Challenging a dogma: antimicrobial susceptibility testing breakpoints for Mycobacterium tuberculosis

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Abstract: The rise in multidrug-resistant tuberculosis makes it increasingly important that antimicrobial susceptibility testing of Mycobacterium tuberculosis produce clinically meaningful and technically reproducible results. Unfortunately, this is not always the case because mycobacteriologists have not followed generally accepted modern principles for the establishment of susceptibility breakpoints for bacterial and fungal pathogens. These principles specifically call for a definition of the minimum inhibitory concentrations (MICs) applicable to organisms without resistance mechanisms (also known as wild-type MIC distributions), to be used in combination with data on clinical outcomes, pharmacokinetics and pharmacodynamics. In a series of papers the authors have defined tentative wild-type MIC distributions for M. tuberculosis and hope that other researchers will follow their example and provide confirmatory data. They suggest that some breakpoints are in need of revision because they either (i) bisect the wild-type distribution, which leads to poor reproducibility in antimicrobial susceptibility testing, or (ii) are substantially higher than the MICs of wild-type organisms without supporting clinical evidence, which may result in some strains being falsely reported as susceptible. The authors recommend, in short, that susceptibility breakpoints for antituberculosis agents be systematically reviewed and revised, if necessary, using the same modern tools now accepted for all other bacteria and fungi by the scientific community and by the European Medicines Agency and the European Centre for Disease Prevention and Control. For several agents this would greatly improve the accuracy and reproducibility of antimicrobial susceptibility testing of M. tuberculosis.

Introduction

The emergence of multidrug-resistant (MDR) and extensively drug-resistant tuberculosis makes it necessary to ensure that antimicrobial susceptibility testing of Mycobacterium tuberculosis produce results that are clinically meaningful and technically reproducible. Unfortunately, this is not always the case. Among the supranational reference laboratories of the World Health Organization (WHO), the accepted minimum performance level (i.e. the proportion of concordant results) for the testing of susceptibility to streptomycin and ethambutol is only 92%. Furthermore, WHO strongly cautions against basing individual treatment for MDR tuberculosis, including ethambutol, pyrazinamide and most second-line drugs, on the results of antimicrobial susceptibility testing. This situation probably stems from the failure of mycobacteriologists to apply the generally accepted criteria for the establishment of susceptibility breakpoints for bacterial pathogens.

For M. tuberculosis, the antimicrobial susceptibility testing breakpoint (also known as the “critical concentration”) is defined as "the lowest concentration of drug that will inhibit 95% (90% for pyrazinamide) of wild strains of M. tuberculosis that have never been exposed to drugs, while at the same time not inhibiting clinical strains of M. tuberculosis that are considered to be resistant (e.g. from patients who are not responding to therapy)". This definition is problematic for two reasons: (i) it automatically categorizes up to 5% of wild-type M. tuberculosis strains as drug-resistant (10% in the case of pyrazinamide); (ii) combination therapy is the standard treatment for tuberculosis and clinical outcome data for individual drugs cannot be readily obtained. Consequently, the critical drug concentrations in current use are largely based on consensus and lack solid scientific support. In fact, the current definition of “critical concentration” may be what prompted WHO to declare that “…the critical concentration defining resistance is often very close to the minimum inhibitory concentration (MIC) required to achieve antitubercular activity, increasing the probability of misclassification of susceptibility or resistance and leading to poor reproducibility of DST results”.

