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Predicting bacterial resistance using the time inside the mutant selection window: Possibilities and limitations☆

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ABSTRACT

The time inside the mutant selection window (T_{MSW}) has been shown to be less predictive of selection of fluoroquinolone-resistant bacteria than the ratio of the area under the concentration–time curve to minimum inhibitory concentration (AUC/MIC). To explore the different predictive powers of T_{MSW} and AUC/MIC, enrichment of ciprofloxacin-resistant mutants of four *Escherichia coli* strains was studied in an in vitro dynamic model at widely ranging T_{MSW} values. Each organism was exposed to twice-daily ciprofloxacin for 3 days. Peak antibiotic concentrations were simulated to be close to the MIC, between the MIC and the mutant prevention concentration (MPC), and above the MPC, with T_{MSW} varying from 0% to 100% of the dosing interval. Amplification of resistant mutants was monitored by plating on medium with 8× MIC of the antibiotic. For each organism, T_{MSW} plots of the area under the bacterial mutant concentration–time curve ($AUBC_M$) exhibited a hysteresis loop: at a given T_{MSW} that corresponds to the points on the ascending portion of the bell-shaped $AUBC_M$–AUC/MIC curve [when the time above the MPC ($T_{>MPC}$) was zero], the $AUBC_M$ was greater than at the same T_{MSW} related to the descending portion ($T_{>MPC} > 0$). A sigmoid function fits these separate data sets well for combined data with the four organisms ($r^2 = 0.81$ and 0.92, respectively), in contrast to fitting the whole data pool while ignoring the AUC/MIC–resistance relationship ($r^2 = 0.61$). These data allow the appropriate use of T_{MSW} as a predictor of bacterial resistance.

1. Introduction

The mutant selection window (MSW) hypothesis [1] allowed an explanation of the specific pattern of concentration-dependent amplification of resistant mutants [2]. Bell-shaped resistance relationships first established with fluoroquinolone-exposed *Staphylococcus aureus* [3] were subsequently reported in studies with *Streptococcus pneumoniae* [4,5] and *Escherichia coli* [6,7]. Although enrichment of resistant mutants was more often linked to the ratio of the area under the concentration–time curve to minimum inhibitory concentration (AUC/MIC), some other predictors of the emergence of bacterial resistance (in this case enrichment of resistant mutants) were also examined. Among them, the ratio of the AUC to the mutant prevention concentration (MPC) was tested [5–9], but there is still no consensus about the advantages of AUC/MPC over AUC/MIC (see a comprehensive analysis of the current data elsewhere [7]). Reports on the relevance of other possible predictors of bacterial resistance, e.g. the time inside the MSW (T_{MSW}), remain even more controversial. Some studies reported sigmoid resistance relationships with T_{MSW} [3,5], but these were less clear than those with AUC/MIC. However, other authors [8,10–12] did not observe any reasonable relationships between the emergence of resistance and T_{MSW}. Moreover, T_{MSW} as a bacterial strain-independent predictor of enrichment of resistant populations has not been tested in any of the above studies including those with more than one organism [5,8,10,11].

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Table 1
Minimum inhibitory concentrations (MICs) and mutant prevention concentrations (MPCs) of ciprofloxacin for the studied *Escherichia coli* strains [7].

Bacterial strain	MIC (mg/L)	MPC (mg/L)	MPC/MIC
E. coli ATCC 25922	0.008	0.11	14
E. coli 4454	0.008	0.25	31
E. coli 4300	0.008	0.10	13
E. coli GM 2995	0.016	0.5	31

To examine the possible reasons for these discrepancies, selection of ciprofloxacin-resistant mutants of four *E. coli* strains that differ in their MPC/MIC ratios was studied at widely ranging T_{MSW} values in an in vitro dynamic model that simulates multiple antibiotic dosing. The primary focus of the study was target mutants resistant to >2× MIC of antibiotic.

2. Materials and methods

2.1. Antimicrobial agents and bacterial strains

Ciprofloxacin powder was purchased from AppliChem BioChemica Chemical Synthesis Services (Darmstadt, Germany). Four strains of *E. coli* [*E. coli* ATCC 25922, *E. coli* 4300, *E. coli* 4454 and *E. coli* GM 2995 (ES1578)] with similar MICs but different MPCs as reported previously [7] were selected for this study (Table 1).

2.2. In vitro dynamic model and simulated pharmacokinetic profiles

A previously described and validated dynamic model [13] was used in this study. Briefly, the model consisted of two connected flasks, one containing fresh BBL™ trypticase soy broth (TSB) (Becton, Dickinson & Co., Le Pont-de-Claix, France) and the other with a magnetic stirrer, the central unit, with the same broth containing a bacterial culture plus antibiotic. Peristaltic pumps circulated fresh nutrient medium to the flasks and from the central 100-mL unit at a flow rate of 17.3 mL/h. The system was filled with sterile TSB and placed in an incubator at 37 °C. The central unit was inoculated with an 18-h culture of *E. coli*. After 2 h of incubation, the resulting exponentially growing cultures reached ca. 10^8 CFU/mL, and ciprofloxacin solutions were injected into the central unit at 12-h intervals.

