15	87						
%	82.2	6.5	11.2	79.2	3.1	17.8						
ertapenem	479	4	7				457	21	12	463	16	11
%	97.8	0.8	1.4				93.3	4.3	2.4	94.5	3.3	2.2
Serratia marcescens												
cefotaxime	81	25	16	72	9	41						
%	66.4	20.5	13.1	59.0	7.4	33.6						
ertapenem	122	0	0				122	0	0	122	0	0
%	100.0	0.0	0.0				100.0	0.0	0.0	100.0	0.0	0.0
Enterobacter cloacae												
cefotaxime	98	8	21	93	5	29						
%	77.2	6.3	16.5	73.2	3.9	22.8						
ertapenem	125	2	0	113	11	3				120	5	2
%	98.4	1.6	0.0	89.0	8.7	2.4				94.5	3.9	1.6
Proteus mirabilis												
cefotaxime	104	8	5	102	2	13						
%	88.9	6.8	4.3	87.2	1.7	11.1						
ertapenem	117	0	0	116	1	0				116	1	0
%	100.0	0.0	0.0	99.1	0.9	0.0				99.1	0.9	0.0
Morganella morganii												
cefotaxime	56	4	4	55	1	8						
%	87.5	6.3	6.3	85.9	1.6	12.5						
ertapenem	64	0	0	64	0	0				64	0	0
%	100.0	0.0	0.0	100.0	0.0	0.0				100.0	0.0	0.0
Citrobacter freundii												
cefotaxime	24	6	33	22	2	39						
%	38.1	9.5	52.4	34.9	3.2	61.9						
ertapenem	62	0	1	58	4	1				60	2	1
%	98.4	0.0	1.6	92.1	6.3	1.6				95.2	3.2	1.6
Citrobacter koseri												
cefotaxime	47	5	3	46	1	8						
%	85.5	9.1	5.5	83.6	1.8	14.5						
ertapenem	55	0	0	55	0	0				55	0	0
%	100.0	0.0	0.0	100.0	0.0	0.0				100.0	0.0	0.0

Table 2. ***(continued)*** Susceptibility of *Enterobacteriaceae* clinical isolates according to CLSI 2009-12

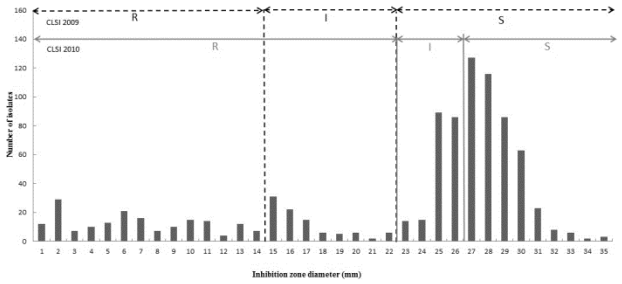
	Assignment of *Enterobacteriaceae* clinical isolates (%) to interpretative category											
	CLSI 2009			CLSI 2010			CLSI 2011			CLSI 2012		
Species/drug	S	I	R	S	I	R	S	I	R	S	I	R
Enterobacter aerogenes												
cefotaxime	29	5	7	27	2	12						
%	70.7	12.2	17.1	65.9	4.9	29.3						
ertapenem	40	1	0	37	2	2				39	1	1
%	97.6	2.4	0.0	90.2	4.9	4.9				95.1	2.4	2.4
Klebsiella oxytoca												
cefotaxime	38	0	2	37	1	2						
%	95.0	0.0	5.0	92.5	2.5	5.0						
ertapenem	40	0	0	40	0	0				40	0	0
%	100.0	0.0	0.0	100.0	0.0	0.0				100.0	0.0	0.0
Proteus vulgaris												
cefotaxime	29	3	0	28	1	3						
%	90.6	9.4	0.0	87.5	3.1	9.4						
ertapenem	32	0	0	31	1	0				32	0	0
%	100.0	0.0	0.0	96.9	3.1	0.0				100.0	0.0	0.0
All Enterobacteriaceae												
cefotaxime	1586	218	272	1529	57	490						
%	76.4	10.5	13.1	73.7	2.7	23.6						
ertapenem	2060	8	8	2006	50	20				2030	30	16
%	99.2	0.4	0.4	96.6	2.4	1.0				97.8	1.4	0.8

