Transwell Tolerance-Resistance (TTR) system: A novel approach to study mycobacterial antibiotic tolerance and emergence of drug resistance.

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ABSTRACT

The requirement for a prolonged antibiotic treatment is one of the reasons that limits the global eradication of tuberculosis (TB), mostly due to the mycobacterial tolerance towards antibiotics. Therefore, understanding the factors that are responsible for the drug tolerance of the bacilli is essential in devising efficient treatment strategies and shortening TB chemotherapy and also to prevent the emergence of drug resistance. However, lack of in vitro methods to study the extended time-kill kinetic assays significantly limits our understanding of antibiotic tolerance in M. tuberculosis. The conventional in vitro time-kill kinetic approach with one time antibiotic addition is often associated with the reduction in the effective concentration of the drug in the medium over the period of long incubation. Using a robotic liquid handler controlled Transwell-Tolerance-Resistance (TTR) system, we exposed M. tuberculosis to a steady state antibiotic concentration over a period of 30 days. Our method is a simplified version of the hollow fiber model, wherein each transwell represents an independent culture. The TTR system in 24-well plate format enables us to perform hollow fiber type experiments with hundreds of parallel cultures at different conditions and drug concentrations. This innovation also enables us to perform statistical analyses that are not possible with hollow fiber systems. We find that a steady state rifampicin exposure using TTR system efficiently eliminate drug tolerant forms and prevented emergence of drug resistance at high concentrations compared to the conventional one time treatment. By combining the TTR system with extrachromosomal barcoding, we were able to trace the rifampicin tolerant clones in the population.

References

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