International Journal of Antimicrobial Agents 53 (2019) 674–677

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Breakpoint to MIC quotient: A parameter to rapidly evaluate the in vitro bactericidal activity of β-lactams on Enterobacteriaceae

Antoine Grillon[a,*], Angeline Chabaud[a], Gilles Zambardi[b], Isabelle Caniaux[c], François Jehl[a]

[a] Laboratory of Bacteriology, University Hospital of Strasbourg, Strasbourg, France
[b] Research and Development, bioMérieux, La Balme-les-Grottes, France
[c] Medical Affairs, bioMérieux, Marcy-l'Etoile, France

ARTICLE INFO

Article history:
Received 10 December 2018
Accepted 27 January 2019

Editor: Jean-Marc Rolain

Keywords:
Enterobacteriaceae
Breakpoint to MIC quotient
Etest
European Committee on Antimicrobial Susceptibility Testing
Minimum Inhibitory Concentration
Minimum Bactericidal Concentration

ABSTRACT

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) do not completely predict the bactericidal nature of the antimicrobial agent. Patients with pathogens having MICs near the clinical breakpoint experience a higher risk of clinical failure. This study defined an indicator, breakpoint to MIC quotient (BMQ), that incorporates MIC and the European Committee on Antimicrobial Susceptibility Testing breakpoint. The BMQ was inversely correlated with MBC in antibiotic combinations against Enterobacteriaceae strains (Spearman coefficient ≤ -0.96). This new parameter may provide timely additional insight for choosing an antibiotic for a severe bacterial infection.

1. Introduction

The treatment of severe bacterial infections warrants the most effective antibiotics in vivo to optimise efficacy and decrease the risk of antibiotic resistance selection [1]. Classically, this choice is based on in vitro susceptibility testing, which identifies the minimum inhibitory concentration (MIC) for specific antibiotics. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides a list of clinical MIC breakpoints, which categorise microorganisms as susceptible or resistant to specific antimicrobial agents and guide their clinical use [2]. The clinical breakpoint of an antibiotic is established according to defined rules [3], including microbiological considerations through the epidemiological cut-off (ECOFF) value, pharmacokinetic/pharmacodynamics (PK/PD) considerations through the PK/PD breakpoint, and clinical considerations through association with clinical cure [4,5]. Antibiotic choice also can be based on the minimum bactericidal concentration (MBC), although this time-consuming method may not provide results in time to assist in choosing the optimal antibiotic.

Patients infected with Gram-negative Enterobacteriaceae or *Pseudomonas aeruginosa* isolates showing borderline MICs (near the breakpoint) may be at higher risk of clinical failure, even mortality, than those with infections having lower MICs [6,7]. Similarly, Falagas et al. found that more treatment failures occurred in patients infected with *Salmonella enterica* strains with high fluoroquinolone MICs (near the breakpoint) than those with Salmonella strains with low MICs, and mortality rate for patients infected by Gram-negative non-fermentative bacilli with high MICs was also higher than for those with low MICs [8].

This study hypothesised that a new ratio, the breakpoint to MIC quotient (BMQ), would inversely correlate with the antibiotic MBC and could bring additional insight for choosing an antibiotic in a timely manner.

2. Methods

To compare two methods of MIC determination, the MICs of 35 Enterobacteriaceae strains (*Klebsiella pneumoniae,* n = 17; *Escherichia coli,* n = 11; *Enterobacter* spp., n = 7) were measured with various resistance mechanisms to the six β-lactam antibiotics listed in Table 1. Imipenem, meropenem, cefepime, cefotaxime, and ceftazidime titrated powders were European Directorate for the Quality of Medicines and Healthcare (EDQM) reference standards; aztreonam titrated powder was purchased from Sigma Aldrich. The

* Corresponding author. Laboratory of Bacteriology, University Hospital of Strasbourg, 1 place de l'hôpital, 67000 Strasbourg, France.
E-mail address: antoine.grillon@chru-strasbourg.fr (A. Grillon).

https://doi.org/10.1016/j.ijantimicag.2019.01.013
0924-8579/

Table 1
Gram-negative strains, resistance mechanisms, the calculated Breakpoint to Minimal Inhibitory Concentration Quotients (obtained by Etest and Broth Microdilution), and Minimal Bactericidal Concentrations.