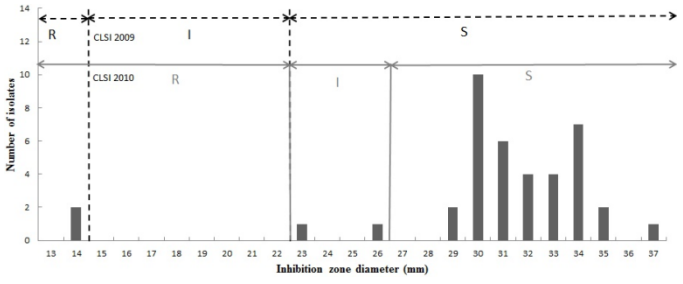
S, susceptible; I, intermediate; R, resistant; Categories without value: interpretation guidelines were not changed compared to the previous version.

Table 3. Susceptibilities of ESBL-positive and ESBL-negative isolates of *E. coli, Klebsiella pneumoniae* to cefotaxime and ertapenem using CLSI 2009-2012 breakpoint criteria

organism(n)	**Percent susceptible (%)**				
	Cefotaxime		Ertapenem		
	CLSI 2009	CLSI 2010	CLSI 2009	CLSI 2011	CLSI 2012
E. coli					
ESBL + (101)	0.9%	0.0%	100.0%	99.0%	99.0%
ESBL - (807)	82.0%	80.0%	99.9%	99.0%	99.4%
K. pneumoniae					
ESBL + (53)	3.7%	0.0%	92.5%	77.4%	81.1%
ESBL - (437)	91.8%	88.6%	98.4%	95.2%	96.1%

Figure 1. Distribution of zone diameters for cefotaxime and CLSI clinical breakpoints in *E. coli* (Fig 1a) and *Klebsiella oxytoca* (Fig 1b). Vertical lines represent the zone diameter breakpoints defined in the CLSI 2009 and CLSI 2010 guidelines.





Results

A total of 2,076 isolates of Enterobacteriaceae were collected during the study period. They comprised 908 *E. coli*, 490 *K. pneumoniae*, 122 *Serratia marcescens*, 127 *Enterobacter cloacae*, 117 *P. mirabilis*, 64 *Morganella morganii*, 63 *Citrobacter freundii*, 55 *Citrobacter koseri*, 41 *Enterobacter aerogenes*, 32 *Proteus vulgaris*, 40 *K. oxytoca*, 6 *Klebsiella ozaenae*, 6 *Providencia stuartii* and 5 *Providencia rettgeri*. Of note, *E. coli* and *K. pneumoniae* together accounted for 67.3% of all *Enterobacteriaceae* isolated.

In aggregate, resistance rates to cefotaxime increased from 13.1% (CLSI 2009) to 23.6% (CLSI 2010-12). The resistance rates to ertapenem were 0.4% (CLSI 2009), 1.0% (CLSI 2011), and 0.8% (CLSI 2012). The most important species-specific changes are described in Table 2.

E. coli

As observed in the trends of *Enterobacteriaceae*, resistance rate to cefotaxime increased from 13.8% with CLSI 2009 to 27.0% with CLSI 2010-12. Resistance rates to ertapenem remained at 0%.

K. pneumoniae

The resistance rate to cefotaxime increased from 11.2% (CLSI 2009) to 17.8% (CLSI 2010-12). The resistance rates to ertapenem increased from 1.4% with CLSI 2009 to 2.4% with CLSI 2011 and to 2.2% with CLSI 2012.

S. marcescens

The resistance rate to cefotaxime increased from 13.1% to 33.6% with CLSI 2009 and CLSI 2010-12, respectively. Resistance rates to ertapenem remained at 0%.

E. cloacae, E. aerogenes

The resistance rate to cefotaxime in *E. cloacae* and *E. aerogenes* increased from 16.5% and 17.1% to 22.8% and 29.3% with CLSI 2009 and CLSI 2010-12, respectively. The resistance rates to ertapenem in *E. cloacae* and *E. aerogenes* changed from 0% with CLSI 2009 to 2.4% and 4.9% with CLSI 2011 and to 1.6% and 2.4% with CLSI 2012, respectively.

