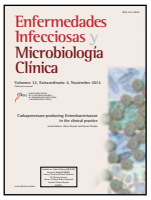
Enferm Infecc Microbiol Clin. 2014;**32(Supl 4)**:33-40

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc



Breakpoints for carbapenemase-producing Enterobacteriaceae: Is the problem solved?

Rafael Cantón[a,b,*], Andrés Canut[c], María Isabel Morosini[a,b] and Antonio Oliver[b,d]

[a]*Departamento de Microbiología, Hospital Universitario Ramón y Cajal e Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain*
[b]*Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain*
[c]*Departamento de Microbiología, Hospital Universitario de Álava, Vitoria, Spain*
[d]*Servicio de Microbiología y Unidad de Investigación, Hospital Universitario Son Espases, Instituto de Investigación Sanitaria de Palma (IdISPa), Palma de Mallorca, Spain*

Keywords:
Carbapenemases
Clinical breakpoints
PK/PD breakpoints
Enterobacteriaceae
Pharmacokinetic/pharmacodynamics

ABSTRACT

The imipenem and meropenem breakpoints for Enterobacteriaceae established by the Clinical and Laboratory Standards Institute (CLSI) are somewhat lower than those established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), but are identical for ertapenem and doripenem. The differences are primarily due to the various pharmacokinetic/pharmacodynamic (PK/PD) approaches used to define these breakpoints. Both approaches use the Monte Carlo simulation with a probability of target attainment (PTA) for reaching the PD target of free drug concentration above the minimum inhibitory concentration (MIC) at least 40% of the time (~40%*f*T >MIC). EUCAST uses PTA mean values with confidence intervals (CIs) of 95% and 99%, whereas the CI used by CLSI is 90%. In addition, CLSI uses an "inflated variance" that takes into account the variability of PK parameters in various types of patients, particularly those who are critically ill. By employing this approach, the susceptible CLSI breakpoint captures a higher number of carbapenemase-producing Enterobacteriaceae (CPE) than EUCAST. EUCAST, however, has recently defined cut-off values for screening CPE. Both committees recommend reporting carbapenem susceptibility results "as tested," demonstrating carbapenemase production only for epidemiological purposes and infection control. New clinical data could potentially modify this recommendation because carbapenemase production also influences specific treatment guidance concerning carbapenems in combination with other antimicrobials in infections due to CPE. This advice should not be followed when imipenem or meropenem MICs are >8 mg/L, which is coincident with the EUCAST resistant breakpoints for these carbapenems.

© 2014 Elsevier España, S.L. All rights reserved.

Puntos de corte para las enterobacterias productoras de carbapenemasas: ¿está resuelto el problema?

Palabras clave:
Carbapenemasas
Puntos de corte clínicos
Puntos de corte PK/PD
Enterobacterias
Farmacocinética/farmacodinamia

RESUMEN

Los puntos de corte de imipenem y meropenem definidos por el Clinical and Laboratory Standards Institute (CLSI) para enterobacterias son ligeramente menores a los del European Committee of Antimicrobial Susceptibility Testing (EUCAST) e iguales para ertapenem y doripenem. Las diferencias son esencialmente debidas a las diferentes aproximaciones farmacocinéticas/farmacodinámicas (PK/PD) utilizadas. Ambos comités utilizan la simulación de Monte Carlo, con una probabilidad de alcanzar el objetivo terapéutico PD (PTA) de una concentración de fármaco libre de, al menos, un 40% por encima del valor de la concentración mínima inhibitoria —CMI— (~40%*f*T > CMI). EUCAST utiliza la media de PTA con intervalos de confianza (IC) del 95 y el 99%, mientras que para el CLSI el IC es del 90%. Además, el CLSI utiliza un "varianza inflada", que tiene en cuenta la variabilidad de los parámetros PK en diferentes pacientes, en particular en los críticos. Con esta aproximación, el punto de corte de sensibilidad del CLSI detecta un mayor número de enterobacterias productoras de carbapenemasas (EPC) que EUCAST. No obstante, EUCAST ha definido recientemente un punto de corte de cribado para detectar EPC. Ambos comités recomiendan informar los resultados de sensibilidad de las carbapenemas sin aplicar reglas de experto y demostrar la producción de carbapenemasas solamente por motivos epidemiológicos y de control de infección. Los nuevos datos clínicos podrían modificar esta recomendación, ya que la producción de carbapenemasas permitiría el tratamiento con carbapenemas en combinación con otros antimicrobianos en las infecciones por EPC. Esta recomendación debe evitarse cuando las CMI de imipenem o meropenem sean > 8 mg/l, valor que coincide con el punto de corte de resistencia de EUCAST para ambas carbapenemas.

© 2014 Elsevier España, S.L. Todos los derechos reservados.

*Corresponding author.
E-mail adress: rafael.canton@salud.madrid.org (R. Cantón).

0213-005X/$ - see front matter © 2014 Elsevier España, S.L. Todos los derechos reservados.

Introduction

In recent years, carbapenem antibiotics have been considered suitable treatment options for infections due to multidrug-resistant Gram-negative bacteria.[1] However, with the increase in resistance mechanisms, particularly carbapenemases in Enterobacteriaceae, their value has been questioned. Nevertheless, due to the lack of new antimicrobials and with the support of clinical data, they are still deemed as major therapeutic options (primarily in combination with other antimicrobials) for treatment of bacterial infections, including those due to carbapenemase-producing Enterobacteriaceae (CPE).[2-6] In this scenario, it is important to identify the infections associated with CPE isolates that can be treated with carbapenems either alone or in combination with other drugs. This therapy should be guided using the clinical microbiology breakpoint values from *in vitro* susceptibility testing studies.

