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Antimicrobial breakpoints for Gram-negative aerobic bacteria based on pharmacokinetic–pharmacodynamic models with Monte Carlo simulation

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***Objectives*: This study describes a comprehensive programme designed to develop pharmacokinetic–pharmacodynamic (PK–PD) breakpoints for numerous antimicrobial classes against key Gram-negative aerobic bacteria.**

***Methods*: A 10 000 subject Monte Carlo simulation was constructed for 13 antimicrobials (21 dosing regimens). Published pharmacokinetic data and protein binding were varied according to log-normal and uniform distributions. MICs were fixed at single values from 0.03 to 64 mg/L. The PK–PD susceptible breakpoint was defined as the MIC at which the probability of target attainment was ≥90%. PK–PD, CLSI and European Committee on Antimicrobial Susceptibility Testing breakpoints were applied to MICs from the 2005 worldwide Meropenem Yearly Susceptibility Test Information Collection database to evaluate the impact of breakpoint discrepancies.**

***Results*: PK–PD breakpoints were within one dilution of the CLSI and European breakpoints for all antimicrobials tested—with a few exceptions. When discrepancies were noted, the PK–PD breakpoint was lower than the CLSI breakpoint [ceftriaxone (0.5 versus 8 mg/L), ertapenem (0.25 versus 2 mg/L), ciprofloxacin (0.125 versus 1 mg/L) and levofloxacin (0.25–0.5 versus 2 mg/L)] and higher than the European breakpoint [ceftazidime (4–8 versus 1 mg/L), aztreonam (4–8 versus 1 mg/L), although ciprofloxacin was an exception to this pattern (0.125 versus 0.5–1 mg/L)]. For Enterobacteriaceae, breakpoint discrepancies resulted in modest (≤10%) differences in the percentages susceptible. In contrast, large (>15%) discrepancies were noted for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.**

***Conclusions*: Breakpoint agreement exists for imipenem, meropenem and the aminoglycosides. In contrast, discrepancies exist for piperacillin/tazobactam, cephalosporins, ertapenem, aztreonam and the fluoroquinolones. These discrepancies are most pronounced for *P. aeruginosa* and *A. baumannii*.**

Keywords: *Pseudomonas*, *Acinetobacter*, β-lactam antibiotics, stochastic, computer modelling

Introduction

The CLSI issues guidance documents for the performance and interpretation of antimicrobial susceptibility testing.[1] These guidelines are used globally to ensure consistent, reproducible methodologies and interpretations. The committee also establishes interpretive criteria or 'breakpoints'. Breakpoints are MIC cut-off values that are used to divide a bacterial population into susceptible, intermediate and resistant categories. These breakpoints are used routinely in the clinical laboratory setting to guide clinical decision-making. CLSI develops preliminary breakpoints based on MIC distributions, pharmacokinetic–pharmacodynamic (PK–PD) studies and mechanisms of antimicrobial resistance. These preliminary breakpoints are later

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confirmed in clinical trials. Additional methodologies are now utilized to evaluate the PK–PD of antimicrobials. PK–PD models with Monte Carlo simulation (PK–PD simulations) have appeared in the literature since the mid-1980s;[2] however, this approach has gained momentum in the last 5–10 years and these studies now pervade the literature.[3–12]

Monte Carlo simulation is a computer modelling process that incorporates the variability in pharmacokinetic parameters and the natural MIC distribution within a bacterial population. This technique can be used to develop interpretive susceptibility criteria based on PK–PD principles (PK–PD breakpoints). Proponents maintain that the resulting breakpoints are more reflective of the true antimicrobial effectiveness in the population compared with traditional approaches.[13] Proponents of PK–PD simulations also maintain that this technique can be used to improve the detection of antimicrobial resistance and facilitate the design of antimicrobial regimens.[13]

CLSI now considers studies that employ PK–PD simulations during the breakpoint determination process for new antimicrobials. However, efforts to use Monte Carlo simulation to re-evaluate breakpoints for older antimicrobials have been met with criticism. Nevertheless, PK–PD simulations have emerged as a widely used methodology for clinical practice, regulatory guidance and drug development. CLSI's European counterpart, The European Committee on Antimicrobial Susceptibility Testing (EUCAST), embraces PK–PD simulations as a chief component of its breakpoint-setting process for old and new antimicrobials.[7,14]

