MIC-based dose adjustment: facts and fables

Johan W. Mouton1*, Anouk E. Muller1,2, Rafael Canton3, Christian G. Giske4, Gunnar Kahlmeter5 and John Turnidge6

1Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands; 2Department of Medical Microbiology, Haaglanden Medical Centre, The Hague, The Netherlands; 3Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYIS), Madrid, Spain; 4Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden; 5Department of Clinical Microbiology, Central Hospital, 351 85, Växjö, Sweden; 6Adelaide Medical School, University of Adelaide, Adelaide, Australia

*Corresponding author. E-mail: jwmouton@gmail.com

Over recent decades, several publications have described optimization procedures for antibiotic therapy in the individual patient based on antimicrobial MIC values. Most methods include therapeutic drug monitoring and use a single MIC determination plus the relevant pharmacokinetics/pharmacodynamics to adjust the dose to optimize antimicrobial drug exposure and antibacterial effects. However, the use of an MIC obtained by a single MIC determination is inappropriate. First, routine clinical laboratories cannot determine MICs with sufficient accuracy to guide dosage owing to the inherent assay variation in the MIC test. Second, the variation in any MIC determination, whatever method is used, must be accounted for. If dose adjustments are made based on therapeutic drug monitoring and include MIC determinations, MIC variation must be considered to prevent potential underdosing of patients. We present the problems and some approaches that could be used in clinical practice.

Introduction

In recent years, several papers have described optimization procedures for antibiotic therapy based on microbial MIC values.1–4 The basis for these is usually that the MICs of relevant antimicrobials are determined and reported to the clinician. It is proposed that concurrently determined antimicrobial serum concentrations are used to determine the individual pharmacokinetic profile of the drug, usually with the help of a computerized population pharmacokinetic program. The program is expected to help the clinician to adjust the dosing regimen to optimize exposure, taking into account pharmacokinetic/pharmacodynamic relationships, patient-specific pharmacokinetic characteristics and the MIC value. Although pharmacokinetic variations are addressed in population pharmacokinetic programs, few clinicians are aware of the performance characteristics of MIC determination and its limitations. The truth is that with the methods at hand and with few exceptions, laboratories are not capable of performing a sufficiently accurate and reproducible determination of an MIC value owing to the inherent assay variation in the MIC test. In addition, semi-automated susceptibility testing machines yield truncated MICs and gradient tests can be quite often problematic. Even with perfectly performed tests the variation may be pronounced, one reason being that the tests produce discontinuous results on an interval scale (i.e. MIC values allocated to discrete values, usually a 2-fold dilution scale), which results in values falling across 2-fold intervals when variation occurs. This may lead to significant dosing adjustment errors, which could ultimately be harmful to patients.

Here we briefly provide the scientific basis for this issue and present a number of examples to underscore our point of view based on current knowledge of MIC measurement characteristics and pharmacokinetic profiling.

What does an MIC represent?

An MIC is obtained using an assay that determines in vitro the concentration that prevents visible growth (significant cell multiplication as determined by the unaided eye) in or on a standardized medium over 18–2 h, at 34°C–37°C using a standardized inoculum.5 The concentrations used are 2-fold dilutions up and down from the concentration 1 mg/L; thus typically 128, 64, 32, etc. down to 0.016 and 0.008 mg/L. Therefore, the MIC is an endpoint measurement and represents the result of growth and bacteriostatic/bactericidal effects over time. In liquid media, visible growth occurs at bacterial densities of ~107–108 cfu/mL. As the initial inoculum in the test is 5×105 cfu/mL, growth may have occurred during the period of incubation but not reached sufficient densities to become visible. The minimum in the MIC is therefore not a strict minimum but rather an observable minimum. Should for some reason the inoculum be larger than that stipulated or the incubation time longer, the MIC will probably be higher and vice versa for shorter incubation and smaller inoculum.

Thus, the MIC does not represent a concentration that can be compared directly with any in vivo concentration found during treatment. In particular, it does not predict the overall bacterial response whether the in vivo concentration is above or below the...
MIC. The MIC is the phenotypic endpoint in a defined and standardized but highly relative system. Importantly, the MIC is measured in broth and not in the range of physiological environments found in the human body. Bacterial growth conditions in the ‘test tube’ are significantly different from those in vivo and no direct inference can or should be made about its relationship to in vivo concentrations. The MIC has value as a standardized measure of activity of the antibiotic against the microorganism.