Breakpoints are the minimum inhibitory concentration (MIC) or inhibition zone values established to separate those isolates from which a high therapeutic success is expected when using an antibiotic classified as "clinically susceptible" from those for which a high probability of therapeutic failure is expected when using an antibiotic classified as "clinically resistant."[7] There has recently been debate on which breakpoints are more suitable for the interpretation of antimicrobial susceptibility testing and patient treatment. The position of considering resistance mechanisms as a primary focus in the breakpoint definition, including those mechanisms expressed at a low level, has been strongly defended.[8,9] However, a more pragmatic position considers correlations of MIC values with clinical outcomes as one of the most important aspects for breakpoint definition. Although there are scarce clinical data for this approach, this position has been supported by pharmacokinetic (PK) and pharmacodynamic (PD) models in which recent breakpoints are primarily influenced.[10,11] Both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the primary committees defining global breakpoints, have been part of this debate. This breakpoint-setting process began with extended-spectrum cephalosporins and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and has been expanded to include carbapenems and CPE. In this article we review breakpoints for carbapenems in Enterobacteriaceae and their relevance for CPE and infections due to these organisms.

Carbapenem breakpoints: CLSI *versus* EUCAST

Current carbapenem breakpoints from both the CLSI and EUCAST committees and their evolution over time are included in Table 1. The CLSI breakpoints for imipenem and meropenem are lower than those established by EUCAST, whereas ertapenem and doripenem breakpoints are currently the same after the review processes in both committees over the past 2 years.[12,13] There were 2 primary reasons for the definition of lower imipenem and meropenem breakpoints by CLSI in 2010 when compared with those previously published by EUCAST.[14] The first was to address the requirement for determining carbapenem breakpoints to capture and efficiently detect all carbapenemase producers (primarily KPC carbapenemases) and to avoid the use of the Modified Hodge Test, a technique with insufficient accuracy to detect all CPEs.[9,15] The second was the use of PK/PD Monte Carlo simulations that take into account the variability of PK parameters in various types of patients (particularly those who are critically ill) when compared with those obtained in volunteers (inflated variance).[16]

The EUCAST carbapenem breakpoints were published in 2006 during the process of harmonizing the breakpoints in Europe, using standard Monte Carlo PK/PD models.[17,18] These breakpoints were established not for detecting resistance mechanisms (e.g., carbapenemase producers) but for maintaining the aim of these values for better definition of patient treatment and prediction of clinical outcomes. In addition, EUCAST defined the epidemiological cut-off values (ECOFFs) to distinguish wild type isolates (those with no resistance mechanism) from non-wild type isolates (those with acquired resistance mechanisms) (Table 1). EUCAST has also recently published guidelines for the detection of resistance mechanisms, including CPEs for which the algorithm for this detection uses imipenem, meropenem and ertapenem screening cut-off values that are different from clinical breakpoints (see below).[19]

The ertapenem breakpoints from CLSI and EUCAST currently have identical values (Table 1). This situation was encouraged by the revision of CLSI breakpoints performed after observing that in June 2010[14] the lower breakpoint values established overcall resistance to

Table 1
Carbapenem breakpoint (mg/L) evolution in Enterobacteriaceae over time

	Year[a]	FDA[b]	CLSI		Year[a]	EMA and EUCAST[c]			
		S	S	R		S	R	ECOFF	Screening cutoffs[d]
Imipenem	≤2010[e]		≤4	≥16					
	2010[f]-2014	≤4	≤1	≥4	2006-2014	≤2	>8	≤0.5; ≤1[g]	≥0.12
Meropenem	≤2010[e]		≤1	≥16					
	2010[f]-2014	≤4	≤1	≥4	2006-2014	≤2	>8	≤0.125	1
Ertapenem	≤2010[e]		≤2	≥8					
	2010[f]		≤0.25	≥1					
	2012-2014	≤2	**≤0.5**	**≥2**	2006-2014	**≤0.5**	**>1**	≤0.06	≥0.12
Doripenem					2008-2013	≤1	>4	≤0.12	
	2010[f]-2014	≤0.5	**≤1**	**≥4**	2014	**≤1**	**>2**	≤0.12	ND

Bold numbers indicate currently identical breakpoints for CLSI and EUCAST. CLSI: Clinical and Laboratory Standards Institute; ECOFF: epidemiological cut-off values; EMA: European Medicines Agency; EUCAST: European Committee on Antimicrobial Susceptibility Testing; FDA: Food and Drug Administration; ND: not defined; R: resistant; S: susceptible.
[a]Year of publication of the breakpoints and included in the corresponding CLSI and EUCAST breakpoint tables.
[b]FDA breakpoints are those included in the drug label information and can be found at http://www.fda.gov/
[c]EMA breakpoints are those defined by EUCAST and can be found in summary of products characteristic (SmPC) of each drug at http://www.ema.europa.eu/ema/
[d]Proposed by EUCAST in 2013.
[e]January 2010.
[f]June 2010.
[g]*E. coli* and *K. pneumoniae*.

this compound in Enterobacteriaceae. This result was particularly observed in CTX-M ESBL-producing isolates that might have slightly increased ertapenem MIC values despite the absence of carbapenemase production.[9,15,20] The doripenem breakpoints are also identical in both committees due to a recent review process completed by EUCAST. This review was performed after the publication of the results of a clinical trial in which, using a fixed 7-day course of doripenem (1 g as a 4-h infusion every 8 h), clinical outcomes were lower than expected when compared with the comparator (imipenem, 1 g as a 1-h infusion every 8 h).[21] Reassessment of the PK/PD data ended with the modification of the resistance breakpoint (Table 1).

Carbapenem epidemiological breakpoints, clinical breakpoints and resistance mechanisms

Although all carbapenemases are characterized by their capacity to hydrolyze carbapenems, carbapenem MICs in CPE isolates might vary considerably, ranging from fully susceptible to highly resistant, according to current CLSI and EUCAST clinical breakpoints. Several factors contribute to this significant MIC variability: the species involved; the total amount of carbapenemase produced; the carbapenemase expression level; the carbapenem tested; the selection of adjuvant chromosomal resistance mechanisms, such as the loss of porins or the overexpression of efflux pumps; and the simultaneous production of multiple beta-lactamases, including several carbapenemases and/or combinations with ESBLs, chromosomal or plasmid AmpC enzymes.[3,9,15,21-26] Concerning the enzyme involved, OXA-48-type carbapenemases are typically associated with lower carbapenem MICs and resistance rates (they are frequently susceptible to imipenem and meropenem), followed by MBL-producing strains, whereas KPC enzymes are associated with the highest MICs and resistance levels.[24]