One limitation of these efforts to date is that the PK–PD analyses often focus on a single antimicrobial class and a few bacterial species. Many argue that the re-evaluation of breakpoints should be done simultaneously for all antimicrobial classes to provide fair comparisons. In response to this criticism, this study describes a comprehensive programme that systematically developed PK–PD breakpoints for numerous antimicrobial classes against key Gram-negative aerobic bacteria. The programme included PK–PD analyses for 13 antimicrobials (21 regimens) from 6 antimicrobial classes against Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. A primary objective of this programme was to systematically model each antimicrobial–bacterium pair in a consistent manner in order to provide a fair comparison of predicted efficacy. This study serves two key purposes. First, it establishes interpretive antimicrobial susceptibility criteria based on PK–PD principles (PK–PD breakpoints). Second, it exposes the degree of disagreement between existing CLSI, EUCAST and PK–PD breakpoints and quantifies the impact of breakpoint discrepancies on global susceptibility patterns.

Methods

Antimicrobials

Thirteen antimicrobials (21 dosing regimens) were chosen based on their routine use for the treatment of Gram-negative aerobic infections. PK–PD parameters, protein binding and the variability of these measurements were obtained from the published literature for penicillins (piperacillin/tazobactam), cephalosporins (cefepime, ceftizoxime, ceftazidime and ceftriaxone), carbapenems (ertapenem, imipenem and meropenem), monobactams (aztreonam), aminoglycosides (gentamicin and tobramycin) and fluoroquinolones (ciprofloxacin and levofloxacin) (Table 1).[15–29] Pharmacokinetic studies were identified using an OVID search engine to query the Medline® database. A Medline® search was performed individually for each antimicrobial by combining the exploded MeSH heading 'pharmacokinetics' with each antimicrobial's generic name. Results were limited to studies of healthy adults published in English between 1970 and 2003. Studies were included if they evaluated clinically relevant dosing regimens and provided the means and standard deviations for the pharmacokinetic parameters of interest. In the event that the AUC_{0-24} was not provided for the fluoroquinolones, it was calculated as follows: $AUC_{0-24}=\text{Dose}/V_{ss}\times K_d$, where V_{ss} was the volume of distribution at steady state (L/kg) and K_d the elimination rate constant (h^{-1}). For the aminoglycosides, the C_{max} was calculated as follows: $C_{max}=\text{Dose}/V_{ss}$.

PK–PD models

Crystal Ball (Decisioneering, Inc., Denver, CO, USA) was used to perform a 10 000 subject Monte Carlo simulation for each antimicrobial using the following PK–PD equations:

β-Lactams[30]

$$f\%T > \text{MIC} = \text{Ln}\left\{\frac{\text{Dose}\cdot(1-\text{PB}_s)}{V_{ss}\cdot\text{MIC}}\right\}\cdot\frac{V_{ss}}{\text{CL}_T}\cdot\frac{100}{\tau}$$

where $f\%T > \text{MIC}$ was the proportion of time that the free serum concentration remained above the MIC (%), Ln the natural logarithm, Dose the dose of antibiotic (mg) administered by intermittent intravenous bolus, PB_s the fraction of drug bound to proteins in human serum, V_{ss} the antimicrobial's volume of distribution at steady state (L/kg), CL_T the total body clearance (L/h) and τ the dosing interval (h).

Aminoglycosides

$$\frac{C_{max}}{\text{MIC}}=\frac{(\text{Dose}/V_{ss})}{\text{MIC}}$$

where C_{max}/MIC was the maximum concentration achieved in the serum (mg/L), Dose the dose of antibiotic (mg) and V_{ss} the antimicrobial's volume of distribution at steady state (L/kg).

Fluoroquinolones

$$\frac{AUC_{0-24}}{\text{MIC}}$$

where AUC_{0-24} was the area under the serum concentration–time curve from 0–24 h (mg·h/L).