What is the accuracy of an MIC?
The accuracy of an MIC measurement is, with a few exceptions for some organism–antimicrobial combinations, not very high. If the MIC for a certain isolate versus an antibiotic is determined repeatedly in one laboratory using the same standardized conditions and by the same lab technician, there will be a log₂ standard deviation of ~0.3–0.5 around the log₂ (the 2-fold dilution series is a base 2-logarithmic series). If the same strain–antimicrobial combination is tested in a range of laboratories, the log₂ standard deviation might increase to ~0.5–1 2-fold dilution and more when media obtained from several manufacturers are used. The variability of an MIC measurement thus consists of several components: inter-strain differences, intra-laboratory variability and inter-laboratory variability. In other words, there is biological variation (strain-to-strain differences) and assay variation as a result of differences in inoculum preparation, media, incubation temperature and incubation time, and variation between laboratories because different laboratories have different facilities and technical skills and degree of training. This variation is also well recognized in the accepted MIC ranges of quality control strains, which often span over two to three dilutions and even four dilutions in some cases. Moreover, the acceptance criteria for reproducibility of an MIC device following the ISO 20776-2 norm indicate an acceptable deviation of one dilution from the mode for 95% of cases, or a range of at least two dilutions in three dilutions and even four dilutions in some cases. Moreover, the acceptance criteria for reproducibility of an MIC device following the ISO 20776-2 norm indicate an acceptable deviation of one dilution from the mode for 95% of cases, or a range of at least two 2-fold dilutions.

Biological variation (strain-to-strain differences) within a species
Individual strains within a species, even when there are no acquired resistance mechanisms, have individual characteristics other than their susceptibility to the agent that will provide differences in MICs. These include the rate of division (growth rate), metabolic status and a variety of antimicrobial receptors. The MIC value summarizes the impact of all characteristics in a single test result. However, it has become apparent that in strains devoid of acquired resistance to the agent in question (the so-called WT) the difference between MIC values within a species is slight; for most species and agents it is only three to five 2-fold concentrations and the major and reproducible difference is between individuals without and with acquired resistance. The upper end of the WT distribution (the highest MIC for isolates devoid of phenotypically detectable resistance) is defined as the epidemiological cut-off value (ECOFF). Under strict standard conditions strain-to-strain differences belonging to the WT can sometimes be determined. In one laboratory, under standard conditions, the reproducibility of the test is better, most likely because part of the variation in phenotype is better controlled, as explained above. However, this requires a number of replicate measurements and not a single measurement.

Assay variation
Variation in MICs not explained by biological variation accounts for a significant part of the total variation observed in the WT distribution. Thus, the greater contribution to observed variation in MIC is assay variation, which can mask any variation in MICs between strains. If MICs are determined multiple times in several labs, it was estimated that at least half the variation observed was due to assay variation, and less than half the variation was due to strain-to-strain differences. Although we often perceive a certain strain as something absolute and not changing, in reality bacteria divide continuously, leading to subtle differences over time of the culture. The definition of a strain is therefore a certain parental lineage but not the same individual bacteria. As the number of generations increases over time, small changes in environment have the impact of variations between individual cultures.

MIC and pharmacokinetic/pharmacodynamic relationships
Pharmacokinetic/pharmacodynamic relationships have been described for most antimicrobial classes, using free serum concentrations as a surrogate for the concentrations at the site of infection. These relationships show a marked consistency, and the pharmacodynamic index values (such as AUC/MIC or %fT>MIC) that result in a certain effect have been determined for most classes of antimicrobials. If the effect is measured in terms of bacterial counts, the ones most often used are the exposures (pharmacokinetic/pharmacodynamic index values), resulting in a static effect over time or a 1 log₁₀ kill effect, often called pharmacodynamic targets. These can be determined in various models, including animal models and hollow fibre in vitro models. Important for the present discussion is the MIC in the equation. The MICs for the strains used in these studies are usually determined multiple times in dedicated laboratories and the assay variability of the MIC is thus restricted to limited intra-laboratory variation. Important, however, is that these targets are derived from a number of strains with a range of MICs and thus the point estimate of the pharmacodynamic index is therefore reasonably well described, provided that enough strains have been tested in the model. When translating this to the clinical setting, this means that the pharmacodynamic target is reasonably accurate for a certain disease setting. However, the application of the pharmacodynamic target to adjustments of dosing regimens based on a single MIC result from an individual strain is not accurate, as explained in the next section.