Strains	Resistance mechanisms	BMQs (Etest/BMD) and MBCs (mg/L)					
		Imipenem	Meropenem	Aztreonam	Cefepime	Cefotaxime	Ceftazidime
E. coli ATCC 25922	Wild type	10.5/21/0.115	143/167/0.013	11/7/0.167	21/21/0.047	13/16/0.073	5/21/0.25
E. coli ATCC 35218	TEM-1	10.5/21/0.110	125/250/0.009	18/21/0.047	31/43/0.023	21/36/0.031	8/14/0.094
E. coli 1310019	*AmpC*	9/19/0.104	125/166/0.012	0.25/0.15/6.67	5/8/0.125	0.3/0.3/3.3	0.17/0.2/9.3
E. coli-A	ESBL	8/11/0.22	62/83/0.016	0.3/< 0.1/> 256	0.25/< 0.1/32	< 0.1/< 0.1 />256	1/0.6/1.5
E. coli-B	ESBL	16/21/0.136	62/166/0.018	0.3/0.25/4	0.25/0.15/6.67	< 0.1/< 0.1/> 256	0.8/0/6/1.5
E. coli-C	ESBL	16/24/0.084	250/285/0.008	11/15/0.063	16/28/0.036	8/< 0.1/> 256	6/7/0.146
E. coli-D	ESBL	10.5/16/0.167	125/91/0.022	0.5/0.3/3.33	0.2/0.1/8	< 0.1/< 0.1/> 256	1.3/0.8/1.25
E. coli 0506040	*Carbapenemase*	2/2/1	5/7/0.3	ND	ND	ND	ND
E. coli 1012303	*Carbapenemase*	0.6/1/2	1/2.7/0.75	ND	ND	ND	ND
E. coli 1104350	Carbapenemase	0.25/0.5/4	0.6/0.5/4	ND	ND	ND	ND
E. coli 1211021	*Carbapenemase*	0.5/0.5/4	1.6/0.6/3	ND	ND	ND	ND
K. pneumoniae 9812031	ESBL SHV	3/4/0.67	87/64/0.031	< 0.1/< 0.1/32	1/0.6/3.3	0.25/ND/ND	< 0.1/< 0.1/32
K. pneumoniae 105102	acquired *AmpC*	1.3/3/0.75	87/87/0.023	0.25/ND/ND	21/21/0.047	0.1//ND/ND	0.25/< 0.1/32
K. pneumoniae 212018	acquired *AmpC*	8/3/0.75	21/32/0.063	0.1/< 0.1/28	5/3/0.375	< 0.1/< 0.1/29.3	< 0.1/< 0.1/32
K. pneumoniae 502051	ESBL TEM	4/4/0.53	2/3/1.5	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32
K. pneumoniae 509019	ESBL CTX-M	8/9.5/0.209	31/87/0.033	1/0.6/1.75	0.3/0.25/4	< 0.1/< 0.1/32	1.75/1.75/0.38
K. pneumoniae 702090	acquired *AmpC*	< 0.1/< 0.1/16	< 0.1/0.25/9.3	< 0.1/< 0.1/25	0.2/0.1/10.7	< 0.1/< 0.1/32	< 0.1/< 0.1/24
K. pneumoniae	ESBL	13/8/0.25	87/87/0.023	0.8/0.4/2.5	0.6/0.1/9.3	< 0.1/< 0.1/> 256	1.3/1.2/1.16
K. pneumoniae 1602110	ESBL CTX-M	3/3/0.75	0.6/1.7/1.5	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/24
K. pneumoniae 1402021	ESBL CTX-M	0.5/1/1.75	1/2/6	< 0.1/< 0.1/32	0.25/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32
K. pneumoniae 1104053	*Carbapenemase*	1.4/1.6/1.5	4/1/1	ND	ND	ND	ND
K. pneumoniae 1109133	*Carbapenemase*	4/1.3/1.5	3/5/0.4	ND	ND	ND	ND
K. pneumoniae 1602112	ESBL CTX-M	3/4/0.5	1.5/2/2	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32
K. pneumoniae 1602107	ESBL CTX-M	0.5/0.6/3.5	0.14/0.2/12	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/22
K. pneumoniae 1508256	ESBL CTX-M	10.5/21/0.136	62/87/0.023	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/25
K. pneumoniae 1008101	Acquired *AmpC*	8/10.5/0.19	31/11/0.175	<0.1/< 0.14/26.7	1.3/1.2/0.83	< 0.1/< 0.1/26.7	< 0.1/< 0.1/32
K. pneumoniae 1102106	ESBL CTX-M	10.5/10/0.25	62/64/0.031	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/32	< 0.1/< 0.1/24
K. pneumoniae 0804168	*Carbapenemase*	4/1.5/1.3	5/5/0.4	ND	ND	ND	ND
E. cloacae 1008085	*Carbapenemase*	0.6/0.125/5	0.2/0.3/6	ND	ND	ND	ND
E. cloacae 1011184	*Carbapenemase*	0.2/0.2/12	< 0.1/0.1/9	ND	ND	ND	ND
E. cloacae 1602081	ESBL + acquired *AmpC*	0.6/0.5/4	0.7/1.2/2.17	< 0.1/< 0.1/26.6	0.6/0.3/5.5	< 0.1/< 0.1/32	0.125/< 0.1/24
E. aerogenes 1602080	ESBL + acquired *AmpC*	0.2/0.7/3	1/1.1/2	< 0.1/< 0.1/24	0.3/0.8/1.3	< 0.1/< 0.1/32	< 0.1/< 0.1/29.3
E. hormaechei 1402023	acquired *AmpC*	0.5/0.7/3	1/0.4/5.3	< 0.1/< 0.1/32	0.25/0.12/6.7	<0 .1/< 0.1/32	< 0.1/< 0.1/32
E. cloacae 1204174	acquired *AmpC*	2.7/3/0.75	21/16/0.125	< 0.1/< 0.1/32	< 0.1/0.6/1.83	0.125/< 0.1/32	< 0.1/< 0.1/32
E. cloacae 1204193	acquired *AmpC*	2.6/3.4/0.58	11/5/0.44	< 0.1/< 0.1/32	1/0.3/3	< 0.1/< 0.1/32	< 0.1/< 0.1/32