The subject weight was fixed at 70 kg for all simulations. Pharmacokinetic data were varied according to log-normal distributions, whereas protein binding was varied according to a uniform distribution ($\pm$10%) and MICs were fixed at single values from 0.03 to 64 mg/L. Table 2 depicts the desired magnitude for each antimicrobial target. In addition, a sensitivity analysis was conducted by increasing and decreasing the desired PK–PD target by 10%. The PK–PD susceptible breakpoint was defined as the MIC at which the probability of target attainment (PTA) was $\geq$90%. Overall, this breakpoint determination study required 252 Monte Carlo simulations (21 regimens $\times$ 12 single-point MICs). Although PK–PD models enable the establishment of regimen-specific breakpoints, the CLSI and the EUCAST have generally advocated only a single set of breakpoints for each antimicrobial–organism pair. For

Table 1. Pharmacokinetic parameters from published studies among healthy adult volunteers[15–29,a]

Antimicrobial and dosing regimen	Cl_T (mL/min)	V_{ss} (L/kg)	$t_{1/2\beta}$ (h)	PB_s (%)[b]	AUC_{0-24}
Piperacillin-tazobactam					
3.375 g every 4 h	184 ± 23	0.15 ± 0.02	0.76 ± 0.11	30	—
3.375 g every 6 h	184 ± 23	0.15 ± 0.02	0.76 ± 0.11	30	—
4.5 g every 6 h	182 ± 20	0.15 ± 0.02	0.76 ± 0.10	30	—
Cefepime					
1 g every 12 h	125 ± 21	0.26 ± 0.04	2.23 ± 0.35	20	—
1 g every 8 h	125 ± 21	0.26 ± 0.04	2.23 ± 0.35	20	—
2 g every 12 h	143 ± 25	0.26 ± 0.05	2.32 ± 0.39	20	—
2 g every 8 h	143 ± 25	0.26 ± 0.05	2.32 ± 0.39	20	—
Ceftizoxime					
1 g every 8 h	161 ± 15	0.40 ± 0.06	1.90 ± 0.10	30	—
Ceftriaxone					
1 g every 24 h	14 ± 1	0.11 ± 0.02	7.65 ± 1.30	90	—
2 g every 24 h	21 ± 4	0.17 ± 0.03	7.50 ± 1.28	90	—
Ceftazidime					
1 g every 8 h	116 ± 18	0.21 ± 0.02	1.87 ± 0.15	10	—
2 g every 8 h	133 ± 20	0.25 ± 0.02	1.96 ± 0.18	10	—
Ertapenem					
1 g every 24 h	30 ± 3	0.12 ± 0.02	4.10 ± 0.30	90	—
Imipenem					
500 mg every 6 h	175 ± 23	0.22 ± 0.05	1.11 ± 0.17	20	—
Meropenem					
1 g every 8 h	240 ± 30	0.27 ± 0.04	1.07 ± 0.11	0	—
Aztreonam					
1 g every 8 h	64 ± 4	0.11 ± 0.01	1.90 ± 0.21	60	—
2 g every 8 h	69 ± 10	0.14 ± 0.04	2.16 ± 0.38	60	—
Gentamicin					
5 mg/kg every 24 h	0.19 ± 0.04	—	—	—	—
Tobramycin					
5 mg/kg every 24 h	0.19 ± 0.04	—	—	—	—
Ciprofloxacin					
400 mg every 8 h	—	—	—	30	33 ± 9
400 mg every 12 h	—	—	—	30	23 ± 3
Levofloxacin					
500 mg every 24 h	—	—	—	30	48 ± 8
750 mg every 24 h	—	—	—	30	82 ± 14

[a]Cl_T, terminal clearance; AUC_{0-24}, area under the antimicrobial concentration–time curve from 0 to 24 h; V_{ss}, volume of distribution at steady state; $t_{1/2\beta}$, terminal half-life; PB_s, protein binding in serum.
[b]PB_s (%) was obtained from the package labelling.