Significant MIC variability and a lack of agreement between various susceptibility testing methods have been reported for clonal *Klebsiella pneumoniae* isolates producing the MBL VIM-1. The presence of heteroresistant populations demonstrating various carbapenemase and/or porin expression levels is considered to be the cause of these discrepancies.[22] Likewise, for KPC enzymes, the gene copy number, the level of production of the enzymes and the presence or absence of Ompk35 and Ompk36 porins have been shown to significantly modify carbapenem MIC values.[23]

Due to this significant MIC variability, the CLSI and EUCAST clinical breakpoints might be useful from a therapeutic perspective, but are not adequate (nor intended, particularly those from EUCAST) for the detection of CPE. For this purpose, the ECOFFs (available on the EUCAST website [http://www.eucast.org]), which are based on wild-type MIC distributions of thousands of strains, are much more helpful. As shown in Table 1, the ECOFFs are significantly lower than the susceptible clinical breakpoints, particularly for ertapenem (0.06 mg/L vs. 0.5 mg/L) and meropenem (0.125 mg/L vs. 2 mg/L) (Fig. 1). The use of ECOFFs dramatically increases the sensitivity of carbapenem MICs to detect CPE, but also significantly reduces the specificity. Overall, the highest specificity has been reported in meropenem, whereas sensitivity is found to be the highest in ertapenem.[9,15,27] Frequent false positive results are caused by the combination of ESBL or AmpC beta-lactamases with the loss of porin expression.[9,15,20] The EUCAST guidelines for the detection of resistance mechanisms have recently established carbapenem MIC screening cut-off values for the detection of CPE, and include MIC values of >0.12 mg/L for meropenem and ertapenem and MIC values of >1 mg/L for imipenem (Table 1).[19] It should be noted that these screening cut-off values are slightly higher than the ECOFFs, increasing the specificity for the detection of CPE; however, false positive results are common. Consequently, the presence of carbapenemases should be confirmed with phenotypic, biochemical or genetic tests.[19]

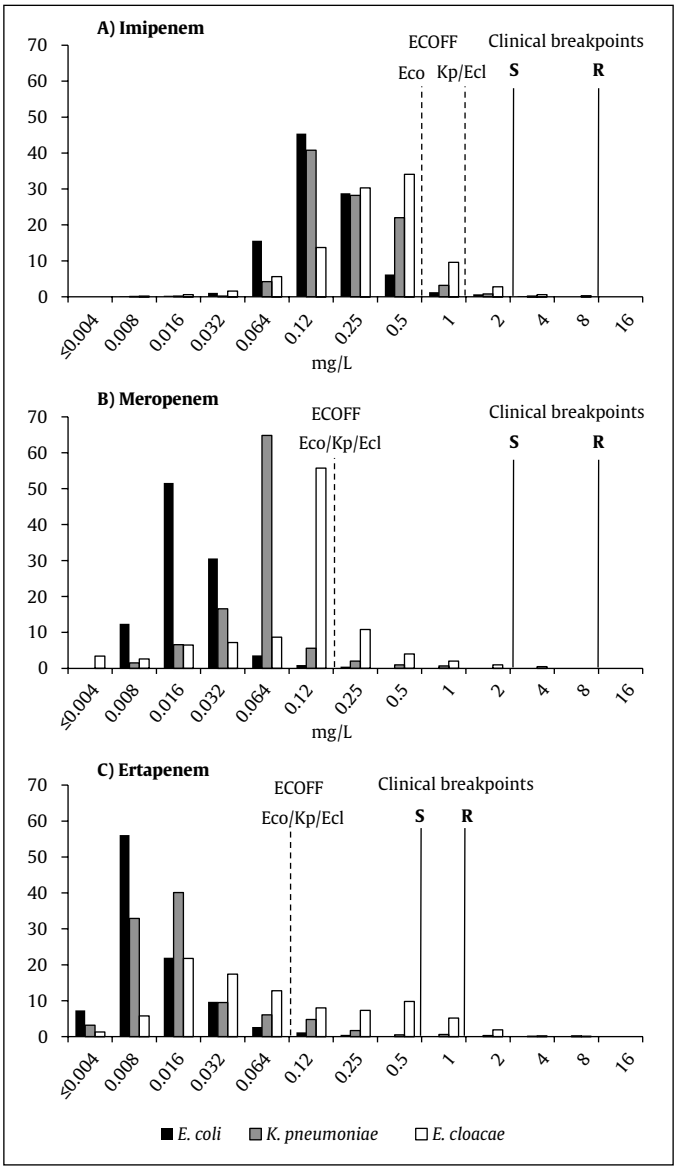


Figure 1. Minimum inhibitory concentration (MIC) distributions for meropenem, imipenem and ertapenem: representation of different epidemiological cut off (ECOFF) values and clinical breakpoints. The data have been obtained from the EUCAST web page (http://www.eucast.org). Ecl: *Enterobacter cloacae;* Eco: *Escherichia coli;* Kp: *Klebsiella pneumoniae.*

PK/PD modeling and carbapenem breakpoints

Carbapenems have time-dependent bactericidal activity. The time, expressed as a percentage of the dosing interval in which the free concentration of the drug remains above the MIC for the microorganism (%*f*T >MIC), is the best PK/PD index that characterizes the carbapenems, and ~40% *f*T >MIC is the best bactericidal target.[28] However, the probable expected exposure in a particular patient is not only dependent on dosing and PK parameters but also on the PK characteristics, which can vary from patient to patient.[29] When a specific PK/PD index value is used as a PD target to predict the probability of a successful treatment outcome, this probability should be true not only for the mean population but also for each individual patient within the population. Therefore, inter-individual variation should be considered in the calculations.

The Monte Carlo simulation is a statistical method that can also be used to integrate population PK and microbiological information.

