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Overcoming Multidrug-Resistance in Bacteria with a Two-Step Process to Repurpose and Recombine Established Drugs

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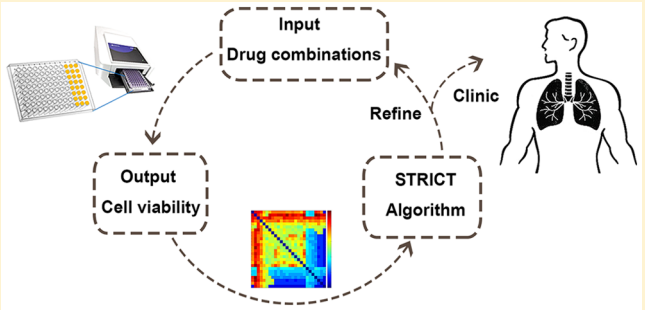
Supporting Information

ABSTRACT: The emergence and ongoing spread of multidrug-resistant (MDR) bacteria is a major global public health threat. MDR has extensively combated the potency of antibiotics. Development of new antibiotics requires several years with prohibitive cost that will not last. An alternative solution is to recombine failed antibiotics, which has been proven to be not only cost-effective, but also potent. However, selection of the optimal combinations of these chemicals through conventional trial-and-error methods is challenging and slow, since M candidates with N doses lead to N^M possible combinations. Herein, we present a artificial intelligence (AI) guided chemical combination optimization technique, namely Streamlined Rapid Identification of Combinatorial Therapies (STRICT), which is phenotype based and can efficiently learn and identify the optimal drug-combinations with minimal experimental efforts. With the guidance of STRICT, we successfully identified potent combinations of five antibiotics from 26 antibiotics that are individually ineffective at inhibiting an artificially induced strain of MDR bacteria. Rather than examine millions of tests, STRICT accomplished this task with only 120 carefully selected tests. Our results indicate that STRICT is a powerful platform to identify efficacious multiantibiotic combinations for the treatment of MDR bacteria. The AI-guided platform introduced here is an effective tool for drug repurposing, beneficial toward large-scale drug screening for other disease models, and also has a broad application in chemical combination optimization to deliver a desired end point for a complex system.



The rapid evolution of antibiotic resistance in bacteria is a growing global healthcare concern.[1,2] Multidrug resistant (MDR) bacteria impede the treatment of many clinical diseases. The prevalence of nosocomial infections caused by MDR Gram-negative bacteria is a worldwide health threat for hospitalized patients and has drastically increased over the past decade.[3] While antibiotics are first-line options to treat infections, they constantly accelerate the emergence and spread of resistance bacteria. Bacteria can be naturally resistant to antibiotics or acquire resistance through spontaneous mutation or horizontal gene transfer.[4,5] When a new generation of bacteria inherits antibiotic resistance genes, the genes are transmitted "vertically" through the child population and "horizontally" when bacteria share or exchange genetic material fragments.[6]

Drug resistance has been reported ever since the advent of antibiotics. Today, many antibiotics are ineffective, but the pace of discovery for new antibiotics has substantially decelerated.[7] Given the rapid emergence of drug-resistant mutations and the limited supply of new antibiotics, new strategies and technological platforms that can overcome this conundrum are critically needed. Combination therapy is one of the mainstream methods to overcome decreasing antibiotic efficacy and mitigate resistance occurrence. By mixing and matching multiple ineffective antibiotics at varying dosages, synergistic combinations can regain efficacy and even result in superior treatments. Finding combinations that provide effective treatments is important to boost clinical therapy efficacy and lower cost to patients.[8] Recent literatures have revealed that combination treatment induced lesser mutation rates of drug resistance compared to individual drugs because mutations that confer resistance to a single drug may not provide advantage in multidrug environments.[9] Therefore, recombination of ineffective antibiotics may provide an alternative solution to treat infections with MDR bacteria and other clinically infectious diseases.

However, optimizing multidrug therapy is tedious and slow. M drugs at N dosage levels result in N^M possible combinations,

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and screening through each possible combination to confirm efficacy becomes resource intensive and impractical when drug candidates are numerous. While existing machine learning algorithms such as ElasticNet (EN),[10] and Neighborhood Component Analysis (NCA)[11] have been used to identify optimal drug-dose combinations, they are more ideal for searching within a small library of drugs. Other recent methods[12−18] provide quicker and better results at finding optimal combinations, yet these approaches become less effective at processing drug libraries with more than 10 candidates.