Table 2. PK–PD targets from the published literature[38–44,a]

Antimicrobials	Indices	Magnitude
Penicillins	$f\%T > MIC$	50
Cephalosporins	$f\%T > MIC$	50
Monobactams	$f\%T > MIC$	50
Carbapenems	$f\%T > MIC$	30
Aminoglycosides	C_{max}/MIC	8
Fluoroquinolones	AUC_{0-24}/MIC	125

[a]$\%T > MIC$, percentage of time that the antimicrobial concentration remains above the MIC; C_{max}/MIC, ratio of the maximum antimicrobial concentration divided by the MIC; AUC_{0-24}/MIC, ratio of the area under the antimicrobial concentration–time curve from 0 to 24 h divided by the MIC.

this reason, common antimicrobial regimens were modelled and the resulting PK–PD breakpoints were reported as ranges.

Susceptibility interpretations

Cumulative MIC distributions were extracted from the 2005 worldwide Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) database.[31] For the purposes of this study, MIC distributions were extracted for all Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* isolates collected during 2005. PK–PD, CLSI and EUCAST breakpoints were applied to these distributions to evaluate the impact of breakpoint discrepancies on the interpretation of global organism susceptibilities.

Results

Breakpoint comparisons

Table 3 depicts the PTA for existing CLSI breakpoints. Further details describing the PTA for each regimen can be found in Figure S1 and Table S1, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/). Table 4 summarizes the PK–PD breakpoints and compares them with existing CLSI and EUCAST breakpoints. Of note, PK–PD breakpoints are regimen-dependent and species-independent (see the Methods section); therefore, the range represents the lowest and highest PK–PD breakpoints achieved with the various regimens.

Table 3. PTA at existing CLSI breakpoints[a]

Antimicrobial and dosing regimen	PTA (%)
Piperacillin-tazobactam	
3.375 g every 4 h	91
3.375 g every 6 h	6
4.5 g every 6 h	21
Cefepime	
1 g every 12 h	2
1 g every 8 h	57
2 g every 12 h	21
2 g every 8 h	87
Ceftizoxime	
1 g every 8 h	5
Ceftriaxone	
1 g every 24 h	0
2 g every 24 h	17
Ceftazidime	
1 g every 8 h	68
2 g every 8 h	98
Ertapenem	
1 g every 24 h	59
Imipenem	
500 mg every 6 h	99
Meropenem	
1 g every 8 h	98
Aztreonam	
1 g every 8 h	26
2 g every 8 h	90
Gentamicin	
5 mg/kg every 24 h	20
Tobramycin	
5 mg/kg every 24 h	20
Ciprofloxacin	
400 mg every 8 h	0
400 mg every 12 h	0
Levofloxacin	
500 mg every 24 h	0
750 mg every 24 h	0

[a]The CLSI breakpoints are consistent for all antibiotics evaluated against Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*, except for piperacillin-tazobactam (breakpoint for *P. aeruginosa*, 64 mg/L). Table 3 reflects the PTA for piperacillin-tazobactam when a breakpoint of 16 mg/L was used. If a breakpoint of 64 mg/L were used instead (i.e. *P. aeruginosa*), the corresponding probabilities of target attainment for piperacillin-tazobactam would be: 3.375 g every 4 h (0%), 3.375 g every 6 h (0%) and 4.5 g every 6 h (0%).

Table 4. Comparison of the PK–PD, CLSI and EUCAST breakpoints (mg/L) for Gram-negative aerobic bacteria[a]

			EUCAST		
Antimicrobial regimen	PK–PD[b]	CLSI[c]	EB	PA	AB
Piperacillin-tazobactam	4/4–16/4	16/4	—	—	—
Cefepime	1–4	8	1	8	—
Ceftizoxime	4	8	—	—	—
Ceftriaxone	0.5	8	1	—	—
Ceftazidime	4–8	8	1	8	—
Ertapenem	0.25	2	0.5	—	—
Imipenem	4	4	4	2	4
Meropenem	4	4	2	2	2
Aztreonam	4–8	8	1	1	—
Gentamicin	2	4	2	4	4
Tobramycin	2	4	2	4	4
Ciprofloxacin	0.125	1	0.5	0.5	1
Levofloxacin	0.25–0.5	2	1	1	1