This method is a standard approach of both EUCAST and CLSI in the process of setting breakpoints.[30] CLSI has based preliminary breakpoints on MIC distributions, PK/PD indexes and mechanisms of antimicrobial resistance, which were later confirmed by clinical trials.[31] EUCAST uses PK/PD simulations as a key component of its breakpoint setting process for old and new antimicrobials, whereas CLSI has only used this approach with new antimicrobials and with the revision of certain breakpoints.[32,33]

When applying the Monte Carlo simulation tool, the PK parameters and their variability (mean value and standard deviation) are used to simulate multiple concentration-time curves. For each of the generated PK curves, which are all slightly different due to the variance of the parameters, the value of the PK/PD index is determined for a range of MICs. However, CLSI and EUCAST use different methods to display the results of the Monte Carlo simulation, and therefore the different breakpoint definition.[30] In both committees, the probability of target attainment (PTA) of a PD breakpoint is calculated as a function of MIC for a particular target. For instance, concerning carbapenems, the PTA measures the probability of reaching the PD target of *%fT* >MIC of at least 40%, a target based on *in vitro* and *in vivo* animal models for every MIC value.[28,34] The breakpoint is the MIC value considered necessary to achieve a PTA of 90%. The value of 90% for PTA is arbitrary, but it is currently accepted by the CLSI when determining MIC breakpoints.[35] However, a PTA of 90% indicates that the MIC used to determine the PD index would likely not be attained in 10% of the population infected by the microorganism.[36]

EUCAST graphically represents the total probability function irrespective of the target, and therefore provides a more complete and comprehensive picture of the data. The values for the mean of the population and the confidence interval (CI) estimations (percentiles) of the mean values are plotted as a function of MIC. If a CI of 80% was chosen, this would correspond with a PTA of 90% (CLSI method). EUCAST has selected the 95% and 99% CI, corresponding to 97.5% PTA and 99.5% PTA, respectively, and uses the MIC values that result from this PTA as the initial value for setting a PK/PD breakpoint. This method has the advantage that the effect of choosing a different PD target can be directly observed and weighed against all the other evidence for setting a breakpoint.[10] Figure 2 describes the %fT >MIC of ertapenem, imipenem, meropenem and doripenem as a function of the MIC.[17,18,37,38] The breakpoint, considered as the MIC value that can theoretically be met with the dosing regimen, can be read directly from the graph at the intersection of the horizontal line at the PD target (%fT >MIC >40%) and the 95% CI.

The different approaches for setting the PK/PD breakpoints between CLSI and EUCAST might justify, among other factors, the difference in the breakpoints for some antimicrobial agents from both committees. For instance, imipenem and meropenem CLSI susceptibility breakpoints are more restrictive than EUCAST breakpoints (Table 1). However, the corresponding ertapenem and doripenem susceptibility breakpoints are the same for both guidelines.

In addition, significant physiological changes can particularly affect PK parameters in critically ill patients. The Monte Carlo simulations in healthy volunteers might underestimate the actual exposures of patients in an intensive care unit. Bhavnani et al[16] proposed the concept of "inflated variance" in such modeling. They conducted a sensitivity analysis by inflating the between-subject variability in population PK parameter estimates to the approximate variances expected for infected patient populations (%CV ≥40%). Table 2 depicts the PK/PD carbapenem breakpoints, based on Monte Carlo simulations, with actual and inflated variance in healthy volunteers and those obtained from critically ill patients without