Modern principles

Antimicrobial susceptibility testing breakpoints are best determined by breakpoint committees composed of specialists in clinical trial science and in pharmacokinetics and pharmacodynamics, population simulation tools, resistance mechanisms, antimicrobial susceptibility testing methods and bacterial population dynamics. Two such committees are the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute of the United States of America. Modern principles for the determination of clinical antimicrobial susceptibility testing breakpoints call for a definition of wild-type MIC distributions (i.e. the Gaussian MIC distributions for organisms of each target species devoid of resistance mechanisms). Strains included within the wild-type MIC distribution are, by definition, devoid of phenotypically detectable acquired or mutational resistance mechanisms, whereas strains outside the wild-type MIC distribution should be suspected of having such resistance mechanisms, although these may or may not be clinically relevant. The highest MIC within the wild-type MIC distribution has been labelled the epidemiological

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cut-off (ECOFF). The ECOFF is used, together with clinical, pharmacokinetic and pharmacodynamic data, to classify a given wild-type MIC distribution as susceptible (S) (high likelihood of clinical success), intermediate (I) (clinical success uncertain) or resistant (R) (low likelihood of clinical success) under what is known as the SIR system. Strains with a MIC above the ECOFF (hence likely to possess resistance mechanisms) are often clinically “R” but could be classified as “I” or “S” if clinically justified. However, this determination is difficult for M. tuberculosis because, since combination therapy is the mandatory treatment for tuberculosis, data on clinical outcomes for individual drugs are difficult to obtain. When this is the case, the clinical breakpoint may have to be based primarily on the clinical outcomes observed for wild-type organisms, and hence on the ECOFF.

### Use of wild-type MIC distributions

Wild-type MIC distributions for M. tuberculosis, considered necessary by many experts, have been largely undetermined. To overcome this gap we recently published tentative wild-type MIC distributions for all major first- and second-line antituberculosis agents. We did so by using a 96-stick replicator and comparing bacterial growth in Middlebrook 7H10 agar containing serial twofold dilutions of drugs with bacterial growth of a control 1:100 dilution in drug-free medium (identical to the routinely used agar proportion method). A fully susceptible H37Rv reference strain and a clinical MDR strain were tested in duplicate in each run for all drugs, and intra- and inter-assay MIC variabilities were very good (i.e. less than ±1 twofold dilutions). MIC distributions can vary depending on the antimicrobial susceptibility testing method used, but preliminary validation data on the same strains in a liquid culture system have shown excellent agreement, both within and between methods (Middlebrook 7H10 versus BACTEC MGIT 960), and a MIC variability of less than ±1 twofold dilutions. Notably, under the current antimicrobial susceptibility testing strategy for M. tuberculosis, the only existing control is the pan-susceptible H37Rv strain, which is normally used only as a control for “S” or “R” classification. No pre-specified MIC ranges exist for the H37Rv strain, although such ranges exist for other bacterial pathogens, and this constitutes another methodological limitation in terms of quality control.

Although most of the tentative ECOFFs were identical or similar to the consensus-based critical concentrations, in this paper we present three unfortunate instances in which they were not. These examples do not involve the most important antituberculosis agents, but they clearly illustrate the usefulness of having defined wild-type MIC distributions and ECOFFs for M. tuberculosis for the setting of antimicrobial susceptibility testing breakpoints.

Testing of susceptibility to the first-line drug ethambutol yields poorly reproducible results. This is probably because the current critical concentration splits the upper end of the wild-type distribution (Fig. 1) and methodological variation (±1 twofold MIC dilutions) produces results that oscillate between “S” and “R.” This problem could be resolved in part by introducing an “I” category, not routinely used in mycobacteriology at present, and/or by ensuring (as for other bacterial and fungal pathogens) that the clinical antimicrobial susceptibility testing breakpoints do not divide the wild-type MIC distributions.

When we defined wild-type MIC distributions for the second-line drug capreomycin, we discovered that the current critical concentration was substantially higher than the epidemiological cut-off (Fig. 2). Thus, non-wild-type isolates would be classified as “S.” Since there is no evidence that strains with MICs above the ECOFF (i.e. strains likely to harbour resistance mechanisms) can be treated with capreomycin, this serves as an example of a potentially hazardous breakpoint that could lead to ineffective therapy and to the development of further resistance.