[a]EB, Enterobacteriaceae; PA, *P. aeruginosa*; AB, *A. baumannii*.
[b]PK–PD breakpoints are regimen-dependent and species-independent (see the Methods section); therefore, the range represents the regimen-dependency of PK–PD breakpoints: piperacillin-tazobactam (3.375 g every 6 h, 4/4 mg/L; 4.5 g every 6 h, 4/4 mg/L; 3.375 g every 4 h, 16/4 mg/L), cefepime (1 g every 12 h, 1 mg/L; 1 g every 8 h, 4 mg/L; 2 g every 12 h, 2 mg/L; 2 g every 8 h, 4 mg/L), ceftazidime (1 g every 8 h, 4 mg/L; 2 g every 8 h, 8 mg/L), aztreonam (1 g every 8 h, 4 mg/L; 2 g every 8 h, 8 mg/L) and levofloxacin (500 mg every 24 h, 0.25 mg/L; 750 mg every 24 h, 0.5 mg/L).
[c]The CLSI breakpoints are consistent for all antibiotics evaluated against Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*, except for piperacillin-tazobactam (breakpoint for *P. aeruginosa*, 64 mg/L). The CLSI does not have breakpoints for the following: *P. aeruginosa* (ertapenem) and *A. baumannii* (ceftizoxime, ertapenem, aztreonam).

The CLSI breakpoints are identical for all of the antimicrobials studied against members of the Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* (except for piperacillin-tazobactam; breakpoint for *P. aeruginosa*, 64 mg/L). The CLSI does not have breakpoints for ertapenem against *P. aeruginosa*. Likewise, there are no CLSI breakpoints for ceftizoxime, ertapenem or aztreonam versus *A. baumannii*. In contrast, the EUCAST has species-specific breakpoints. Missing breakpoints are denoted as dashes in Table 4.

When the PK–PD breakpoint range was considered, the PK–PD breakpoints were within one dilution of the CLSI and EUCAST breakpoints for all antimicrobials tested—with a few exceptions. When discrepancies were noted, the PK–PD breakpoint was generally lower than the CLSI breakpoint [ceftriaxone (PK–PD, 0.5 mg/L; CLSI, 8 mg/L), ertapenem (PK–PD, 0.25 mg/L; CLSI, 2 mg/L), ciprofloxacin (PK–PD, 0.125 mg/L; CLSI, 1 mg/L) and levofloxacin (PK–PD, 0.25–0.5 mg/L, CLSI, 2 mg/L)] and higher than the EUCAST breakpoint [ceftazidime (PK–PD, 4–8 mg/L; EUCAST Enterobacteriaceae, 1 mg/L) and aztreonam (PK–PD, 4–8 mg/L; EUCAST, 1 mg/L)]. Ciprofloxacin was an exception to this trend (PK–PD, 0.125 mg/L; EUCAST, 0.5–1 mg/L). Of note, cefepime and piperacillin-tazobactam PK–PD breakpoints were regimen-dependent. For cefepime, the breakpoints ranged from 1 mg/L

with the 1 g every 12 h regimen to 4 mg/L with the 1 g every 8 h and 2 g every 8 h regimens. For piperacillin-tazobactam, the breakpoints ranged from 4/4 mg/L with the 3.375 g every 6 h and 4.5 g every 6 h regimens compared with 16/4 mg/L with the 3.375 g every 4 h regimen.

Impact of divergent breakpoints on susceptibility interpretations

The 2005 worldwide MYSTIC database contained thousands of MICs for the antimicrobials of interest. Table 5 depicts the

Table 5. Cumulative frequency distribution for Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* isolates from the 2005 MYSTIC worldwide database with the corresponding percentage susceptible using PK–PD (P), CLSI (C) and EUCAST (E) breakpoints[a]