Herein, we introduce STRICT, a fast and accurate optimal drug combination searching technique, to systematically search for optimal antibiotic combinations from millions of possible antibiotic combinations with minimal experimental effort. To demonstrate the application of STRICT, we investigated an antibiotic library with 26 drugs. The selection of optimal antibiotic combinations is based on the resistance level of the bacteria, which is specific and correlates to clinical phenotype outcomes. We artificially induced five multiantibiotic-resistant mutants. The "strongest" mutant was resistant for 26 antibiotics from 10-fold to 300 fold. With the guidance of STRICT, we experimentally investigated 120 drug combinations in two rounds of tests and recombined several subgroups of these 26 ineffective antibiotics to potent drug combinations for the "strongest" mutant treatment. This approach is phenotype based and does not require professional knowledge of drug action mechanisms. By simultaneously cross-referencing multiple drugs, STRICT systematically scores and ranks every drug and their interactions across multiple combinations based on minimal experimental effort and data, making STRICT highly suitable for identifying effective multidrug combinations. Therefore, it increases the frequency of identifying novel synergistic effects, leading to economical drug repurposing. Since only minimal experimental efforts are required to optimize the recombination of large-scale ineffective antibiotics, the presented technique could be employed to rapidly identify efficacious multiantibiotic combinations that inhibit MDR bacteria for personalized medicine.

METHODS AND MATERIALS

Data Processing. In each iteration of optimization, the doses of antibiotics and the corresponding bacteria viability data was imported into MATLAB software and processed via original code with built-in functions. To compare the STRICT score of each drug, normalization was conducted. The dosage of each antibiotic was divided by the maximal dose of the antibiotic in every iteration. Thus, the antibiotic combination is denoted as a vector:

$$X_m = (x_{m,1}, x_{m,2}, ..., x_{m,n}) \tag{1}$$

Where $x_{m,n} \in [0,1]$ denotes the normalized dose of antibiotic n in combination m of the iteration. The corresponding bacteria viability of combination X_m is denoted as Y_m.

Scoring with STRICT. STRICT is based on projection distance. In high dimensional space, if the point represented by vector X_m is closer to a certain axis a, its corresponding Y_m will be considered more affected by the factor represented by axis a, as long as the projection is not zero. Specifically, consider a combination $k = (x_{k,1}, x_{k,2}, \ldots, x_{k,n})$, its projection p on axis i is $p = (0, \ldots, x_{k,i}, \ldots, 0)$, such that the normalized Euclidean distance from point k to the projection p is

$$D_{k,i} = \sqrt{\frac{\sum_{s\neq i} x_{k,s}^2}{n-1}} \tag{2}$$

Where $D_{k,i}$ is normalized to $[0,1]$. As a shorter distance of the projection means $x_{k,i}$ contributes more to the output Y_k, the distance $D_{k,i}$ is mapped with a decreasing function:

$$f(D_{k,i}) = (1 - D_{k,i})^q \tag{3}$$

Where q is adjustable; greater q value makes the points farther from their projections, which would weigh less in the calculation of the STRICT score. Theoretically, any decreasing function that has definition on the domain $[0,1]$ should satisfy the criteria for the mapping function. Here we choose only one of the simplest forms with an adjustable parameter.

The single drug STRICT score is defined as the sum of the productions of the outputs, projections, and the function of the distance, denoted as

$$\text{score}_i = \sum_{k=1}^{m} Y_k \cdot x_{k,i} \cdot f(D_{k,i}) \tag{4}$$

By this definition, STRICT score can easily expand to drug pair interactions, if we project point k to plane i,j instead of axis i. The projection is denoted as $p = (0, \ldots, x_{k,i}, \ldots, x_{k,j}, \ldots, 0)$, and the normalized distance is

$$D_{k,i} = \sqrt{\frac{\sum_{s\neq i,j} x_{k,s}^2}{n-2}} \tag{5}$$

Thus, the drug-pair STRICT score is defined as

$$\text{score}_{i,j} = \sum_{k=1}^{m} Y_k \cdot \sqrt{x_{k,i} \cdot x_{k,j}} \cdot f(D_{k,i,j}) \tag{6}$$

By this definition, STRICT score can easily apply to calculate the interactions of three, four, or even more drugs.

***E. coli* Strain, Culture, And Antibiotics.** *E. coli* strain ATCC25922 (American type culture collection) was inoculated in Luria−Bertani (LB) medium (10% Peptone, 5% yeast extracts and 10% sodium chloride). Peptone, yeast extracts, and sodium chloride were obtained from Sinopharm Chemical Reagent Co., Ltd. LB liquid inoculated with a single colony (starting from a single cell) was allowed for bacteria growth overnight (24 h, 37 °C, shaking at 150 rpm). Aliquots of this culture were stored in 10% (v/v) glycerol at −80 °C and used for all ancestral strain related experiments. All experiments were initiated from a freshly thawed aliquot from this single batch.