Abbreviations: BMQs: Breakpoint to Minimal inhibitory concentration Quotients; MBCs: Minimal bactericidal Concentrations; BMD: Broth microdilution; ESBL: Extended-spectrum-beta-lactamase; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae; E. cloacae*: *Enterobacter cloacae*; *E. hormaechei*: *Enterobacter hormaechei.*

MICs were measured simultaneously using the same broth suspension (0.5 McFarland units) for each strain by two methods: (i) the broth microdilution (BMD) method, as described in the EUCAST recommendations, in agreement with the International Organization for Standardization ISO 20776-1 [2]; and (ii) gradient-strip tests (Etest, bioMérieux, La-Balme-les-Grottes, France) according to the manufacturer recommendations. The MBCs were measured by first determining the MIC by the BMD method [2], followed by plating 10 microliters from all tubes at or above MIC onto Mueller-Hinton agar. MBC was defined as the lowest concentration leaving ≤ 0.01% bacteria compared with the control [9].

3. Results

Both methods of MIC measurement were compared (171 pairs): the BMD method showed a statistically significant direct linear correlation with the gradient-strip method, with a Spearman coefficient of correlation 0.9708 (95% confidence interval [CI] 0.9612–0.9781; $P < 0.001$; Fig. 1A). The MBCs are shown in Table 1.

For the new potential indicator, the BMQ was defined as the quotient of the susceptible (lower) breakpoint divided by the MIC value for the pathogen [2]. To test the hypothesis that a higher BMQ value would predict a lower MBC, the BMQ values were calculated and compared with the bactericidal effect of the antibiotic of each strain measured by the MBC. The BMQ calculated with MICs measured by the Etest showed a statistically significant inverse correlation with the MBC, with a Spearman correlation coefficient of –0.9631 (95% CI –0.9722 to –0.9512; $P < 0.001$; Fig. 1B). Similarly, BMQ calculated with MICs measured by BMD showed a statistically significant inverse correlation with the MBC, with a Spearman correlation coefficient of –0.9778 (95% CI –0.9833 to –0.9706; $P < 0.001$; Fig. 1C). Based on these data, BMQ seems to be a good indicator of the potential in vitro bactericidal activity of tested antibiotics.

