Wild-Type MIC Distributions Must Be Considered To Set Clinically Meaningful Susceptibility Testing Breakpoints for All Bacterial Pathogens, Including *Mycobacterium tuberculosis*

We thank Pasipanodya and Gumbo for their comprehensive review on pharmacokinetics and pharmacodynamics (PK/PD) for antituberculosis agents (5). Although they summarize a neglected area, we believe that setting susceptibility breakpoints solely using PK/PD data, without considering wild-type MIC distributions and clinical outcome data if available, could lead to susceptibility reporting with considerable problems in clinical interpretation and reproducibility.

First, the authors' suggested susceptibility breakpoints, most notably for the first-line agents isoniazid (INH) and rifampin (RIF), differ significantly from those used successfully for a long time. If suggested breakpoints are compared to MIC distributions of clinical isolates from different geographical regions (Fig. 1) (6), these breakpoints would define large proportions of wild-type isolates (i.e., isolates lacking resistance mechanisms) (2) as resistant and most of the patients as having multidrug-resistant tuberculosis. This contradiction is presumably due to PK/PD data that were derived from studies in which INH or RIF were given in monotherapy, but these findings are hardly relevant for the multidrug regimen used today. Even though the authors have pointed out exceptions, the WHO has concluded after a meta-analysis that patients with pansusceptible strains respond well to the standard short-course regimen used today, with the relapse rate being only 3.1% (9).

Second, the suggested susceptibility breakpoints split the wild-type MIC distribution, which introduces serious reproducibility problems since susceptibility results of wild-type strains with MICs around the breakpoint would deviate between susceptible and resistant in strains which by definition do not have an acquired resistance mechanism. According to modern principles for the setting of susceptibility testing breakpoints, splitting the wild-type MIC distribution should be avoided to ensure proper reproducibility (1, 6).

Finally, the authors claim that wild-type MIC distributions vary between different regions. The three studies referred to hardly support this notion, as they were not designed for that purpose. Two of them evaluated new methods (Etest and broth microdilution), one of which had an inferior agreement with the reference method, and the third used the radiometric macrodilution method. Additionally, one of them included resistant, non-wild-type isolates and could therefore by definition not define the wild-type for *M. tuberculosis*. Different phylogenetic groups of *M. tuberculosis*, being abundant in different regions, could have minor variations in wild-type MIC distributions, but this remains to be proven. For other bacterial pathogens, wild-type isolates have similar MIC distributions regardless of geographical origin or source (human, animal, or environmental) (4, 7), and there is no obvious reason why *M. tuberculosis* should be different. Just like how wild-type distributions show small variations, which could be dependent on sample size and methodology, there is an intersubject variation in PK variables, which is greater than a 10-fold range for rifampin and which is highly dependent on the distribution of fast and slow acetylators for isoniazid (3, 8).

Concisely, the breakpoints for INH and RIF suggested by the authors are not likely to be clinically meaningful for the treatment of tuberculosis, and their use is expected to introduce serious reproducibility problems. All together, bas-

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**FIG. 1.** Wild-type MIC distributions of rifampin and isoniazid compared to current and suggested susceptibility testing breakpoints. Reproduced with permission from Oxford University Press (6). Wild-type MIC distributions were obtained on Middlebrook 7H10 medium using a replicator and compared to previous Bactec 460 susceptibility results (6). The bars with vertical lines represent the wild-type MIC distributions, and filled bars represent isolates that had previously been categorized as resistant. The current breakpoints and the breakpoints suggested by the authors (5) are indicated with arrows. Clearly, the suggested breakpoints would classify most of the wild-type strains (by definition lacking resistance mechanisms) as resistant and most of the corresponding patients as cases of multidrug-resistant tuberculosis. Also, the suggested breakpoints split the wild-type MIC distribution, which could lead to serious reproducibility problems. (a) Rifampin; (b) isoniazid.