Twenty-six antibiotic solutions were prepared from powder stocks (Sangon Biotech) and dissolved in either Dimethyl sulfoxide (DMSO) or ultrapure water. Then, they were diluted in the LB medium to the target concentration. The concentration of DMSO used in this study was less than 0.1%. Fresh antibiotic stocks were used on a weekly basis.

***E. coli* Growth Conditions.** Bacterial samples were stored at −80 °C in 10% (v/v) glycerol. Before tests, frozen samples were recovered by 1000× dilution into LB medium, and left growing overnight. The colony was transferred into fresh LB for 24 h (37 °C, shaking at 150 rpm). The bacterial concentration was adjusted in LB to 2×10^5 CFU/mL for further analysis. 100 μL bacteria were transferred into

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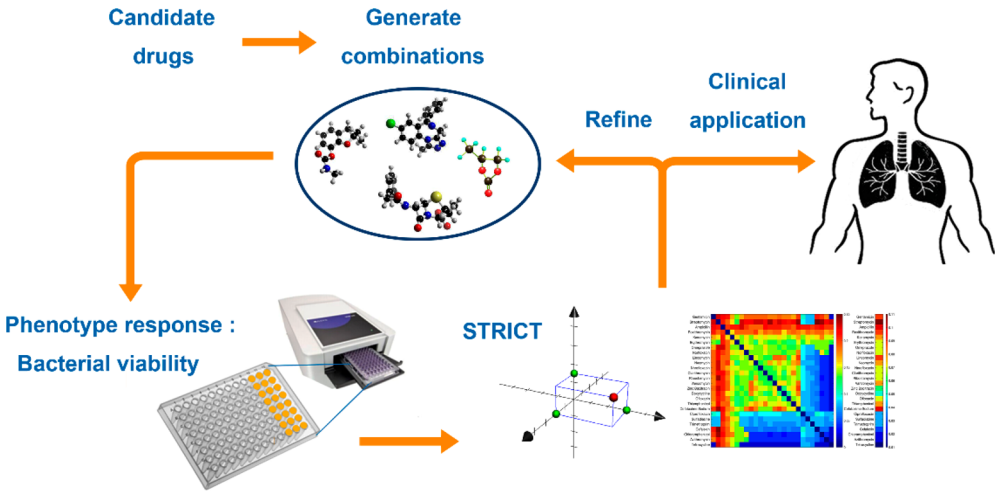

Figure 1. A schematic illustration of the STRICT optimization platform. Drug combinations are generated from a drug candidate library and administered to biological systems, and then the phenotype response (bacterial viability) measurements are input into STRICT, which scores the drugs and their interactions. STRICT results are either further refined or applied clinically.

Erlenmeyer flask with 50 mL fresh LB and incubated for 60 h. Every 6 h (with a first time point at 0 h growth), 1 mL content was taken to measure OD_{600} with a Microplate reader (BioTek) until the concentration was stable. This process was repeated at each time point in sextuplet to deliver the appropriate power for statistical analysis with the Origin8© software.

Minimal Inhibitory Concentration (MIC) test. The minimum inhibitory concentration (MIC) indicates the lowest concentration of an antibiotic which prevents visible growth of a bacteria. MIC of these 26 antibiotics were measured by a standard overnight growth assay in liquid media.[19] Twenty-six antibiotics were diluted to 2× the initial concentration in LB media and then we prepared a 2x gradient of antibiotic concentration in 96-well microtiter plates (Costar 3792; Corning). Two $\times 10^5$ CFU/mL bacteria were inoculated into each well with a 96-pin replicator. Inoculated 96 well plates were incubated for 12 h and then OD_{600} was measured by Microplate reader (six replicates per antibiotic concentration). The lowest drug concentration at which $OD_{600} < 0.1$ after background subtraction was considered the MIC.