4. Discussion

Since isolates with MICs near the breakpoint have lower clinical response to the antibiotic [6-8], the choice between two antibiotics with the same MIC for the same pathogenic isolate but with two different clinical breakpoints would likely benefit from consideration of this ‘distance’ to the breakpoint. This is especially true for immunocompromised patients, who need antibiotics with high bactericidal activity because of their impaired immune system [10]. Although measurement of MBC should be useful in these situations, it is not routinely used because the method is time-consuming and precise. Moreover, the advantages of the BMQ over the MBC are the more rapid results and the adjustments for ECOFF value, PK/PD considerations through the PK/PD breakpoint, and clinical considerations by using the well-established, clinically used, periodically updated breakpoints of EUCAST [2,3,11,12]. Clinical considerations for the antibiotic breakpoint include the MIC

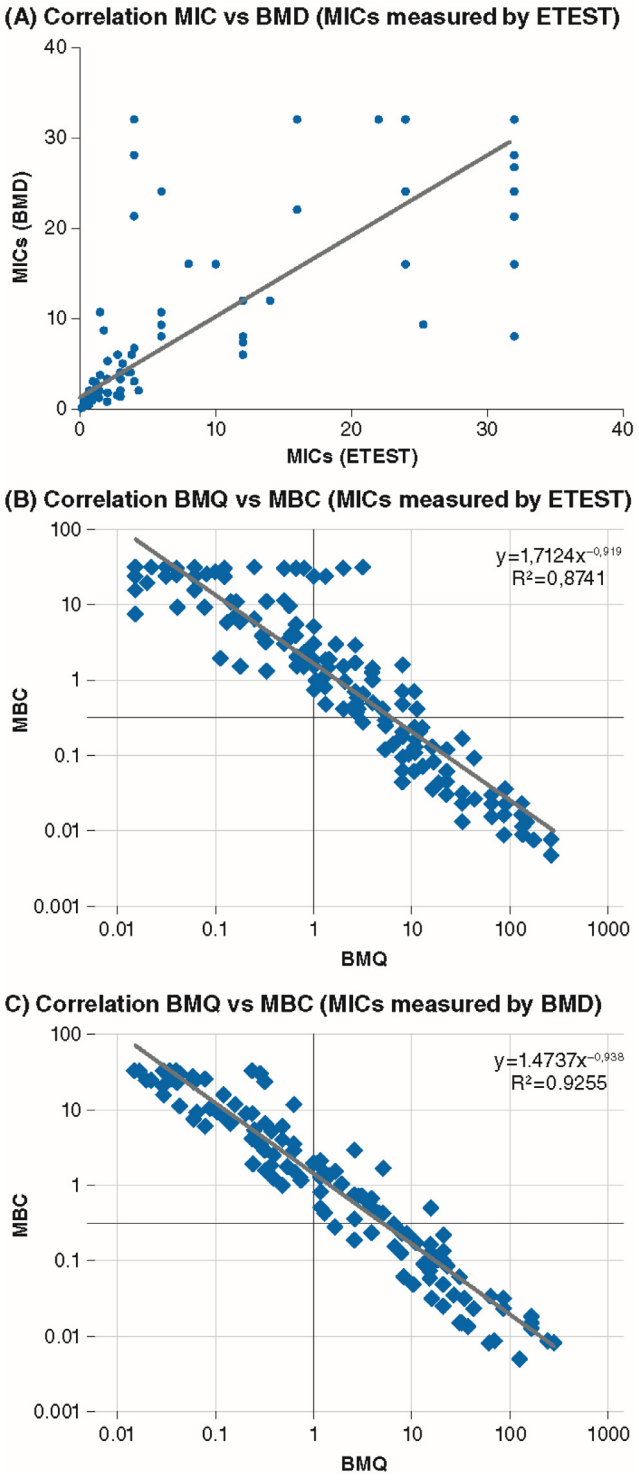

Fig. 1. (A) Linear correlation between MICs as measured by Etest (*x*-axis) and BMD (*y*-axis) for all the strains tested and all antibiotics (171 XY pairs): Spearman $r = 0.9708$ (95% confidence interval 0.9612–0.9781; $P < 0.001$). (B) Inverse correlation between BMQ (with MIC measured by Etest) and MBC for the 35 strains and the six antibiotics tested. Semi-log plot comparing BMQ (with MIC measured by gradient-strip test) and MBC. (C) Inverse correlation between BMQ (with MIC measured by BMD) and MBC for the 35 strains and the six antibiotics tested. Semi-log plot comparing BMQ (with MIC measured by BMD) and MBC.

values for the wild-type bacterial population through the ECOFF value. The PK/PD clinical breakpoint of β-lactam antibiotics means that the target concentration will be reached in approximately 90% of patients for 30–70% of the time between antibiotic administrations. Antibiotic treatment at breakpoint concentrations should provide one or two logs of bactericidal activity, sometimes bacteriostatic activity; pathogens with lower MICs may experience greater declines, as suggested in animal experiments [3].