A series of monoexponential profiles that mimic twice-daily dosing of ciprofloxacin with a half-life ($t_{1/2}$) of 4 h was simulated for 3 consecutive days. The simulated $t_{1/2}$ represented weighted means of values reported for humans (3.2–5.0 h) [14]. The concordance between measured and designed concentrations of ciprofloxacin has been reported elsewhere [7]. For each organism, simulated pharmacokinetic profiles were designed to provide T_{MSW} values ranging from 0% to 100% of the dosing interval. To ensure the desired T_{MSW} values, the ratio of 24-h AUC (AUC_{24}) to the MIC was varied over ≥50-fold range.

2.3. Quantitation of ciprofloxacin-resistant mutants

To reveal resistant *E. coli* mutants, multiple specimens of bacteria-containing medium were sampled from the central unit of the model over the entire observation period. Each sample was serially diluted if necessary and was plated manually onto BBL™ trypticase soy agar (Becton, Dickinson & Co.) plates containing 8× MIC of ciprofloxacin. The choice of this level of resistance was based on our previous observations [7] demonstrating similar time courses of mutants resistant to 4× and 8× MIC; mutant selection at these levels was more reproducible than at either 2× or 16×

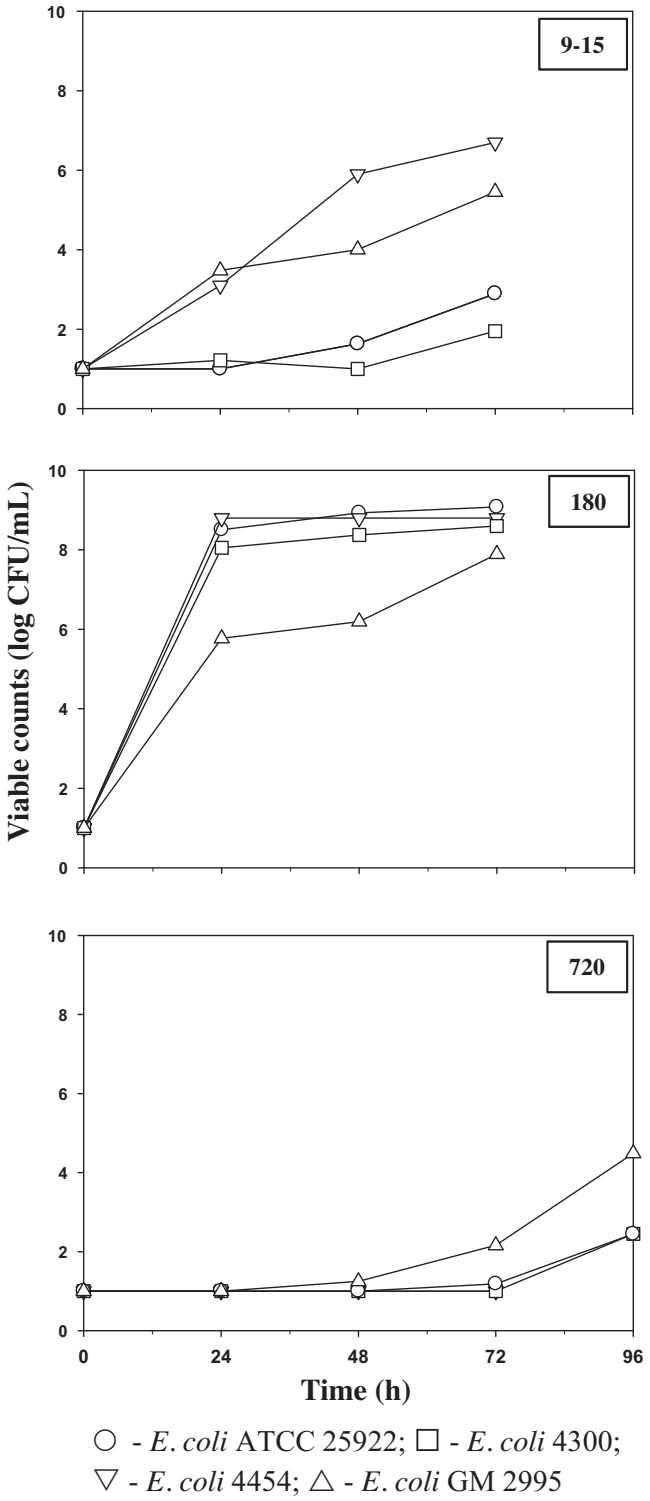

Fig. 1. Time courses of resistant subpopulations of *Escherichia coli* exposed to ciprofloxacin. The simulated ratios of the area under the concentration–time curve to minimum inhibitory concentration (AUC/MIC) (in h) are indicated by boxed numbers.

MIC of ciprofloxacin. The lower limit of detection was 10 CFU/mL (equivalent to at least one colony per plate).

With each simulated dosing regimen, the area under the bacterial mutant concentration–time curve ($AUBC_M$) [9] was determined from the beginning of treatment to 72 h. The $AUBC_M$ values were corrected for the areas under the lower limit of detection over the