MICs, therapeutic drug monitoring and dose adjustments
Therapeutic drug monitoring is increasingly being used for antimicrobial agents, particularly in patients in the ICU with unpredictable clearance. Several studies have shown that preset pharmacokinetic/pharmacodynamic targets were not met in such patient groups, one of the major reasons being augmented
clearance.\textsuperscript{17} MICs for strains that are cultured from patient specimens and likely to be the cause of the infection are increasingly used to determine individual pharmacokinetic/pharmacodynamic targets.\textsuperscript{2} It should be clear from the arguments above that this is not justified, because the measurement of an MIC is not sufficiently accurate. Thus, there is a significant risk of underdosing if by chance the measured MIC was at the low end of values that would have been observed if the test had been performed repeatedly. Thus, even if the MIC varies over only two dilutions, the pharmacokinetic concentrations to be reached vary 4-fold because the pharmacokinetic/pharmacodynamic target such as the AUC/MIC may vary 4-fold.

**Is there a solution? Practical guidance from an MIC point of view**

Given the inaccuracy of an individual MIC measurement, there is no universal solution for MIC-guided therapy as will be demonstrated by the following examples. Nevertheless, some practical guidance can be given as discussed below to avoid errors and misinterpretations in dosing adjustment.

First, consider MICs from a distribution point of view. In general, three sections of an MIC distribution can readily be distinguished (Figure 1). The first is the WT distribution (white area) representing MICs for isolates considered devoid of phenotypically detectable resistance mechanisms. The second (striped area) represents MICs for strains just outside the WT distribution with low-level resistance and in which isolates may or may not be different from each other. An example here is the so-called plasmid-mediated quinoline resistance mechanism such as the qnr or aac(6')-Ib-cr genes in *Escherichia coli*.\textsuperscript{18} or a low-level expression of certain β-lactamases.\textsuperscript{19} Sometimes resistance to the agent is so discrete that another related agent is better suited to disclose the presence of the resistance mechanism.\textsuperscript{20} Finally, there are the MICs for strains with a phenotypical high-level resistance (black area). The questions that arise are: Given the poor accuracy of an individual MIC measurement for a particular isolate, to which of the three MIC distributions does the strain belong? Which MIC value could be used for pharmacodynamic attainment in the individual patient? As the MIC for a certain species and/or agent may well have a higher rate of accuracy than that obtained for another, does this have a major impact on decision making? From a practical point of view there are four possibilities, and in each case the MIC used to determine the pharmacodynamic target in the individual patient warrants a different approach.

The first scenario is that the MIC for the strain indicates that it is within the WT distribution. In this case, it should be clear that the MIC is not a fixed number but is within the range of values with the ECOFF as the upper limit. The WT range of values follows a log-normal distribution and is a composite of strain-to-strain variation (biological variation) and both intra- and inter-laboratory variation (assay variation). It follows that in this situation the ECOFF should be taken as the value to determine the pharmacokinetic/pharmacodynamic target for the individual. ECOFF values are readily available at the web site of the EUCAST.\textsuperscript{21} In many situations, the ECOFF is similar to the clinical breakpoint; the isolate is reported as susceptible by the laboratory and it is therefore primarily the pharmacokinetic and disease characteristics of the patient that will determine adjustment of the dosing regimen. This is particularly true for new drugs with a new mechanism of action, against which low- and high-level resistance mechanisms are infrequent.