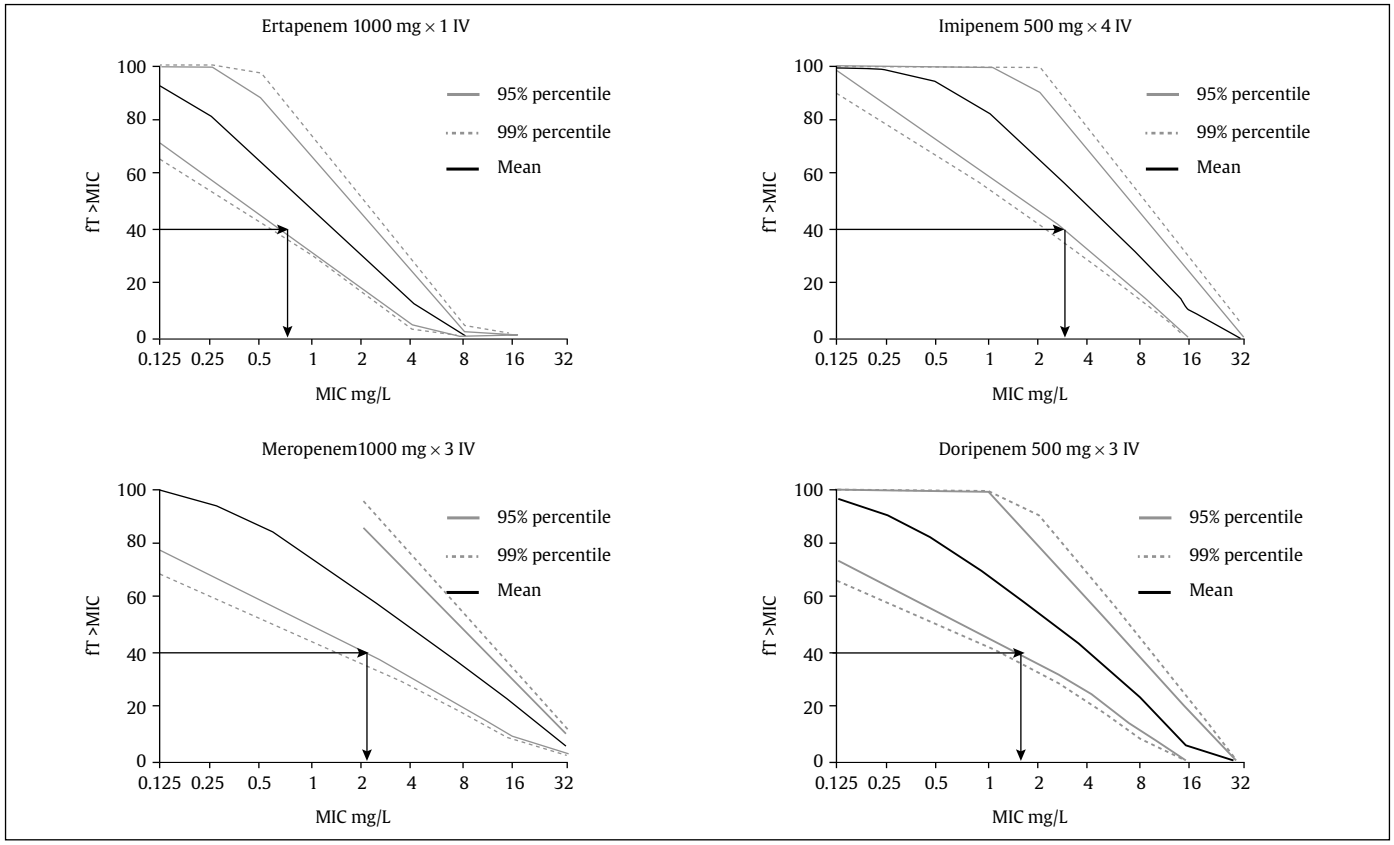


Figure 2. %fT> MIC of ertapenem, imipenem, meropenem and doripenem as a function of the minimum inhibitory concentration (MIC) for different dosing regimens. (http://www.eucast.org/documents/rd/). Representations have been obtained from EUCAST rationale documents (references 17, 18, 37 and 38) and modified to include the intersection of the horizontal line at the PD target and the 95% CI representing the PK breakpoint.

Table 2
PK/PD breakpoints (expressed as mg/L) based on Monte Carlo simulations in healthy volunteers and in critically ill patients without renal dysfunction

Agent	Dosing regimen	Actual variance*	Inflated variance*	Critically ill patients without renal dysfunction	Reference
		40%*f* T >MIC	40%*f* T >MIC	40%*f* T >MIC	
Ertapenem	1000 mg × 1 IV	0.25	0.125	0.25	41
Imipenem	1000 mg × 3 IV	1	1	2	42
Meropenem	1000 mg × 3 IV	1	0.5	2	39
Doripenem	500 mg × 3 IV	1	0.5	2	43

IV: intravenous.
*Data from reference 16.

renal dysfunction. The results show that the breakpoints based on simulations with actual variance in healthy subjects are in agreement with current CLSI breakpoints, except for ertapenem (0.25 mg/L vs. 0.5 mg/L). However, there are relevant differences between the breakpoints of meropenem and doripenem calculated with inflated variance and those obtained for critically ill patients without renal dysfunction. Special care should be taken with this type of patient, in whom renally cleared drugs are frequently underdosed, and therefore the probability of achieving PD targets diminishes.[39]

Ertapenem administered as a rapid 5-minute infusion provides a regimen pharmacodynamically equivalent to the 30-minute infusion for MIC values of 0.25 and 0.5 mg/L, suggesting that PTA should be similar for both dosing regimens in healthy adult volunteers.[40] However, the bactericidal target (40%*f*T >MIC) could not be attained for MIC values of ≥0.5 mg/L in critically ill patients;[41] therefore, the 0.25 mg/L breakpoint —established in June 2010 by CLSI— (Table 1) appears to be more suitable when considering these patients. According to the literature, the administration of imipenem, meropenem and doripenem as intermittent bolus appears to achieve a PD target for MIC values of 1-2 mg/L.[39,42,43] However, the administration of intermittent boluses of imipenem and meropenem in critically ill patients results in robust empirical coverage up to MICs of 2 mg/L, in agreement with the EUCAST breakpoints for Enterobacteriaceae. Administration by extended or continuous infusion improves imipenem, meropenem and doripenem exposures and should be recommended for treating infections due to those microorganisms for which high MICs have been obtained, particularly in critically ill patients without renal dysfunction. According to Mouton et al,[36] the use of PK parameters from different populations (healthy volunteers or patients) in the Monte Carlo simulations for established dosing regimens will result in different breakpoints.

Clinical correlation of carbapenem breakpoints and clinical outcome

The increasing number of CPEs in various countries has led to the publication of correlations between MIC values and clinical breakpoints. In a recent review, Tzouvelekis et al[3] investigated 301 patients infected with KPC- or MBL-producing *K. pneumoniae* isolates, primarily from bloodstream infections. Combination therapy with at least 2 agents, including a carbapenem, had the highest rates of clinical success with the lowest failure rate (8.5%). Furthermore, 50 of these patients received a carbapenem in monotherapy, and a relationship between clinical outcome and carbapenem MIC values was established. A lower percentage of success was found in the isolates with carbapenem MIC values higher than 8 mg/L (Fig. 3). These results reinforce previous PK/PD and experimental infection models in which carbapenems exhibited a time-dependent bactericidal killing effect when the free drug concentration remained above the MIC value 40%-50% of the time between dosing intervals. In these models, the PTA increases with a high-dose prolonged-infusion regimen (e.g., 3-h infusion of 2 g tid for meropenem).[3]

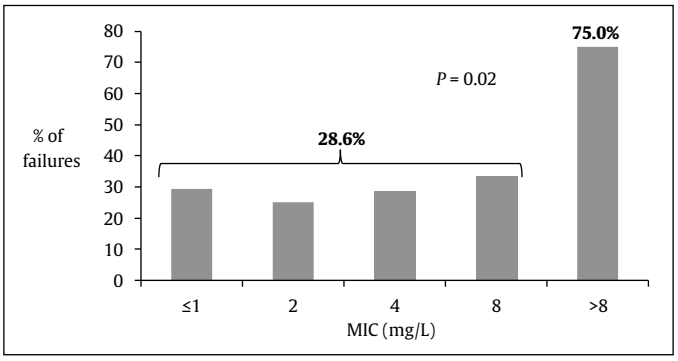


Figure 3. Percentage of success in 50 patients infected with carbapenemase-producing *Klebsiella pneumoniae* receiving meropenem or imipenem monotherapy (data have been obtained from reference 3). A decreased percentage of success is found in isolates with carbapenem minimum inhibitory concentration (MIC) values higher than 8 mg/L.