Experimental Evolution. Evolution experiments were conducted in Erlenmeyer flask with final volume of 100 mL. Ancestral strain was inoculated into a series of Erlenmeyer flask with a 2-fold antibiotic concentration gradient, beginning with 50% MIC. After 24h, OD_{600} of each flask was measured. The flask at the highest antibiotic concentration and with $OD_{600} > 0.2$ after background subtraction was propagated into a fresh higher antibiotic gradient. This round of selection lasted for 15 days, specific antibiotic-resistant mutants were induced, and the corresponding antibiotic concentration was recorded. Then, a new antibiotic was introduced to start a new round of screening. For the first round, TMP was used to induce TMP-resistant mutant. Then, we kept the highest concentration of TMP in the culture and added the next antibiotic to repeat this procedure. Five antibiotics were applied to induce bacteria resistance in the following order, TMP, TMP + ERY, TMP + ERY + AMP, TMP + ERY + AMP + NOR, TMP + ERY + AMP + NOR + KAN. Eventually, we obtained five antibiotic-resistant mutants with various resistances, T-, TE-, TEA-, TEAN-, and TEANK-resistant mutant. After each antibiotic selection, mutants were propagated into a fresh LB for 24 h (37 °C, shaking at 150 rpm) and were stored at −80 °C in 10% (v/v) glycerol for further analysis. This procedure lasted for a total of 75 days.

RESULTS AND DISCUSSION

The STRICT Technique. STRICT is a type of feature selection, designed specifically to evaluate the synergy in drug combinations. STRICT technique consists of two iterative steps forming a feedback loop (Figure 1). The first step is to use uniform design of experiment to generate drug combinations with uniform distribution in the parametric space,[20] followed with measurement of their output phenotype responses (e.g., cell viability). Then, in the second step, the results are input into STRICT, where it scores the efficacy and ranks the interactions of every drug in the combinations. STRICT analysis is used to generate combinations for the next iteration for testing or directly applied for treatment (see Methods and Materials for detailed description).

A notable problem with combining drugs is toxicity. Drug-drug interactions will likely enhance toxicity and reduce the usefulness of this approach. To optimize a combination with the lowest accumulative dose, we defined the total dose (TD) of a combination as the sum of the dosage of each drug in the combination divided by its corresponding minimal inhibitory concentration (MIC) dose over the wild type (WT) strain:

$$\mathrm{TD} = \sum \frac{\mathrm{dose}_i}{\mathrm{WTMIC}_i} \tag{7}$$

TD value demonstrates the minimal dose required to inhibit the bacteria when they become multidrug resistant from WT.

Selection of Typical Antibiotics. a recent study showed that the evolution of resistance to a single antibiotic is frequently accompanied by increased resistance to multiple other antibiotics in particular that have the same mechanism of action.[21,22] Here, we applied an integrated laboratory evolution approach to artificially induce five multiantibiotic-resistant mutants.

Through literature search, 26 typical antibiotics with diverse action mechanisms were selected in this study. These antibiotics are well characterized, widely employed in clinic, and have various modes of actions (Supporting Information

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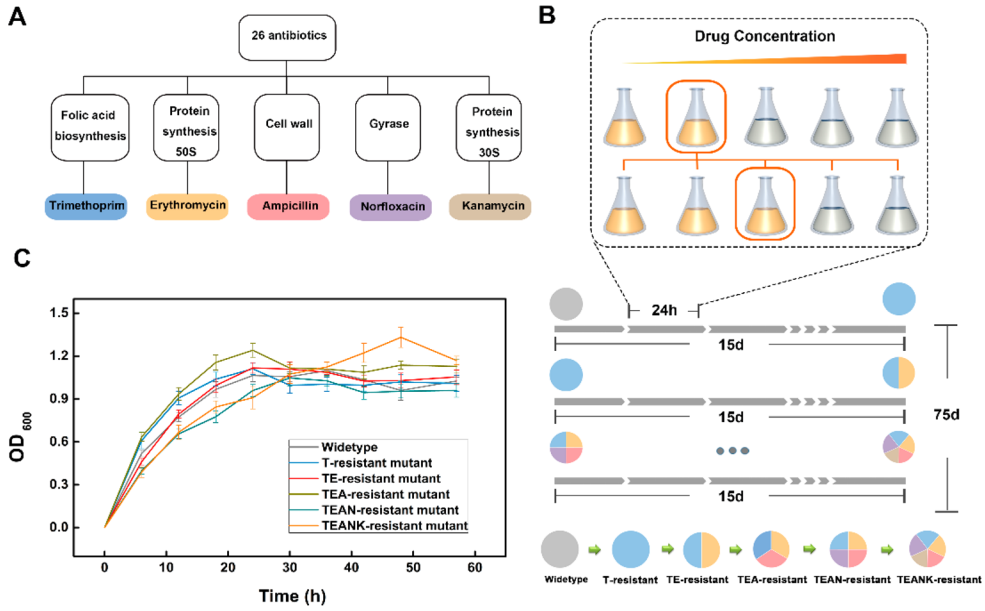