<table>
<thead>
<tr>
<th>Breakpoint</th>
<th>Current Breakpoint</th>
<th>Suggested Breakpoint</th>
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<tbody>
<tr>
<td>Rifampin</td>
<td>0.2 mg/L</td>
<td>0.05 mg/L</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.03 mg/L</td>
<td>0.02 mg/L</td>
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</tbody>
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**TABLE 1.** Number of isolates (n) for rifampin and isoniazid compared to current and suggested susceptibility testing breakpoints. Reproduced with permission from Oxford University Press (6). Wild-type MIC distributions were obtained on Middlebrook 7H10 medium using a replicator and compared to previous Bactec 460 susceptibility results (6). The bars with vertical lines represent the wild-type MIC distributions, and filled bars represent isolates that had previously been categorized as resistant. The current breakpoints and the breakpoints suggested by the authors (5) are indicated with arrows. Clearly, the suggested breakpoints would classify most of the wild-type strains (by definition lacking resistance mechanisms) as resistant and most of the corresponding patients as cases of multidrug-resistant tuberculosis. Also, the suggested breakpoints split the wild-type MIC distribution, which could lead to serious reproducibility problems. (a) Rifampin; (b) isoniazid.
ing susceptibility testing breakpoints solely on PK/PD data is a questionable strategy for most pathogens, including *M. tuberculosis*.

REFERENCES


Kristian Ångeby*
Department of Clinical Microbiology, Karolinska Institute
Karlinska University Hospital
Stockholm, Sweden

Pontus Jurén
Department of Preparedness
Swedish Institute for Communicable Disease Control
Stockholm, Sweden

Thomas Schön
Department of Clinical Microbiology
Kalmar County Hospital
Kalmar, Sweden

*Phone: 46-8-517 728 77
Fax: 46-8-30 80 99
E-mail: kristian.angeby@karolinska.se

Authors’ Reply

We thank Ångeby and colleagues for their interest in our review. However, we would like to take issue with their view of PK/PD-derived breakpoints, namely, that this approach only uses PK/PD data. In fact, our approach uses not only PK/PD data but also drug dose, population pharmacokinetics, and wild-type MIC distribution, as can be seen from examination of Fig. 2 to 6 in the original publication (3). It is beyond dispute that dose, pharmacokinetic variability, and PK/PD relationships are also of paramount importance in determining whether a pathogen will respond to an antibiotic or not. On the other hand, the epidemiologic cutoff method uses only wild-type MIC distribution. It makes no clinical sense not to take dose, pharmacokinetic variability, and drug penetration indices into account.

We bristle at the notion that the normative treatment success rates should be defined by data from predominantly low-burden countries, or some similar arbitrary aggregate, while calling treatment outcomes from high-burden countries such as South Africa, and indeed most of Africa, “exceptions.” We contend that greater weight should be given to outcomes from areas of the world with the greatest TB burden and that these are indeed normative and not the outliers.

Third, Ångeby et al. say that breakpoints derived for monotherapy “are hardly relevant for the multidrug regimen used today.” Yet, the same epidemiologic cutoff breakpoints that they uphold so much suffer from the very same predicament, i.e., they are designed to measure resistance to one drug and do not say anything about multiple-drug therapy. Surely, equal standards should be applied. If resistance is found to one drug, say a rifampin MIC of 64 μg/ml with an *rpoB* mutation, what physician would go ahead and administer that drug in combination therapy?

The question of classifying isolates as resistant in the absence of known mechanisms of resistance or the argument that our proposed susceptibility breakpoints would split wild-type distributions and introduce reproducibility problems is neither here nor there. Both merely reflect limitations of science at a particular point in time, but those limitations can be overcome. For example, chromosomal mutations have been the prototypic mechanism associated with resistance for decades; recently we learned that efflux pumps are also important (4, 5).

We are puzzled by the insistence that there should be little variability in *Mycobacterium tuberculosis* MIC distribution in isolates from around the world. The human strain of *M. tuberculosis* is known to have spread to all continents in ancient times, e.g., to Borneo before European contact, to ancient Egypt, and to the Americas >17,000 years before Amerigo Vespucci (1). Recently, Fortune and colleagues infected macaques with a single bacterial strain and then sequenced isolates from active, latent TB, and reactivation TB pulmonary lesions (2). They identified 14 new single-nucleotide polymorphisms that had already developed in 265 to 507 days of no antibiotic treatment, leading to mutation rates of 10−6 to 10−8 mutations/bp/day. Thus, it would be astounding if passage of bacteria through millions of humans over tens of thousands of years had introduced changes in many other genes and generated different genotypes in different locales but had not introduced variability in genes associated with drug effect. Intersubject variability, whether of pharmacokinetics or MICs, is the point of evolution; it makes no sense to pretend it does not exist.

REFERENCES


Jotam G. Pasipanodya
Tawando Gumbo
Division of Infectious Diseases
UT Southwestern Medical Center
5323 Harry Hines Blvd., Dallas, Texas 75390-9113

Phone: (214) 648–9914
Fax: (214) 648–2741
E-mail: Tawanda.Gumbo@UTSouthwestern.edu