The current results are obviously theoretical and only represent in vitro considerations. Evaluation of this parameter on an immunocompromised animal model will be necessary to its in vivo validation. However, BMQ showed an excellent correlation with MBC in strains exhibiting various resistance mechanisms, including β-lactamases (TEM-1 β-lactamase, n = 1; expanded-spectrum β-lactamases [ESBLs], n = 14; de-repressed or plasmid-acquired *ampC*, n = 7; ESBL + acquired *amp*C, n = 2; and ESBL Sulfhydril-variable (SHV) and carbapenemases, n = 9).

In summary, the BMQ is an easy-to-calculate parameter for the majority of routinely detected bacteria once the MIC is known, and may predict the bactericidal activity. This indicator may represent an easy-to-use method for clinicians to interpret the MICS, and it warrants further study such as in animal models. Of particular interest would be to extend the concept to other antibiotic families and other microorganisms such as fluoroquinolones on Gram-negative bacteria, and glycopeptides on Gram-positive bacteria.

Acknowledgments

Editorial support, including medical editing, was provided by The Curry Rockefeller Group, LLC, and funded by bioMérieux.

Funding

This study was funded by bioMérieux, Inc. AG and AC have no disclosure for this work.

Competing Interests

GZ and IC are employees of bioMérieux. FJ is coordinator of the 'CA-SFM' (for Comité de l'Antibiogramme de la Société Française de Microbiologie, the French National Antimicrobial Committee) and has received payments from bioMérieux for expert testimony, equipment, and writing assistance.

Ethical Approval

Not required.

References

[1] Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44(Suppl 2):S27–72. doi:10.1086/511159.

[2] EUCAST. European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters, 7.1; 2017.

[3] Mouton JW, Brown DFJ, 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. doi:10.1111/j.1469-0691.2011.03752.x.

[4] Lee NY, Lee CC, Huang WH, Tsui KC, Hsueh PR, Ko WC. Cefepime therapy for monomicrobial bacteremia caused by cefepime-susceptible extended-spectrum beta-lactamase-producing enterobacteriaceae: MIC matters. Clin Infect Dis 2013;56:488–95. doi:10.1093/cid/cis916.

[5] Patel TS, Nagel JL. Clinical outcomes of enterobacteriaceae infections stratified by carbapenem MICs. J Clin Microbiol 2015;53:201–5. doi:10.1128/JCM.03057-14.

[6] Torres E, Delgado M, Valiente A, Pascual A, Rodriguez-bano J. Impact of borderline minimum inhibitory concentration on the outcome of invasive infections caused by Enterobacteriaceae treated with β-lactams: a systematic review and meta-analysis. Eur J Clin Microbiol Infect Dis 2015;34:1751–8.

[7] Su T, Ye J, Yang C, Huang C, Chia J, Lee M. Influence of borderline cefepime MIC on the outcome of cefepime-susceptible Pseudomonas aeruginosa bacteremia treated with a maximal cefepime dose: a hospital-based retrospective study. Ann Clin Microbiol Antimicrob 2017;24:52.
[8] Falagas ME, Tansarli GS, Rafailidis PI, Kapaskelis A, Vardakas KZ. Impact of antibiotic MIC on infection outcome in patients with susceptible gram-negative bacteria: A systematic review and meta-analysis. Antimicrob Agents Chemother 2012;56:4214–22. doi:10.1128/AAC.00663-12.
[9] Thrupp L. Susceptibility testing of antibiotics in liquid media. Baltimore: V. Lorian; 1986.
[10] DeGirolami P, Eliopoulos G. Antimicrobial susceptibility tests and their role in therapeutic drug monitoring. Clin Lab Med 1987;7:499–512.
[11] Brown D, Canton R, Dubreuil L, Gatermann S, Giske C, MacGowan A, et al. Widespread implementation of EUCAST breakpoints for antibacterial susceptibility testing in Europe. *Euro SurveillBull Eur Sur Les Mal Transm = Eur Commun Dis Bull* 2015;15 pii: 21008. doi:10.2807/1560-7917.ES2015.20.2.21008.
[12] EUCAST.org. The European Comitee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameter, version 8.0 2018.