The second situation is an MIC immediately above the ECOFF. Here, the actual distribution of MICs for the particular strain that would be observed with repeated testing is unknown. In most of the cases and provided the laboratory is proficient in the MIC determination, it could be argued that the reported MIC could be correct within a range of three 2-fold dilutions. However, if the laboratory is less proficient or if the initial value represented the outer boundaries of the distribution obtained with repeat testing, the reported value could be as much as four dilutions off. In addition, there is a paucity of published data in the literature on the reproducibility outside the WT population. However, there is no clear reason to assume a priori that the variability would be higher or lower. An overly conservative system could lead to a risk of overdosing and assuming a 2-fold variation even outside the WT distribution could be a reasonable compromise. Thus, although there is no consensus on this issue, one suggestion could be to use a margin of at least two 2-fold dilutions if used for individual target attainment calculations. This would significantly increase the margin of safety.

The third situation is an individual MIC value that is clearly outside the WT population and clearly (far) above the clinical resistance breakpoint. There is little chance of increasing exposure to reach the desired pharmacodynamic target. MIC-guided therapy is not an option.

The fourth situation sometimes arises as a complication of the first two. Owing to emerging resistance and because of the inaccuracy of measurement of the MIC, a strain with a low-level resistance mechanism could be assigned to the WT, particularly if it is based on a single measurement. As the probability of this is dependent on the frequency of low-level resistance, this is unlikely to occur in areas where these isolates are rare (as illustrated in Figure 1a) but may become an issue where they are common (Figure 1b). In that case, an MIC with a phenotype of WT borderline MIC represents a non-WT strain in a significant fraction of occurrences. It is not clear at present at which frequency level such a situation should be taken into account; it will be different for each drug–species combination and local or region specific. This clearly needs further study.

The implications for MIC interpretation discussed above are summarized in Table 1. It should be noted, however, that the number of dilutions used as a safety margin, two in Table 1, are dependent on laboratory proficiency and the extent of the WT distribution. For narrow drug–species distributions such as Staphylococcus aureus and vancomycin, the margin of error is likely much lower, as demonstrated by the studies showing a prominent pharmacodynamic relationship.\textsuperscript{22}

**Conclusions**

We conclude that the use of an individual MIC value to modify a dosing regimen because a target is or is not likely to be attained is not justified. The MIC should be interpreted in the context of assay variation, and species identification and WT distributions need to be taken into account. This is not always straightforward, in particular if there is an abundance of local strains with low-level resistance mechanisms.
lower targets is appropriate owing to variation in MIC measurement. As discussed, all the evidence summarized in the EUCAST WT distributions indicate that even though there are MIC differences between specific strains, these cannot be readily detected or reproduced in the routine laboratory owing to the shortcomings of the system we employ. Moreover, for WT MIC distributions categorized as susceptible by the clinical breakpoints it is most likely that the difference between isolates, even when there, is so small that it is doubtful as to the usefulness in a clinical situation. Furthermore, small changes to the test system (in pH, incubation time, media, etc.) will drastically change the MIC of many agents. We have otherwise deliberately not discussed clinical breakpoints in relation to ECOFFs. The intention of the paper is to describe problems arising from accepting individual MIC determinations to modify dosing regimens.

Placing the MIC in the context of assay variation is particularly important if it is used to optimize therapy in the individual patient using target attainment. It could very well be that the current paradigm to increase the pharmacodynamic target in the critically ill to 100% \( f_{T_{\text{MIC}}} \) of \( \beta \)-lactams because of decreased efficacy at lower targets\(^a\)\(^b\) is appropriate owing to variation in MIC measurements as discussed in this paper. Alternatively, it explains why multiples of the MIC trough concentration are sometimes found to be predictors of efficacy or emergence of resistance.\(^a\)\(^b\) This clearly suggests the need for more specific research in this area. Finally, as isolates with low-level resistance become more frequent, generally or locally, specific solutions need to be found to interpret MICs of borderline susceptibility.

### Table 1. Suggested interpretation of the MIC for target attainment under various conditions

<table>
<thead>
<tr>
<th>MIC found</th>
<th>Interpretation for target attainment</th>
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<tbody>
<tr>
<td>Within WT, ( &lt; ) ECOFF</td>
<td>ECOFF</td>
</tr>
<tr>
<td>( &gt; ) ECOFF</td>
<td>MIC + two 2-fold dilutions(^a)</td>
</tr>
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\(^a\)Number of dilutions could be higher or lower than two depending on the proficiency of the lab and the drug–species distribution.

### Transparency declarations

None to declare.

### References

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