ANTIMICROBIAL RESISTANCE

Transient antibiotic resistance calls for attention

Clinicians have long observed that infections diagnosed as susceptible to antibiotics can sometimes resist treatment. New studies show that such treatment failures can be explained by subpopulations of transiently resistant cells that are often missed by standard clinical diagnostics, offering new therapeutic avenues.

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Antibiotic resistance is a growing problem for effective antimicrobial therapy. Classically, we think of resistance to antibiotics as a stable trait mediated by mutations in existing genes, or by acquisition of dedicated resistance genes[1]. Yet, resistance can also be unstable, where a small fraction of cells becomes transiently resistant to an antibiotic. Bacterial cells within a clonal population can differ in their response to antibiotics, either due to phenotypic variability among genetically identical cells (known as 'persistence'), or rapidly emerging genotypic variability (known as 'heteroresistance')[2–4]. Persistence appears when a small subpopulation of cells arrest growth and thereby become more resistant to antibiotics. In contrast, when heteroresistance occurs, the small subpopulation of surviving cells is capable of growing and dividing in the presence of the drug. Such phenomena have long been observed in the laboratory[5,6] but have been difficult to track in the clinic. Implementing en masse systematic screening approaches, two new studies in this issue of *Nature Microbiology* uncover a surprisingly high prevalence of heteroresistance among a wide range of clinical isolates and highlight avenues to exploit this phenomenon for potential therapeutic benefits[7,8].

Nicoloff and co-workers systematically tested a panel of clinical isolates from multiple bacterial species against 28 clinically used antibiotics[7]. By measuring the fraction of cells that can grow at a range of antibiotic concentrations, they detected a high prevalence of heteroresistance among clinical isolates, even in strains characterized as antibiotic sensitive, by conventional clinical methodologies. The authors further showed that these resistant subpopulations often became more susceptible after growing in media without antibiotics, underscoring the transient and unstable nature of these phenotypes (Fig. 1). Using whole genome sequencing, they unravelled the mechanism of these observations, showing that heteroresistance was associated with spontaneous tandem gene amplification of known antibiotic-specific resistance genes. Following a relaxed selection in the absence of antibiotic stress, these heteroresistant strains rapidly lost or reduced the number of amplified genes and reverted back to antibiotic sensitivity (Fig. 1). By genetically deleting amplified loci, or adding additional copies of these antibiotic resistance genes, the authors confirmed that gene amplifications are a rapid and common mechanism for transient heteroresistance.