Figure 2. Experimental evolution of antibiotic resistance under multidrug treatments. (a) The 26 typical antibiotics were divided into five categories via their diverse modes of actions. In each category, a typical antibiotic was selected to represent its category, namely, Trimethoprim (TMP), Erythromycin (ERY), Ampicillin (AMP), Norfloxacin (NOR), and Kanamycin (KAN). (b) *Escherichia coli* populations were serially inoculated into a series of Erlenmeyer flasks with a 2-fold gradient of antibiotic concentrations. After 24 h, the Erlenmeyer flask with the highest antibiotic concentration permitting bacterial growth (OD_{600} > 0.2) was propagated into another round of drug resistance incubation using higher antibiotic concentration ladders. Five antibiotics were studied in order, that is, TMP, TMP + ERY, TMP + ERY + AMP, TMP + ERY + AMP + NOR, and TMP + ERY + AMP + NOR + KAN. (c) Daily growth curves of the five mutants and wild type bacteria. These curves can be considered as a saturation curve with a relatively fast initial increase in fitness followed by a plateau with little or no additional adaptation afterward. There are no significant differences in the growth trend between the curves.

(SI) Table S1). These antibiotics are divided into five categories based on their action targets: protein synthesis 30S, protein synthesis 50S, cell wall, DNA gyrase, and dihydrofolic acid (Figure 2A). The most commonly used antibiotic of each category is chosen to induce MDR bacteria in order to encompass the action mechanisms of all 26 antibiotics. The antibiotics chosen are Trimethoprim (TMP), Erythromycin (ERY), Ampicillin (AMP), Norfloxacin (NOR), and Kanamycin (KAN).[23,24]

TMP binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). THF is an essential precursor in the thymidine synthesis pathway and the interference with this pathway inhibits bacterial DNA synthesis biosynthesis.[25] ERY is a macrolide that binds the 23S rRNA molecule in the 50S subunit and also inhibits protein synthesis.[26] AMP acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make the cell wall.[27] NOR is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.[28] KAN is a aminoglycoside antibiotic used to treat both Gram-negative and Gram-positive infections; it inhibits protein synthesis by blocking amino-acyl−tRNA binding at the A-site in the 30S ribosomal subunit.[29] These five selected antibiotics represent a wide range of action mechanisms covered by the 26 antibiotics mentioned above.

Evolutionary MDR Bacteria Induction. We followed literature protocols with minor modifications to induce drug-resistant bacteria by propagating ancestral strain in batch cultures in the presence of successively increased antibiotic concentration (see Methods and Materials).[30,31] To eliminate the effect of DMSO concentration on bacterial growth, the growth rate of bacteria at different DMSO concentrations was measured (SI Figure S1). DMSO can inhibit bacterial growth at concentrations higher than 3%, while no effect on bacterial growth was observed when DMSO concentration is less than 3%. The concentration of DMSO used in this study is below 0.1%.

We artificially induced a strain of *E. coli* resistant to 26 antibiotics ranging from 10- to 300-fold compare to its WT (see Methods and Materials)[32] (Figure 2B). This procedure lasted for 75 days. We obtained five antibiotic-resistant mutants with various resistances, T-resistant mutant, TE-resistant mutant, TEAN-resistant mutant, and TEANK-resistant mutant. SI Figure S2 shows the changes in resistance. Each population's resistance at each time point was inferred from the drug concentration in the well-chosen for propagation.[33] The strain that is simultaneously resistant to all five antibiotics is denoted as TEANK-resistant.

Daily growth curves of these five mutants and WT bacteria were measured every 6 h until the bacterial concentration was stable. From growth curves, we estimated the growth rate change for each mutant as it evolved (Figure 2C). In general, these curves can be considered as saturation curves with relatively fast initial increase in fitness followed by a plateau with little or no further additional adaptation. There are no significant differences in the growth trend between these curves. MICs of these 26 antibiotics for WT bacteria were measured to select the drug-dose input for each antibiotic in evolutionary experiments (SI Table S2). The MIC values for WT bacteria is consistent with those in the literature.[34]

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Then, MICs of these 26 antibiotics for TEANK-resistant mutant were measured to evaluate the evolution of resistance. In order to facilitate the analysis, we took $\log_2$ (MIC+1) for visualization. Comparisons between the MIC of TEANK-resistant mutant and WT bacteria are shown in Figure 3 and SI Figure S3. As expected, the result indicated a roughly 10× to 300× increase in MIC for these 26 antibiotics, which means that within a certain concentration range, individual antibiotics are ineffective at suppressing the growth of TEANK-resistant mutant.