Organisms	No of isolates	Cumulative MIC (mg/L) distribution										Divergence in % susceptible[b]
		0.125	0.25	0.5	1	2	4	8	16	32	64	
Enterobacteriaceae												
Piperacillin-tazobactam	7099	2	5	20	33	60	75^{P}	81^{P}	85^{PC}	89	91	10
Cefepime	4486	68	74	78	81^{PE}	84^{P}	86^{P}	88^{C}	91	93	97	7
Ceftizoxime[c]	965	82	86	90	91	93	93^{P}	94^{C}	94	95	100	1
Ceftriaxone	1517	84	84	87^{P}	88^{E}	88	89	91^{C}	92	95	100	4
Ceftazidime	7104	39	59	71	76^{E}	79	82^{P}	84^{PC}	86	92	94	8
Ertapenem	1517	94	96^{P}	97^{E}	98	98^{C}	99	99	99	99	100	2
Imipenem	7104	36	67	82	91	97	98^{PCE}	99	99	100	100	0
Meropenem	7104	92	94	97	99	99^{E}	99^{PC}	99	100	100	100	0
Aztreonam	1602	4	4	87	87^{E}	87	87^{P}	90^{PC}	93	100	100	3
Gentamicin	5173	3	13	62	78	83^{PE}	85^{C}	87	92	94	96	2
Tobramycin	5421	3	8	47	68	79^{PE}	83^{C}	85	91	95	97	4
Ciprofloxacin	7103	70^{P}	73	76^{E}	80^{C}	82	87	89	90	93	99	10
Levofloxacin	1517	73	76^{P}	80^{P}	83^{E}	84^{C}	86	90	100	—	—	8
P. aeruginosa												
Piperacillin-tazobactam	2395	1	1	5	9	22	47^{P}	58^{P}	68^{PC}	75	80	21
Cefepime	1833	1	1	3	17^{P}	35^{P}	51^{P}	65^{CE}	74	79	95	48
Ceftizoxime[c]	298	—	<1	<1	1	2	2^{P}	4^{C}	7	21	100	2
Ceftriaxone	589	<1	<1	1^{P}	3	6	10	18^{C}	29	47	100	17
Ceftazidime	2397	1	2	5	24	51	65^{P}	72^{PCE}	78	86	88	7
Imipenem	2397	2	4	19	44	62^{E}	69^{PC}	76	83	88	98	7
Meropenem	2398	22	37	52	63	69^{E}	75^{PC}	81	85	89	99	6
Aztreonam	609	<1	1	11	11^{E}	11	11^{P}	74^{PC}	88	99	100	63
Gentamicin	1835	1	3	24	36	57^{P}	67^{CE}	73	80	83	84	10
Tobramycin	1916	2	5	44	60	70^{P}	73^{CE}	75	80	82	84	3
Ciprofloxacin	2398	41^{P}	49	59^{E}	65^{C}	70	79	81	83	87	99	24
Levofloxacin	589	4	32^{P}	52^{P}	61^{E}	69^{C}	78	83	100	—	—	37
A. baumannii												
Piperacillin-tazobactam	669	8	10	14	16	18	22^{P}	27^{P}	33^{PC}	36	46	11
Cefepime	509	1	2	3	6^{P}	14^{P}	20^{P}	27^{C}	44	59	79	21
Ceftriaxone	88	—	—	—^{P}	2	3	5	18^{C}	42	51	100	16
Ceftazidime	669	1	2	3	5	10	23^{P}	30^{PC}	36	59	67	7
Imipenem	669	9	25	39	54	63	67^{PCE}	71	75	85	99	0
Meropenem	669	8	19	35	54	61^{E}	66^{PC}	72	77	88	100	5
Gentamicin	424	2	7	26	38	43^{P}	49^{CE}	57	72	77	81	6
Tobramycin	578	2	6	24	34	43^{P}	49^{CE}	54	66	75	83	6
Ciprofloxacin	669	18^{P}	27	32	34^{CE}	36	47	49	50	64	97	16
Levofloxacin	88	36	41^{P}	42^{P}	43^{E}	47^{C}	58	69	100	—	—	2