We finally come to the case of rifampicin and its analogue rifabutin. It has long been thought that some rifampicin-resistant strains were susceptible to rifabutin, although the clinical evidence is minimal. However, the wild-type MIC distribution shows that the current critical concentration of rifabutin is set three twofold MIC dilutions higher than the ECOFF (Fig. 3). The scientific grounds for the establishment of this breakpoint remain unclear. Moreover, strains with rifabutin MICs above the ECOFF, which would have been categorized as rifabutin-susceptible but rifampicin-resistant using the current critical concentration, were shown to have rpoB mutations associated with resistance. We therefore believe that previous reports of rifampicin-resistant but rifabutin-susceptible strains resulted...
strains are treatable with rifabutin. Data are available to suggest that these pharmacokinetic or pharmacodynamic no controlled clinical trial results or

Kristian Ängeby et al.

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current critical concentrations used to

Discussion

On the whole, our data suggest that the current critical concentrations used to test the susceptibility of M. tuberculosis to antimicrobials should be reviewed and in some cases revised in accordance with modern principles for setting antimicrobial susceptibility testing breakpoints, specifically the use of wild-type MIC distributions and defined ECOFFs, together with any available data on clinical outcomes and on pharmacokinetics and pharmacodynamics. These methods have been followed to set susceptibility breakpoints for non-fastidious and fastidious organisms, including anaerobic and Helicobacter and Listeria spp., and both Candida and Aspergillus spp. They rest on principles accepted by the European Medicines Agency, the European Centre for Disease Prevention and Control and the scientific community at large.

To ensure the accuracy and reproducibility of the SIR classification, one must make certain that antimicrobial susceptibility testing breakpoints do not divide the wild-type MIC distributions. This will guarantee that patients with drug-resistant tuberculosis receive effective chemotherapy and will prevent further resistance from developing.

Some may argue that M. tuberculosis strains in different regions could have different wild-type MIC distributions. To our knowledge, this is not supported by any scientific evidence. In fact, data for other bacterial pathogens confirm that wild-type MIC distributions are the same, whether an isolate comes from India or the Arctic or from birds or human beings.

Wild-type MIC distributions for M. tuberculosis should be defined whenever new antituberculosis agents are investigated, as is routinely done for other pathogens. Moreover, the use of wild-type MIC distributions and defined ECOFFs improves the chances of detecting newly acquired resistance. In the case of M. tuberculosis, acquired resistance is expected to be mutational, since the only important mechanism whereby this pathogen acquires resistance is by natural selection of drug-resistant mutants. Hence, if mutations are detected in strains with MICs higher than the ECOFF, these mutations can then be included in the recently recommended molecular kits for the rapid detection of drug resistance.

In conclusion, we hope to trigger a discussion on susceptibility testing and the setting of susceptibility breakpoints for M. tuberculosis, and we encourage others to support or challenge our MIC distributions for relevant drugs so that mycobacteriology specialists can also access MIC distributions like the ones on the EUCAST MIC-distribution web site, which contains, for instance, 8,005 values from 68 investigators for susceptibility of Escherichia coli to meropenem. Our MIC-distributions for M. tuberculosis are now on the MIC web site.
Challenge to a doctrine: susceptibility breakpoints for Mycobacterium tuberculosis

Kristian Ängeby et al.

Susceptibility testing for Mycobacterium tuberculosis

MLHCS. The challenge: a new susceptibility breakpoint needs to be established in a feasible test that has a high accuracy and reproducibility. This is crucial for the diagnosis of tuberculosis and the selection of appropriate treatment. The authors propose a new breakpoint for Mycobacterium tuberculosis that is based on the MIC of the wild-type strain. They suggest that this breakpoint can be used in clinical practice and that it can be validated by further research.

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Kristian Ängeby et al.