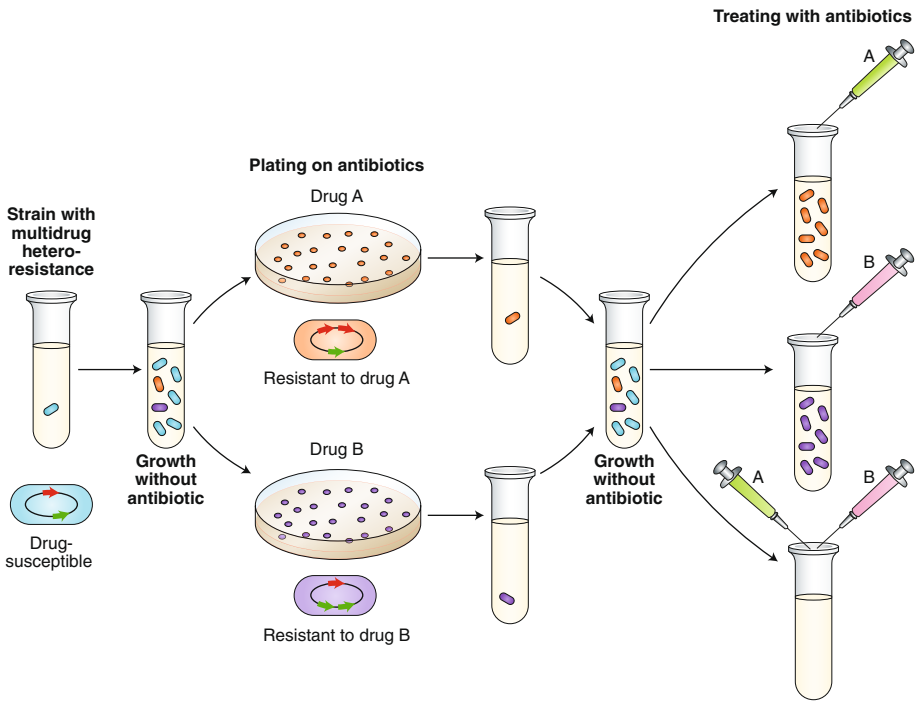

Fig. 1 | Heteroresistance emerges from gene amplifications, calling for combined antimicrobial therapy. Nicoloff and co-workers[7] and Band and co-workers[8] found that clinical isolates with multidrug heteroresistance can produce cells with differing drug susceptibilities, including cells sensitive to antibiotics (indicated by blue colouring), as well as subpopulations that are resistant to, and grow on, different drugs (indicated by orange and purple colouring). This phenomenon is mainly facilitated by rapid and reversible tandem amplification of antibiotic resistance genes (indicated by arrows) specific for each antibiotic. Growing these cells without antibiotics leads to the emergence and reversion of antibiotic resistance through amplification and loss of antibiotic resistance genes, respectively. While treating bacteria with individual antibiotics to which the strain is heteroresistant (indicated by orange and purple colouring) fails to kill the bacterial population, drug combinations targeting multidrug heteroresistance lead to efficient eradication in vitro and even in vivo in mouse models.

With heteroresistance emerging as a prevalent phenomenon and a plausible cause of treatment failures, the study by Band and co-workers investigated how drugs might be used in combination to effectively treat heteroresistant infections[8]. Testing a set of carbapenem-resistant clinical isolates, the authors found that individual

strains were commonly heteroresistant to several different drugs. Next, the authors asked whether the mechanisms underlying heteroresistance to the different drugs were independent. They found that treating bacteria with one of the antibiotics selected for a subpopulation of cells resistant only to the applied drug, and that deleting a gene conferring resistance to a given antibiotic only reduced the fraction of cells showing heteroresistance to this particular antibiotic. Together, these findings suggested that multidrug heteroresistance arises independently in different cells, potentially by amplification of different antibiotic resistance genes, as detailed by Nicoloff and co-workers[7].

Given that heteroresistances to different antibiotics were independent, the authors asked whether treatment can be improved with the simultaneous administration of two drugs, to which the strains encoded heteroresistance. Starting with in vitro experiments, they found that drug combinations were indeed much more effective in clearing a population than any of the single drugs alone (Fig. 1). These drug combinations worked even against clinical isolates that were misdiagnosed as multidrug- or pan-resistant by conventional clinical tests. Shifting to a mouse model, the authors showed that a combination of drugs to which a multidrug-resistant strain was heteroresistant protected mice from lethality, while monotherapy led to treatment failure[8]. These findings suggest that accounting for heteroresistance may open up avenues for drug combinations that were originally thought of as ineffective due to stable resistance.

While the two studies highlight heteroresistance as a clinical challenge, they also present opportunities for new types of clinical diagnostics and therapy that can better detect, and perhaps even exploit, heteroresistance. Starting with diagnostics, both studies showed that current clinical diagnostics are hard-pressed to identify rare subpopulations of unstable resistant cells, so new tools are needed with high sensitivity to allow detection of low-frequency, unstable, resistant cells, while maintaining the high reproducibility and low complexity typically required for clinical testing. We can envision such diagnostics connected with model-based tools to predict treatment failure caused by heteroresistance.

Therapy may also develop into tools that exploit heteroresistance mechanisms — specifically, by tailoring more effective mono- and multidrug therapy. The discovery that unstable amplification underlies much of the clinically observed heteroresistance presents an opportunity to develop novel strategies that either target the emergence of these heteroresistant cells or directly target their inherent vulnerabilities. Indeed, a better understanding of the molecular basis of these frequent spontaneous tandem amplifications may suggest novel ways to combat the emergence of heteroresistance. Given that these spontaneous amplifications may be important stepping stones for the evolution towards even higher, more stable antibiotic resistance, such approaches may perhaps even help in slowing the emergence of classical stable resistance. Even if the emergence of heteroresistance is not by itself preventable, it is possible that the small fraction of resistant cells within the population have some collateral weaknesses that can be exploited[9]. For example, as heteroresistance appears through gene amplification, it is likely associated with increased metabolic or genome replication burdens. Through these avenues, it is beautiful to see how basic innovative science can directly link from molecular mechanistic discoveries to important insights for clinical therapy. ❐

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