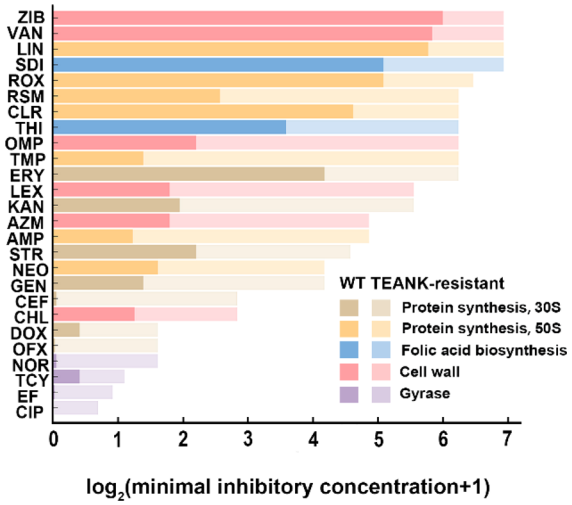

Figure 3. Comparison of MIC between WT and TEANK-resistant bacteria for the 26 antibiotics.

Recombination of Ineffective Antibiotics into Potent Regimens with STRICT. In order to rapidly identify efficacious multiantibiotic combinations to inhibit TEANK-resistant mutant, STRICT was introduced to systematically search for optimal antibiotic combinations from the 26 ineffective antibiotics.

Iteration 1. The initial round of experiment started with 80 combinations of the 26 antibiotics (Supplementary Data 1). For each antibiotic, three doses were chosen according to the dose–response curves of WT bacterial, namely 0, 0.5 × MIC_{50} and 0.5 × MIC. Individual antibiotics of these concentrations were far from suppressing the evolution of TEANK-resistant mutant, but we expected that they synergistically work together to inhibit the growth of the mutated bacteria. The distribution of the initial combinations was nearly uniform as each combination contained roughly 10 antibiotics and the 80 combinations were chosen with the largest sum of pairwise distances from millions of randomly generated experiment designs.

The bacterial viability was used to assess the efficacy of the antibiotics and antibiotic combinations on overall bacteria activity. The concentration resulting with bacterial viability <0.1, after background subtraction, was considered as the lethal concentration for bacteria. The bacterial viability readouts were then used to analyze individual drug performance and drug–drug interactions via the STRICT algorithm (Figure 4). The algorithm calculated the scores of individual antibiotics and their pairwise interactions, and visualized them by heat maps (Figure 5A, B). The sequence of the antibiotics was rearranged by hierarchical cluster based on their pairwise interaction matrix to facilitate selection of synergistic combinations. Five effective antibiotics that scored highest were promoted into the next iteration.

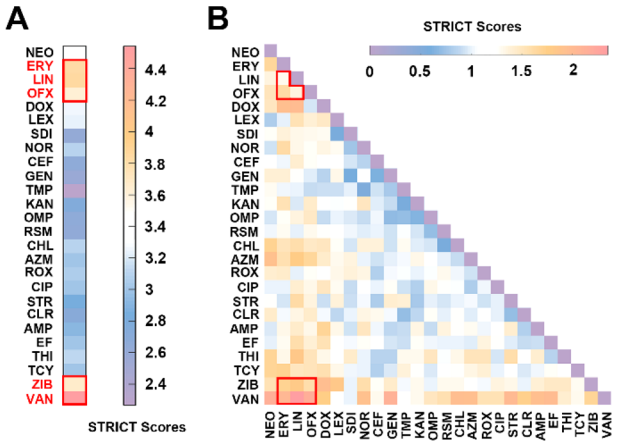

Figure 5. Heat maps of (a) individual-drug effects and (b) pairwise-drug interaction effects show the STRICT scores of each drug and drug–drug combination. The top five scoring antibiotics (red mark) are selected for refinement in the next iteration of optimization.

Iteration 2. During the second iteration, 40 drug-dose combinations were generated in the same way as the first iteration (i.e., largest pairwise distances). Supplementary Data