Susceptibility testing for Mycobacterium tuberculosis

Policy & practice

Resumen

Desafiar un dogma: valores límite en pruebas de sensibilidad antimicrobiana para Mycobacterium tuberculosis

El aumento en la tuberculosis resistente a la medicación hace que resulte cada vez más importante que las pruebas de sensibilidad antimicrobiana de Mycobacterium tuberculosis produzcan resultados clínicamente significativos y técnicamente reproducibles. Desgraciadamente, este no es siempre el caso, porque los especialistas en micobacteriología no han seguido los principios modernos generalizados para establecer los valores límite de la sensibilidad para patógenos bacterianos y micóticos. Estos principios requieren específicamente una definición de las concentraciones inhibitorias mínimas (CIM) aplicables a los organismos sin mecanismos de resistencia (también conocidos como distribuciones de CIM naturales), para usarla en combinación con los datos sobre resultados clínicos, farmacocinéticos y farmacodinámicos. En una serie de artículos, los autores han definido de manera provisional las distribuciones de CIM naturales para M. tuberculosis y esperan que otros investigadores sigan en el futuro su ejemplo y proporcionen datos que lo confirmen. Sugieren que algunos valores límite necesitan una revisión porque o (i) bisecan la distribución natural, lo que provoca una reproducibilidad pobre en las pruebas de sensibilidad antimicrobiana, o (ii) son considerablemente más elevados que los CIM de los organismos naturales sin aportar pruebas clínicas, lo que puede provocar que algunas cepas sean consideradas de manera equivocada como susceptibles. En resumen, los autores recomiendan que se estudien y se revisen sistemáticamente los valores límite de la sensibilidad de los agentes antituberculosos, si fuera necesario, usando las mismas herramientas modernas aceptadas actualmente por la comunidad científica, la Agencia Europea de Medicamentos y el Centro Europeo para la Prevención y Control de Enfermedades para el resto de bacterias y hongos. Según varios agentes, esto mejoraría considerablemente la precisión y reproduccibilidad de las pruebas de sensibilidad antimicrobiana de M. tuberculosis.

Referencias


Rезюме

Вызов догма: критические точки в проверке антимикробной чувствительности Mycobacterium tuberculosis

Рост числа случаев лекарственно-устойчивого туберкулеза увеличивает важность получения клинически значимых и технически воспроизводимых результатов при проверке антимикробной чувствительности Mycobacterium tuberculosis (микобактерий туберкулеза). К сожалению, это не всегда так, поскольку специалисты в области микобактериологии не следовали общепринятым современным принципам установления критических точек восприимчивости для бактериальных и грибковых возбудителей. Эти принципы, в частности, требуют, чтобы определение минимальных подавляющих концентраций (МПК), применяемых к организмам без механизмов сопротивления (также известных как распределения концентраций подавления (МПК) для дикого типа), применялось в сочетании с данными о клинических исходах, фармакокинетике и фармакодинамике. В ряде работ авторы определяли вероятные распределения МПК для дикого типа M. tuberculosis и выразили надежду, что другие исследователи также последуют их примеру и будут предоставлять подтверждающие данные. Они полагают, что некоторые критические точки нуждаются в пересмотре, поскольку они либо (i) делают распределение дикого типа наполненным, что приводит к плохой воспроизводимости проверки антимикробной чувствительности, либо (ii) являются существенно более высокими, чем МПК для организмов дикого типа (без доказательных клинических данных), что может привести к ложной классификации некоторых штаммов как восприимчивых. Резюмируя, авторы рекомендуют при необходимости проводить систематический анализ и пересмотр критических точек чувствительности для противотуберкулезных средств с использованием тех же современных инструментов, которые в настоящее время применяются для всех остальных бактерий и грибков научным сообществом, Европейским агентством по лекарственным средствам и Европейским центром по контролю и профилактике заболеваний. Для нескольких агентов это позволит значительно повысить точность и воспроизводимость проверки антимикробной чувствительности M. tuberculosis.
Policy & practice

Susceptibility testing for Mycobacterium tuberculosis

Kristian Ångeby et al.