P. mirabilis, P. vulgaris, C. koseri, M. morganii

The resistance rate to cefotaxime in *P. mirabilis*, *P. vulgaris*, *C. koseri* and *M. morganii* increased from 4.3%, 0%, 5.5%, and 6.3% to 11.1%, 9.4%, 14.5% and 12.5% using CLSI 2009 with CLSI 2010-12, respectively. Resistance rates to ertapenem remained at 0%.

ESBL

Overall, ESBL-producers accounted for 11.1% (101/908) of *E. coli* isolates and 10.8% (53/490) of *K. pneumoniae* isolates. For cefotaxime, none of the ESBL-producing *E. coli* and *K. pneumoniae* was regarded as susceptible using the CLSI 2010 recommendation.

Depending on the bacterial species, there were different effects on susceptibility. Similar susceptibility patterns were noted for both ESBL-positive and ESBL-negative *E. coli* (Table 3). Only minor reductions in susceptibility of *E. coli* to cefotaxime and ertapenem were observed when comparing CLSI 2009 and CLSI 2010-12 breakpoints. However, the rates of susceptibility to cefotaxime and ertapenem among *K. pneumoniae* changed to a greater extent, particularly in ESBL-positive *K. pneumoniae*. Susceptibilities of cefotaxime against ESBL-positive *K. pneumoniae* were 3.7% and 0% for CLSI 2009 and CLSI 2010 breakpoints, respectively. Susceptibilities of ertapenem against ESBL-positive *K. pneumoniae* were 92.5%, 77.4%, and 81.1% for CLSI 2009, CLSI 2010 and CLSI 2012 breakpoints, respectively. In addition, three of the 40 *K. oxytoca* isolates and two of the 117 *P. mirabilis* isolates were ESBL-positive. One of the three ESBL-positive *K. oxytoca* isolates were susceptible to cefotaxime with CLSI 2009 breakpoints and became intermediate susceptible to cefotaxime with CLSI 2010-12 breakpoints. Two ESBL-positive *P. mirabilis* isolates were intermediate susceptible to cefotaxime with CLSI 2009 breakpoints and were classed as resistant with CLSI 2010-12 breakpoints. All ESBL-positive *K. oxytoca* and *P. mirabilis* isolates were susceptible to ertapenem.

Discussion

In this study, we found that breakpoint changes in the CLSI guidelines resulted in higher resistance rates to cefotaxime and ertapenem. In addition, the effects vary among individual *Enterobacteriaceae* species.

Our findings, in agreement with other studies, suggest that the application of new CLSI standards results in significantly more *Enterobacteriaceae* being reported resistant to extended-spectrum cephalosporins and carbapenems [12]. As a consequence, clinicians will most likely prescribe other antimicrobial classes. As the amount of antibiotic used and the development of resistance are generally linked, increased reports of *Enterobacteriaceae* resistant to third-generation

cephalosporins and carbapenems may cause greater usage of other antibiotics and changes in antibiograms [13, 14]. Further clinical studies are required to determine the long-term effects on the antimicrobial stewardship policy and the trends in antibiotic resistance.

In addition, application of higher diameter breakpoints will increase the number of multipledrug-resistant *Enterobacteriaceae*, which is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, including carbapenems and extended spectrum cephalosporins [15]. With the implementation of CLSI 2010-12 guidelines, *Enterobacteriaceae* will increasingly be reported as multidrug-resistant and carbapenem-resistant, resulting in higher rates of patient isolation as well as higher costs arising from increased hospital hygiene measures and the requirement for confirmatory tests in the laboratory for multidrug-resistant *Enterobacteriaceae*.

The effects of the breakpoints changes from CLSI 2009 to CLSI 2010-2012 differ among *Enterobacteriaceae* species: for instance, while the resistance rate for cefotaxime in *K. oxytoca* remains almost unchanged, isolates of *E. coli* are more frequently reported as resistant to cefotaxime using the CLSI 2010/2011 criteria (Table 2). This finding is probably related to the different distribution in natural diameter among individual *Enterobacteriaceae* species as compared with the breakpoints, which are defined for the whole *Enterobacteriaceae* family (Figures 1a and 1b). For instance, 13.2% of *E. coli* had intermediate susceptibility to cefotaxime using the CLSI 2009 guideline, and were classified as resistant in the 2010-12 guidelines. In contrast, none of the *K. oxytoca* isolates had a zone diameter of 15-22 mm. Regarding the uniform resistant breakpoint definitions for both drugs and species, the lower average diameters of *E.coli* readings explain the greater increase in cefotaxime resistance rates compared to those of *K. oxytoca*. Other investigators also found a more limited change in the susceptibility to cefotaxime among *K. oxytoca* between the CLSI 2009 and CLSI 2010-12 recommendations [6]. We suggest that species-adapted breakpoints for *Enterobacteriaceae* species would eliminate artifacts and improve interpretation of antibiotic susceptibility testing.