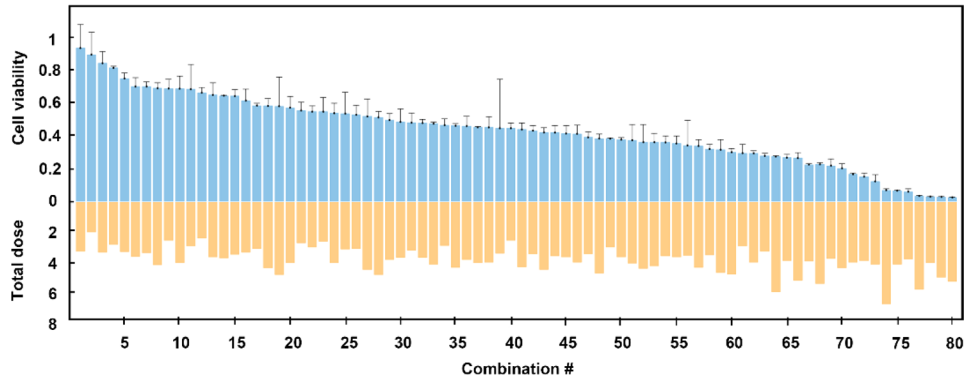

Figure 4. Cell viability and total dose ranking of the first iteration for TEANK-resistant mutant, combinations are arranged from high to low cell viability. Total dose is the dosage summation of each drug in the combination divided by its corresponding MIC of the WT strain. Error bars denote s.d. values across n = 3 set of experiments.

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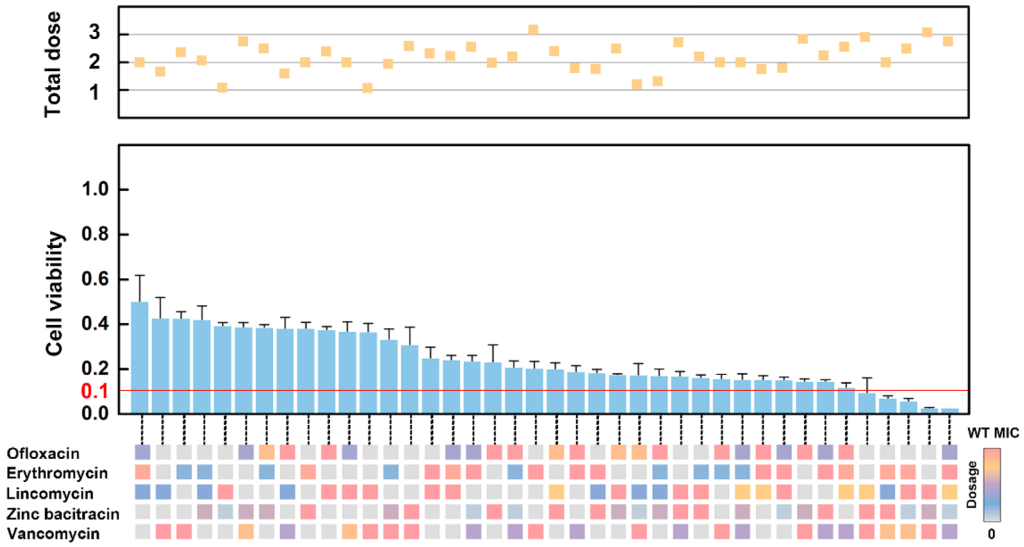

Figure 6. Five drugs are further optimized in a second iteration to locate the drug-dose combination with the strongest inhibitory effect. Combinations resulting in cell viability <0.1 are considered as lethal to bacteria. Error bars denote s.d. values across n = 3 set of experiments.

2\ shows the dosage levels and combinations used in this iteration. Each combination contained five antibiotics, four doses were chosen for each antibiotic, namely 0, MIC_{20}, MIC_{50}, and 0.5 × MIC. The bacterial viability for each combination is shown in Figure 6. Thus, with only two iterations, we narrowed 26 candidate antibiotics down to a practical 5-drug regimen. Experimentally, it could take less than 48 h to identify a useful antibiotic combination against infection of a multiple-drug resistant (MDR) strain with this process.

Verification of Optimization Results (Iteration 3). To further justify this regimen, a second order polynomial model (see Supporting Information: Polynomial model) was built by stepwise regression based on the 40 combinations from the second iteration. The model predicted that there were regimens potentially capable of inhibiting the MDR strain to zero viability ($OD_{600} \leq 0.1$) with lower TD values (TD < 2.0). Thus, we chose totally 20 such regimens predicted by the model for another iteration of experiments, where 19 of them had TD value ≤2.0 and one had higher TD value (TD = 2.4) as positive control (Supplementary Data 3). Our results showed, though the TD values of first 19 combinations varied between 1.6 and 2.0, none of them succeeded in inhibiting the MDR strain to zero viability ($OD_{600} \leq 0.1$), while the positive control did (SI Figure S4).