[a]The multiple annotations for PK–PD breakpoints represent their dose-dependent nature: piperacillin-tazobactam (3.375 g every 6 h, 4/4 mg/L; 4.5 g every 6 h, 4/4 mg/L; 3.375 g every 4 h, 16/4 mg/L), cefepime (1 g every 12 h, 1 mg/L; 1 g every 8 h, 4 mg/L; 2 g every 12 h, 2 mg/L; 2 g every 8 h, 4 mg/L), ceftazidime (1 g every 8 h, 4 mg/L; 2 g every 8 h, 8 mg/L), aztreonam (1 g every 8 h, 4 mg/L; 2 g every 8 h, 8 mg/L) and levofloxacin (500 mg every 24 h, 0.25 mg/L; 750 mg every 24 h, 0.5 mg/L). The CLSI does not have breakpoints for the following: *P. aeruginosa* (ertapenem) and *A. baumannii* (ceftizoxime, ertapenem and aztreonam), whereas the EUCAST does not have breakpoints for the following: Enterobacteriaceae (piperacillin-tazobactam and ceftizoxime), *P. aeruginosa* (piperacillin-tazobactam, ceftizoxime, ceftriaxone and ertapenem) and *A. baumannii* (piperacillin-tazobactam, cefepime, ceftizoxime, ceftriaxone, ceftazidime, ertapenem and aztreonam).

[b]This column reflects the difference in percentage susceptible among the three different breakpoints (CLSI, EUCAST and PK–PD). Large discrepancies indicate that the percentage susceptible varies greatly depending upon which breakpoint is applied.

[c]The most current MIC data available for ceftizoxime were from 2001.

species-specific cumulative frequency distribution for each antimicrobial. For Enterobacteriaceae, breakpoint discrepancies resulted in modest differences ($\leq$10%) in the percentage susceptible that resulted from the application of PK–PD, CLSI and EUCAST breakpoints: piperacillin-tazobactam (10%), ciprofloxacin (10%), levofloxacin (8%), ceftazidime (8%) and cefepime (7%). All others had differences of 4% or less.

In contrast, large discrepancies (>15%) were noted for *P. aeruginosa* and *A. baumannii*. For *P. aeruginosa*, the largest differences existed for: aztreonam (63%), cefepime (48%), levofloxacin (37%), ciprofloxacin (24%), piperacillin-tazobactam (21%) and ceftriaxone (17%). All others were 10% or less. Likewise, for *A. baumannii*, large differences existed for cefepime (21%), ciprofloxacin (16%), ceftriaxone (16%) and piperacillin/tazobactam (11%). The remaining susceptibilities differed by 7% or less.

Discussion

Infectious disease practitioners have a unique opportunity to deliver pathogen-directed therapy because they can remove the offending pathogen from the patient's body and examine it in the laboratory. In doing so, they are able to identify the exact microbial species and determine its responsiveness to antimicrobial therapy. Antimicrobial susceptibility testing has long been recognized as a vital process in the management of patients with infectious illnesses. Such testing enables clinicians to customize therapy and presumably enhances the probability of a successful treatment outcome. Antimicrobial susceptibility testing essentially involves three processes: (i) testing; (ii) interpretation; and (iii) reporting. Testing and reporting have received much attention, while less has been discussed regarding interpretation. This may be due to the fact that the art of breakpoint setting has largely been the responsibility of a concentrated group of clinicians, microbiologists and scientists who have historically established criteria for the international community. Many still view the breakpoint-setting process as a 'black box', clouded by complexity, bureaucracy and special interests and therefore are unsure how they might contribute to this important process. In stark contrast to this view, the CLSI strives for transparency and welcomes input from practice and the scientific community in the form of microbiology data, clinical outcomes studies and PK–PD information.

This study provides such information by systematically evaluating existing susceptibility breakpoints for several antimicrobial classes considered clinically useful for the treatment of patients infected with Gram-negative aerobic bacteria. Overall, the PK–PD simulations described in this paper support the conservation of existing breakpoints for most of the older antimicrobials used clinically for the treatment of Gram-negative aerobic infections. When differences were noted, the PK–PD breakpoints were generally lower than the CLSI breakpoints and higher than the EUCAST breakpoints. Since both EUCAST and PK–PD breakpoints are heavily dependent upon PK–PD simulations, these comparisons provide some indication that the widespread inclusion of PK–PD simulations in the CLSI breakpoint-setting process might lead to the derivation of lower breakpoints than presently exist for the older antimicrobials. This represents the worst possible scenario because it suggests that the current CLSI breakpoints might lead the user to incorrectly conclude that an isolate is susceptible when the PK–PD simulation predicts failure.