The CLSI 2010 guidelines have led to a fundamental change of antibiotic susceptibility testing results for ESBL-producing isolates. With the updated interpretive criteria, it is no longer necessary to perform routine ESBL testing and edit results in confirmed ESBL-producers. The adoption of the new CLSI guidelines raises the question on how sensitive and specific CLSI 2010-12 breakpoints are for third-generation cephalosporins in the detection of ESBL. Overall, the study shows that CLSI 2010-12 breakpoints successfully designated a larger number of ESBL-positive isolates as cefotaxime-resistant. Other studies also demonstrated the high sensitivity of the CLSI ESBL screening breakpoint for cefotaxime [16].

Implementation of the CLSI 2011/12 interpretive criteria may have increased the carbapenem resistance rate in some *Enterobacteriaceae* species. Generally, ertapenem were active against more than 90% of *Enterobacteriaceae* isolates; however, there were wide variations in the ertapenem susceptibility between *Enterobacteriaceae* species in this study. Previous studies showed that the rate of ertapenem resistance in *Enterobacteriaceae* isolates worldwide was low based on the CLSI 2011 criteria [17]. More recently, Huang *et al*. reported that the resistance rate may be higher in some *Enterobacteriaceae* species and certain countries [18]. In our study, only 77.4% of ESBL-positive *K. pneumoniae* isolates were susceptible to ertapenem according to the CLSI 2011 criteria, while ertapenem was active in more than 99% of *E. coli* isolates. Some *Enterobacteriaceae* species demonstrated a substantial change with different criteria. The rates of susceptibility to ertapenem among *Enterobacteriaceae* as measured by the CLSI 2009 and 2011 criteria differed by more than 5% among ESBL-positive *K. pneumoniae* (15.1%), *E. cloacae* (9.4%), *C. freundii* (6.3%) and *E. cloacae* (7.4%); however, when the results were interpreted by the CLSI 2012 criteria, only ESBL-positive *K. pneumoniae* differed by more than 5%. There are wide variations in the species-dependent reduction in susceptibility among countries. In Hong Kong, *Proteus* spp., *Enterobacter* spp., *Providencia* spp. and *Morganella* spp. had the greatest reduction in the imipenem susceptibility rate with the new CLSI breakpoints [19].

This study was limited to the moderate to high antimicrobial resistance found in Taiwan [20]. The increase in resistance rates due to changes in the guidelines here reported, may be more obscure compared to changes seen in populations with lower levels of resistance, since diameter distributions are shifted to lower mean values. Another limitation of this study was the inclusion of only cefotaxime and ertapenem. In order to better understand the importance of other antibiotics in clinical practice, more studies are needed to investigate the impact of the breakpoint change in a complete antibiogram.

Furthermore, in this study the underlying resistance mechanisms were not identified. Some studies have reported data suggesting that different resistance mechanisms can have varying effects on categorization based on the old and new interpretive criteria [21, 22]. In addition, the identification of ESBL production was performed by using only the combination disk diffusion method. Some ESBL-producing organisms may be missed with the phenotypic confirmatory test. The application of genotypic confirmatory tests or Etest ESBL strips is useful for *in vitro* confirmation of ESBL [23].

Conclusions

Widespread implementation of the new CLSI standards for antibiotic susceptibility testing make results more comparable worldwide. However, further evidence-based clinical studies should validate the proposed guideline changes. In the implementation stage of the new CLSI standards, laboratories and clinicians need to be aware of the implications of modified antibiotic susceptibility testing reports in clinical practice, such as the effects on antibiotic prescription. In addition, to ensure early detection of emerging resistance, species-related zone breakpoints should be considered.

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Conflict of interests: No conflict of interests is declared.