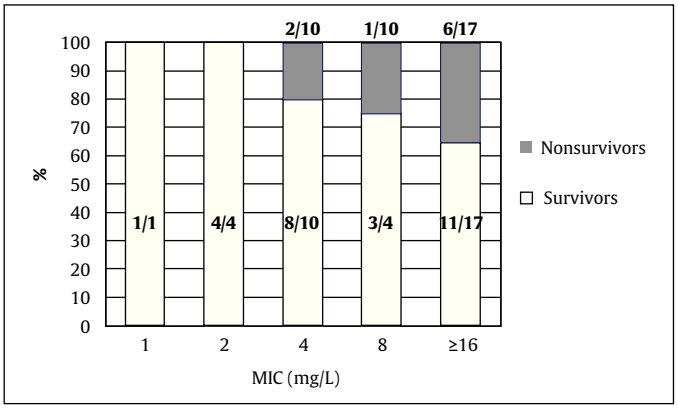


Figure 4. Thirty-day mortality rate in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* treated with combination therapy including meropenem and stratified by meropenem minimum inhibitory concentration (MIC) values (data have been obtained from reference 2).

In 2 recent studies, higher mortality was observed in patients who received monotherapy than in those treated with combination schemes.[2,6] Mortality rose when carbapenem MIC values in KPC-producing *K. pneumoniae* isolates were higher than 8 mg/L, a concentration value coincident with the EUCAST resistant breakpoints for imipenem and meropenem. In one of these studies, however, despite the low number of patients evaluated, mortality was absent with a combination therapy of an active agent and meropenem for infections from isolates displaying MIC values for meropenem ≤2 mg/L (Fig. 4).[2] Nevertheless, more clinical data are needed to support this therapy. These studies should also be expanded to include other CPEs, such as MBL and OXA-48 carbapenemases.

Implication of carbapenem breakpoints on antimicrobial susceptibility testing reports and surveillance

The varying carbapenem clinical breakpoints recommended by CLSI and EUCAST affect antibiotic susceptibility testing reports and, consequently, non-homogeneous resistance rates might be reported.[44-46] Susceptibility reports classifying bacteria either as carbapenem susceptible or carbapenem resistant will influence clinicians to whether to use these drugs or alternative agents. Thus, when implementing a given breakpoint guideline, laboratories should be aware of the implication of their report and their antibiotic prescription policies.[47]

The imipenem and meropenem breakpoint values are still different for both committees, whereas the ertapenem and doripenem categories have been harmonized and fully coincide (Table 1). These discrepancies might lead to significant differences in worldwide surveillance results, particularly in the case of CPEs that display high geographic variability and differences in the prevalent types of enzymes. The extent to which breakpoint changes influence carbapenem resistance rates remains unknown due to few studies reflecting this problem. These studies have primarily been performed on routine clinical isolates, but not in specific collections of CPE. Nevertheless, the studies demonstrated differences according to the breakpoint version (year of publication) used for comparison. In a study comparing the effect of changing breakpoints from CLSI 2009 to those from EUCAST 2011, it was shown that this comparison slightly affected the carbapenem susceptibility patterns of routine isolates, with ertapenem the most affected carbapenem.[44]

Breakpoint discrepancies also influenced the susceptibility patterns of a collection of ESBL- and AmpC beta-lactamase-producing clinical Enterobacteriaceae isolates. With the exception of ertapenem, similar rates of susceptibility to carbapenems were demonstrated when using the breakpoints published in 2013.[45] This outcome would not have been in agreement with the data published in 2014 because ertapenem now has identical breakpoint values from both committees. Other authors studied the influence of breakpoints on cumulative routine antibiograms, showing that there were no differences when considering imipenem and meropenem in *E. coli*, and there were small differences (less than 0.5%) in *K. pneumoniae* isolates.[48] These differences might be higher when considering CPE alone due to differences in breakpoints and the difficulties in establishing accurate susceptibility results with these isolates.

Two aspects preventing accurate reports of antimicrobial susceptibility testing (AST) values are the heterogeneous synthesis of carbapenemases (notoriously variable) and the controversial precision and adequacy of various routine tests to determine the susceptibility of carbapenems.[22,49] Carbapenem MICs obtained primarily from automated systems or gradient strips are variable in the case of CPE. The use of the ECOFFs defined by EUCAST for establishing susceptibility is insufficient to precisely define a truly carbapenem-susceptible isolate, whether or not it produces a carbapenemase.[8,28]

Microbiological report of carbapenemase-producing isolates

Antimicrobial susceptibility testing reports must circumvent potential misunderstandings in interpretation and should be accompanied by the clarification of the scientific rationale behind the given results and the presumed clinical implications of the report. Uncertainties in the interpretation of susceptibility reports must be avoided and the recommendations to clinicians should be homogeneous to ensure uniform interpretations.[30]

The current interpretation of the susceptibility reports is to be "reported as found," irrespective of whether there is carbapenemase production. Classification of susceptible, intermediate or resistant is primarily based on the reading of the inhibition zone and/or the MIC value and not on an interpretive reading, i.e., considering the underlying resistance mechanism and applying an expert rule. Thus, based on the current EUCAST and CLSI recommendations, CPE must be considered as susceptible to any or all carbapenems if the value obtained reflects such a category.

In summary, editing of *in vitro* AST results for carbapenems in CPE is no longer recommended. However, screening for their production is useful (or even mandatory in some countries) for epidemiological and infection control purposes. This procedure is based upon the rationale that revised breakpoints for Enterobacteriaceae are sufficiently adjusted to detect the majority of carbapenemases, and although categorized as susceptible with these breakpoints, the results should be "reported as tested," i.e., the presence or absence of a carbapenemase does not in itself influence the categorization of susceptibility. This statement is currently included in the EUCAST breakpoint tables.[12]

The possible clinical impact of CPE being reported as susceptible to carbapenems must not be minimized. Recent studies recommend the use of a carbapenem in combination with other active compounds in bloodstream infections due to CPE.[2,6] However, more evidence-based clinical studies are necessary to validate this approach.[50] Given that in many countries the number of CPEs isolated in the clinical laboratories is increasing, performance of longitudinal surveillances of carbapenems' susceptibility patterns is strongly recommended because they are extremely useful to guide recommendations for antibiotic use, to aid infection control and to provide clear epidemiological data.[51]