The results indicated that the polynomial model was biased, which we considered was caused by lack of data points for regression modeling. When the data from iteration 2 and 3 are combined together to provide enough data points for modeling, another polynomial model was constructed (see Supporting Information: Polynomial model) and predicted that no combination, other than the optimal combination that STRICT identified, could inhibit the MDR strain to zero viability ($OD_{600} \leq 0.1$) with TD value ≤2. Therefore, the third iteration justified the regimen identified in Iteration 2 as the global optimal regimen.

Furthermore, to ensure the second model's accuracy, a series of statistical analysis was performed, including the following statistics (SI Figure S5): residual distribution versus fitted value, Cook's distance, normal probability plot, and residual histogram. Residuals are the deviations between model-predicted values and true experimental values, which is a common terminology in statistics. The distribution of residuals can be used to judge whether the regression model explains the data properly. All results indicated the reliability of regression model-based predictions.

Validation of STRICT in Silico. The robustness and optimization efficiency of STRICT, has been investigated on MDR bacteria. To further the compare STRICT with established optimization algorithms, specifically EN, NCA, and stepwise regression for large drug libraries optimization, a series of in silico simulation was conducted.

The ability of noise resistance was considered as an indication of robustness and efficiency. To test this, the 26 real drug data was padded with random irrelevant variables forming semisynthetic data sets. These random irrelevant variables, acting as "dummy drugs", are mutually independent and do not interact with any other drug. Thus, these "dummy drugs" do not affect the efficacy of the real drugs or their drug−drug interactions. We adopted Normalized Root Mean Squared Error (NRMSE) to compute the deviations between assessments with and without the random irrelevant variables (see Supporting Information: NRMSE calculation). Smaller NRMSEs signifies that the algorithm is more robust, indicating better efficiency at identifying effective drug regimens.

The in silico results, for assessing individual-drug effects within a combination of 26 real drugs and 0−100 random irrelevant variables, show the NRMSE of STRICT is up to 7.87× smaller than EN, 7.24× smaller than NCA, and 6.70× smaller than stepwise regression (SI Figure S6A). Similarly, the in silico results, for assessing pairwise-drug interaction effects within a combination of 26 real drugs and 0−100 random irrelevant variables, show the NRMSE of STRICT was up to 5.45× smaller than EN, 4.68× smaller than NCA, and 5.90× smaller than stepwise regression (SI Figure S6B).

Runtime is another critical factor in optimizing large number of drug-dose combinations, which is largely affected by two variables: the number of examined combinations and the number of candidate-drugs. We assess the runtime with synthetic data sets (see Supporting Information: Comparison of runtime) when either variable is set as constant. Runtime for examining varying number of combinations is performed with

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20 candidate-drugs generating from 5 to 1000 combinations (SI Figure S6C). Runtime for varying number of drugs is performed with 200 combinations generated from 5 to 160 different drugs (SI Figure S6D). Within both settings, the runtime for STRICT is up to 4617× faster than stepwise regression, 258× faster than NCA, and 124× faster than EN.

CONCLUSION

The ability to screen for synergistic combinations, for drug repurposing and other applications, is gaining more importance as it is increasingly difficult to develop new antibiotics. To bypass the requisite of testing a daunting drug combination search space, we developed STRICT to screen for the optimal antibiotic combinations to treat MDR bacteria from a large number of ineffective antibiotics with minimal experimental efforts. Through literature search, 26 antibiotics were selected in this experiment. We artificially cultured *Escherichia coli* that were individually resistant to the 26 antibiotics from 10-fold to 300-fold. With STRICT guidance, we experimentally investigated 120 drug combinations rather than millions of tests to identify an effective combination of antibiotics from these 26 ineffective antibiotics to combat MDR bacteria.

The approach presented here can be attributed to several unique features. First, STRICT is phenotypically driven, so one does not need profession background or knowledge of optimization mechanisms. Second, a phenotypic approach increases the likelihood of finding unexpected synergistic drugs or chemicals. Drug repurposing allows for novel potent regimens, which serve as important complement for conventional time-consuming and labor-intensive process of new drug development. Our results showed that STIRCT not only provided a reliable screening platform for rapid and effective antibiotics combination to inhibit the development of bacterial resistance or other disease models, but also has a broad application in chemical combination optimization to deliver a desired end point for a complex system.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b02690.

Supporting figures for bacterial viability and DMSO concentrations, individual trajectories of resistance vary, comparison of MIC between WT and TEANK-resistant bacteria, confirmation of STRICT results, statistical analysis of STRICT combinatorial data, validation and NRMSE calculation results, supplementary table for employed antibiotics and their mode of actions, concentrations for the 26 antibiotics used in STRICT (PDF)

Supplementary data (XLSX)

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Notes

The authors declare no competing financial interest.

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