Resistant Plus Susceptible Tuberculosis: The Undiscovered Country

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(See the major article by Zetola et al on pages 1754–63.)

Before the emergence of molecular epidemiology techniques, the conceptual approach to tuberculosis treatment seemed straightforward. The clinical laboratory sent the patient’s isolate (singular) for testing at a reference laboratory, which would perform a drug-susceptibility test (DST) whose findings were reported as a categorical result (ie, susceptible or resistant). This information then guided selection of appropriate antimicrobial therapy, and in clinical trials, this approach worked in the vast majority of cases. With the advent of molecular typing methods, a core premise of the classical paradigm was challenged. Genetic analyses of cultures, and even patient samples, provided evidence that a minority of patients with tuberculosis harbor >1 strain of bacteria at the same time [1–6] and that some of these mixed infections involve both drug-susceptible and drug-resistant organisms [7, 8]. What then are the consequences for patient management? [9].

In previous reports, mixed infection has been identified as the cause of discrepant DST results in pretreatment isolates [8] and of results that change during the course of therapy [7]. Thus, in patients whose initial phenotypic DST identifies only susceptible isolates, mixed infection can explain the subsequent growth of resistant organisms, offering an alternative explanation to reinfection or acquired resistance. In this situation, a patient would typically receive only first-line medications. Given that these antibiotics have little activity against the subpopulation of resistant organisms, one can readily envision how treatment failure could ensue. In the converse situation, the initial DST identifies drug-resistant organisms, but because of mixed infection there is subsequent growth of susceptible isolates. Is it possible that this situation would also result in an adverse treatment outcome if first-line drugs are withheld from patients with a subpopulation of drug-susceptible organisms? In this issue of the Journal, Zetola et al ask precisely this question.

To define mixed infection, Zetola et al identified sputum cultures from patients with MDR tuberculosis that demonstrated the presence of isoniazid- and rifampin-susceptible strains within the first 3 months of treatment; the authors referred to this situation as phenotypic DST heterogeneity. In this cohort in Botswana, phenotypic DST heterogeneity was observed in 7% of patients (33/475) and was not associated with baseline patient characteristics. In multivariable analyses, the rate of poor clinical outcomes (death, treatment failure, or treatment default) was 2-fold higher in patients with phenotypic DST heterogeneity, achieving statistical significance overall, and also among people living with human immunodeficiency virus (HIV). Together, these observations led the investigators to ponder whether the addition of the most potent tuberculosis drugs, isoniazid and rifampin, might have improved outcomes in these patients with mixed infections.

The authors presented a strong argument in favor of a link between phenotypic DST heterogeneity and poor treatment outcomes by controlling for several potential confounders, such as correlates of tuberculosis severity (smear status) and the degree of immunosuppression. However, whether this link is causal requires further investigation. The DST results at the time of culture conversion or of treatment failure and/or death did not obviously explain how the presence of isoniazid- and rifampin-susceptible isolates could have led to poor outcomes. Among patients with phenotypic DST heterogeneity, drug-susceptible isolates were detected in the last positive culture in 9 of 16 patients who experienced culture conversion, yet in only 5 of 19 patients who experienced poor outcomes. This suggests the possibility of alternative explanations for...
their observation. As in any observational study, there is the possibility of underadjustment; for example, the authors did not control for resistance to injectable agents or fluoroquinolones, which can negatively impact treatment success for MDR tuberculosis [10]. Differential exposure misclassification could also explain some of the association: if sicker (or less adherent) patients had a greater number of cultures performed in the first 3 months of treatment than those who were less sick (or more adherent), then phenotypic DST heterogeneity might have been detected more readily among those more likely to fail, die or default. Likewise, genetic susceptibility might vary between those with phenotypic DST heterogeneity and those without; perhaps polyclonal infection is a marker of a host with a diminished capacity to control Mycobacterium tuberculosis.

Therefore, the precise reason why patients with phenotypic DST heterogeneity had a worse outcome remains unknown and is something that might be best addressed in an experimental model of antibiotic treatment.

BEYOND MIXED INFECTIONS: WHAT IS DRUG RESISTANCE?

Another challenge stemming from genetic studies is whether isolates can be unambiguously classified as drug susceptible or drug resistant. Using phenotypically resistant organisms and gene disruption studies, investigators have been able to identify many tuberculosis resistance genes over the past 2 decades. These findings have translated into the development of commercial kits that enable one to test isolates directly for resistance-associated mutations, such as the Line-Probe assay (Hain) or the rifampin resistance assay in the GeneXpert test (Cepheid). In many parts of the world, phenotypic DST results are not available, so these new assays offer clinicians unambiguous results to guide patient treatment. However, in settings where these tests are done in addition to phenotypic DST testing, reports are emerging in which resistance mutations are detected in isolates that are phenotypically drug susceptible [11, 12]. These discordant results beg the question of whether phenotypic resistance occurs as a quantum event, due to the acquisition of a single mutation, or whether the resistance phenotype is the result of a more complex, multistep process [13]. Indeed, it has recently been reported that certain so-called resistance mutations have not been formally linked to the phenotype via allelic exchange experiments [14] and that resistance emerges at different rates in bacteria from different lineages [15]. Thus, certain mutations may be associated with resistance, without being causal, and of those that predispose to resistance, the penetrance depends on genetic variants elsewhere in the genome. Which test best predicts the optimal treatment: a genetically confirmed mutation or dead bacteria in a vial of antibiotics? This area of research remains a mostly undiscovered country whose exploration could lead to practice- and paradigm-altering knowledge.

Until now, the catalogue of readily available genotype-based resistance tests was restricted to mutations that had been preselected for inclusion in kits. Likewise, the capacity to detect mixed infections was limited, as most genetic studies extracted DNA from a population of bacteria instead of conducting colony-by-colony analysis [15]. With the advent of whole-genome sequencing, which is rapidly migrating from a research tool into the clinical arena, one now can obtain genetic evidence of drug-resistance and mixed infections in a single run [6]. In general, more data are better than fewer data. However, converting these data into actionable intelligence remains daunting. If tuberculosis is not always monoclonal and M. tuberculosis strains are not simply sensitive or resistant, do we envision the deployment of personalized medicine, on a global scale, in resource-challenged settings? Or should we carry on as if the newer findings are outliers that are predicted to have little impact on tuberculosis control? While it is tempting to suggest we were better off not knowing, the current burden of tuberculosis worldwide provides empirical evidence that what has been tried over the past decades has not been entirely successful, especially in settings where tuberculosis and HIV infection intersect. More studies, like those of Zetola et al, will hopefully add more data and, ultimately, more guidance as we reconsider the definition and treatment of drug-resistant tuberculosis.

Note

Potential conflicts of interest. All authors: No reported conflicts.

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