Conclusions

Both CLSI and EUCAST have revised and updated carbapenems' breakpoints for Enterobacteriaceae based on MIC distributions, PK/PD data, available clinical data and results from animal models. Despite a similar approach, CLSI and EUCAST carbapenem breakpoints are different for imipenem and meropenem, but identical for ertapenem and doripenem. These differences are primarily based on the interpretation and the use of PK/PD tools. In the case of the CLSI, the final goal of the susceptible carbapenem breakpoint is to capture as many CPE isolates as possible, whereas in the case of EUCAST the goal is to appropriately guide carbapenem therapy rather than detect CPE isolates. However, both committees recommend reporting the carbapenem susceptibility result "as tested." They also recommend demonstrating carbapenemase production in Enterobacteriaceae for epidemiological purposes and for the implementation of infection control measures. New clinical data with infection due to CPE would potentially modify these recommendations. The production of a carbapenemase also affects specific treatment advice. Using carbapenems in combination with other antimicrobials in infections due to CPE, which also takes into account specific MIC values and reinforces the interpretation of the antibiogram, would be necessary from a clinical point of view.[52] This might resolve the dilemma of detecting carbapenemases in CPE not only for infection control purposes but for the establishment of a suitable antimicrobial therapy that should be guided by breakpoint values.

Funding

The research of Rafael Cantón at the Microbiology Department of Ramón y Cajal University Hospital is funded by the European Commission (grants R-GNOSIS-FP7-HEALTH-F3-2011-282512 and MON4STRAT-FP7-HEALTH-2013-602906) and the Instituto de Salud Carlos III of Spain grants PI12/00734 and REIPI RD12/0015 (Spanish Network for Research in Infectious Diseases) is co-financed by the European Development Regional Fund (A Way to Achieve Europe program).

Conflicts of interest

R.C. is currently chairman of EUCAST (European Committee on Antimicrobial Susceptibility Testing). A.C., A.O. and R.C. are members of the executive committee of COESANT (Comité Español del Antibiograma). M.I.M. has no conflicts of interest to declare.