Another group has also evaluated susceptibility breakpoints for Gram-negative bacteria.[32] Similar to the present study, DeRyke *et al.*[32] utilized PK–PD simulations to develop susceptibility breakpoints. Then they compared the percentage susceptible achieved with PK–PD and CLSI breakpoints. Despite minor differences in model assumptions, PK–PD indices selection and modelling equations, both studies reported similar PK–PD-based breakpoints and concluded that PK–PD and CLSI breakpoints resulted in similar susceptibilities for Enterobacteriaceae, but not *P. aeruginosa* and *A. baumannii*. The CLSI has discussed breakpoint revisions for Enterobacteriaceae for several years, but these studies suggest that the most urgent area may be the revision of breakpoints for *P. aeruginosa* and *A. baumannii*. As stated in the introduction, the EUCAST has already established separate breakpoints for these problematic bacterial species. In several instances, the EUCAST and CLSI breakpoints are widely divergent.

While PK–PD simulations can assist with the establishment of antimicrobial breakpoints, it is important to remember that these simulations are based on a number of assumptions. First, the basic justification for PK–PD modelling is that prior studies have identified correlations between PK–PD indices and health outcomes. These relationships have typically been derived from immunocompromised murine models; however, some have been validated in clinical populations.[33] Second, the PK–PD equation used to construct the β-lactam models represents a one compartment iv bolus model and does not consider the $\%T > \text{MIC}$ contributed by the time of infusion.[30] While the PK–PD impact is minimal for short intravenous infusions ($\leq$30 min), prolonged or continuous infusions would enhance the ability of a given β-lactam regimen to achieve PK–PD targets.[10,34,35] With regard to the pharmacokinetic data, the parameters chosen were selected from healthy adults rather than patients. Since many antimicrobials are renally eliminated and patients may have compromised renal function, these models may predict lower drug exposures and consequently lower PK–PD-based breakpoints. In defence of this strategy, CLSI breakpoints are used for patients with both normal and compromised renal function; therefore, the use of pharmacokinetics from healthy volunteers constitutes the most conservative approach. In addition, a recent study has demonstrated that the PTA is similar whether the pharmacokinetic parameters are obtained from healthy volunteers or patients.[36]

The pharmacokinetic parameters used in this study pertain to values measured in serum; therefore, these PK–PD-based breakpoints are most readily applicable to bloodstream infections. However, many of the antimicrobials mentioned in this study are used clinically for diseases such as pneumonia and urinary tract infections and it is well recognized that some antimicrobials, including the fluoroquinolones, have higher concentrations in the epithelial lining fluid and in the urine than in the blood. The reader should recognize that neither the CLSI nor the EUCAST endorse disease-specific breakpoints. Furthermore, it is customary for the CLSI to only consider PK–PD models based on serum pharmacokinetics. Finally, clinicians should recognize the regimen-dependent nature of PK–PD breakpoints. Higher doses can be used to improve the PK–PD and enhance the probability of clinical success. Remember that the CLSI establishes breakpoints for the global community; however, drug regimens differ

greatly among countries or even within geographic regions of the same country.

As PK–PD targets continue to be refined, it is important to acknowledge that minor changes in the desired target could have a large impact on the PTA; therefore, we have provided the PTA for various PK–PD targets in the Supplementary data [Figure S1 and Table S1, available at *JAC* Online (http://jac.oxfordjournals.org/)]. It is also worth mentioning that the adoption of a breakpoint that dissects a wild-type MIC distribution may negatively impact the reliability of laboratory testing; therefore, when this occurs, breakpoint-setting committees generally adopt a breakpoint near the upper end of the wild-type MIC distribution. In addition to the laboratory testing concern, recent evidence suggests that this practice 'places patients in harms way and likely undermines clinician confidence in susceptibility breakpoints'.[37]

Certainly, PK–PD simulations are not the only data to consider when establishing antimicrobial breakpoints. Regulatory authorities and breakpoint-setting bodies must also consider microbiology data, known resistance mechanisms and clinical data. However, when any of these types of data unveil a potential signal—as is the case with the PK–PD simulations in this study—these bodies have a civic and ethical duty to be responsive and revisit existing breakpoints in light of contemporary information.

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