References

1. Lynch TJ. Choosing optimal antimicrobial therapies. Med Clin North Am. 2012; 96:1079-94.
2. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae:* importance of combination therapy. Clin Infect Dis. 2012;55:943-50.
3. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25:682-707.
4. Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother. 2014;58:2322-8.
5. Van Duin D, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant Enterobacteriaceae: a review of treatment and outcomes. Diagn Microbiol Infect Dis. 2013;75:115-20.
6. Daikos GL, Markogiannakis A, Souli M, Tzouvelekis LS. Bloodstream infections caused by carbapenemase-producing *Klebsiella pneumoniae:* a clinical perspective. Expert Rev Anti Infect Ther. 2012;10:1393-404.
7. International Organization for Standardization (ISO). Clinical laboratory testing and *in vitro* diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Reference method for testing the *in vitro* activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. International Standard 20776-1, ISO. Geneva. 2006.
8. Livermore DM, Andrews JM, Hawkey PM, Ho PL, Keness Y, Doi Y, et al. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? J Antimicrob Chemother. 2012;67:1569-77.
9. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother. 2013;68:487-9.
10. Mouton JW, Brown DF, Apfalter P, Cantón R, Giske CG, Ivanova M, et al. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clin Microbiol Infect. 2012;18:E37-45.
11. Dudley MN, Ambrose PG, Bhavnani SM, Craig WA, Ferraro MJ, Jones RN; Antimicrobial Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute. Background and rationale for revised clinical and laboratory standards institute interpretive criteria (Breakpoints) for Enterobacteriaceae and *Pseudomonas aeruginosa*: I. Cephalosporins and Aztreonam. Clin Infect Dis. 2013; 56:1301-9.
12. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0, 2014. Available at: http://www.eucast.org
13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 24th Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement (June 2010 update). CLSI document M100-S20-U. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
15. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect. 2012;18:432-8.
16. Bhavnani SM, Dudley MN, Landersdorfer L, Drusano GL, Craig WA, Jones RN, et al. Pharmacokinetic-pharmacodynamic basis for CLSI carbapenem susceptibility breakpoint changes. 50th Intersci. Conf. Antimicrob. Boston, MA; Agents Chemother; 2010. Abstract A-1382.
17. European Committee on Antimicrobial Susceptibility Testing. Imipenem: Rationale for the clinical breakpoints, version 1.3, 2009. Available at: http://www.eucast.org
18. European Committee on Antimicrobial Susceptibility Testing. Meropenem: Rationale for the clinical breakpoints, version 1.5, 2009. Available at: http://www.eucast.org
19. Giske C, Martínez-Martínez L, Cantón R, Stefani S, Skov R, Glupczynski Y, et al. EUCAST subcommittee for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0. November 2013. Available at: http://www.eucast.org
20. Endimiani A, Pérez F, Bajaksouzian S, Windau AR, Good CE, Choudhary Y, et al. Evaluation of updated interpretative criteria for categorizing *Klebsiella pneumoniae* with reduced carbapenem susceptibility. J Clin Microbiol. 2010;48:4417-25.
21. Kollef MH, Chastre J, Clavel M, Restrepo MI, Michiels B, Kaniga K, et al. A randomized trial of 7-day doripenem versus 10-day imipenem-cilastatin for ventilator-associated pneumonia. Crit Care. 2012;16:R218.
22. Tato M, Morosini M, García L, Albertí S, Coque MT, Cantón R. Carbapenem heteroresistance in VIM-1-producing *Klebsiella pneumoniae* isolates belonging to the same clone: consequences for routine susceptibility testing. J Clin Microbiol. 2010;48:4089-93.
23. Kitchel B, Rasheed JK, Endimiani A, Hujer AM, Anderson KF, Bonomo RA, et al. Genetic factors associated with elevated carbapenem resistance in KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2010;54:4201-7.
24. Oteo J, Sáez D, Bautista V, Fernández-Romero S, Hernández-Molina JM, Pérez-Vázquez M, et al; Spanish Collaborating Group for the Antibiotic Resistance Surveillance Program. Carbapenemase-producing Enterobacteriaceae in Spain in 2012. Antimicrob Agents Chemother. 2013;57:6344-7.
25. Tsai YK, Liou CH, Fung CP, Lin JC, Siu LK. Single or in combination antimicrobial resistance mechanisms of *Klebsiella pneumoniae* contribute to varied susceptibility to different carbapenems. Plos One. 2013;8:e79640.
26. Antunes NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB. Class D β-lactamases: are they all carbapenemases? Antimicrob Agents Chemother. 2014; 58:2119-25.
27. Vading M, Samuelsen Ø, Haldorsen B, Sundsfjord AS, Giske CG. Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems. Clin Microbiol Infect. 2011;17:668-74.
28. Nicolau DP. Pharmacokinetic and pharmacodynamic properties of meropenem. Clin Infect Dis. 2008;47 Suppl 1:S32-40.
29. Dalhoff A, Ambrose PG, Mouton JW. A long journey from minimum inhibitory concentration testing to clinically predictive breakpoints: deterministic and probabilistic approaches in deriving breakpoints. Infection. 2009;37:296-305.
30. Jenkins SG, Jerris RC. Critical assessment of issues applicable to development of antimicrobial susceptibility testing breakpoints. J Clin Microbiol. 2011;49 Suppl:S5-10.
31. Frei CR, Wiederhold NP, Burgess DS. Antimicrobial breakpoints for gram-negative aerobic bacteria based on pharmacokinetic-pharmacodynamic models with Monte Carlo simulation. J Antimicrob Chemother. 2008;61:621-8.
32. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, et al. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother. 2003;52:145-8.
33. Marchese A, Esposito S, Barbieri R, Bassetti M, Debbia E. Does the adoption of EUCAST susceptibility breakpoints affect the selection of antimicrobials to treat acute community-acquired respiratory tract infections? BMC Infect Dis. 2012;12: 181.
34. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of "bug and drug". Nat Rev Microbiol. 2004;2:289-300.
35. Maglio D, Kuti JL, Nicolau DP. Simulation of antibiotic pharmacodynamic exposure for the empiric treatment of nosocomial bloodstream infections: a report from the OPTAMA program. Clin Ther. 2005;27:1032-42.
36. Mouton JW. Setting clinical MIC breakpoints from a PK/PD point of view: it is the dose that matters. In: Vinks AA, Derendorf H, Mouton JW, editors. Fundamentals of antimicrobial pharmacokinetics and pharmacodynamics. New York: Springer; 2014. p. 45-61.
37. European Committee on Antimicrobial Susceptibility Testing. Doripenem: Rationale for the clinical breakpoints, version 1.0, 2009. Available at: http://www.eucast.org
38. European Committee on Antimicrobial Susceptibility Testing. Ertapenem: Rationale for the clinical breakpoints, version 1.3, 2009. Available at: http://www.eucast.org
39. Roberts JA, Kirkpatrick CMJ, Roberts MS, Robertson TA, Dalley AJ, Lipman J. Meropenem dosing in critically ill patients with sepsis and without renal dysfunction: intermittent bolus versus continuous administration? Monte Carlo dosing simulations and subcutaneous tissue distribution. J Antimicrob Chemother. 2009;64:142-50.
40. Wiskirchen DE, Housman ST, Quintiliani R, Nicolau DP, Kuti JL. Comparative pharmacokinetics, pharmacodynamics, and tolerability of ertapenem 1 gram/day administered as a rapid 5-minute infusion versus the standard 30-minute infusion in healthy adult volunteers. Pharmacotherapy. 2013;33:266-74.
41. Burkhardt O, Kumar V, Kielstein JT, Welte T, Derendorf H. Underdosing of ertapenem in critically ill patients with pneumonia confirmed by Monte Carlo simulations. Int J Antimicrob Agents. 2010;35:93-9.
42. Sakka SG, Glauner AK, Bulitta JB, Kinzig-Schippers M, Pfister W, Drusano GL, et al. Population pharmacokinetics and pharmacodynamics of continous versus short-term infusion of imipenem-cilastatin in critically ill patients in a randomized controlled trial. Antimicrob Agents Chemother. 2007;51:3304-10.
43. Roberts JA, Lipman J. Optimal doripenem dosing simulations in critically ill nosocomial pneumonia patients with obesity, augmented renal clearance, and decreased bacterial susceptibility. Crit Care Med. 2013;41:489-95.
44. Hombach M, Bloemberg GV, Böttger EC. 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. 2012;67:622-32.
45. Hombach M, Mouttet B, Bloemberg GV. Consequences of revised CLSI and EUCAST guidelines for antibiotic susceptibility patterns of ESBL- and AmpC β-lactamase-producing clinical Enterobacteriaceae isolates. J Antimicrob Chemother. 2013;68: 2092-8.
46. Hombach M, Wolfensberger A, Kuster SP, Böttger EC. Influence of clinical breakpoint changes from CLSI 2009 to EUCAST 2011 antimicrobial susceptibility testing guidelines on multidrug resistance rates of Gram-negative rods. J Clin Microbiol. 2013;51:2385-7.
47. Weisenberg SA, Morgan DJ, Espinal-Witter R, Larone DH. Clinical outcomes of patients with *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*

after treatment with imipenem or meropenem. Diagn Microbiol Infect Dis. 2009; 64:233-5.
48. Wolfensberger A, Sax H, Weber R, Zbinden R, Kuster SP, Hombach M. Change of antibiotic susceptibility testing guidelines from CLSI to EUCAST: influence on cumulative hospital antibiograms. PLoS One. 2013;8:e79130.
49. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, et al. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in Enterobacteriaceae. J Clin Microbiol. 2007;45:2723-5.
50. Esterly JS, Wagner J, McLaughlin MM, Postelnick MJ, Qi C, Scheetz MH. Evaluation of clinical outcomes in patients with bloodstream infections due to Gram-negative bacteria according to carbapenem MIC stratification. Antimicrob Agents Chemother. 2012;56:4885-90.
51. Jean SS, Hsueh PR, Lee WS, Yu KW, Liao CH, Chang FY, et al. Carbapenem susceptibilities and non-susceptibility concordance to different carbapenems amongst clinically important Gram-negative bacteria isolated from intensive care units in Taiwan: results from the Surveillance of Multicentre Antimicrobial Resistance in Taiwan (SMART) in 2009. Int J Antimicrob Agents. 2013;41:457-62.
52. Cantón R. Lectura interpretada del antibiograma: una necesidad clínica. Enferm Infecc Microbiol Clin. 2010;28:375-85.