

Physiologically structured population model (PSP) of heterogeneity of target binding to describe resistance of Gram-negative bacteria to polymyxin B (PB)



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Objectives

To set up a framework for application of PSP models [1] for studying resistance of cell populations to cytotoxic drugs based on heterogeneity of distribution of target binding properties among cells and to apply it to explain resistance of Gram-negative bacteria to PB.

Background

General Physiologically Structured Population (PSP) Model [1]

A PSP model describes dynamics of a population in terms of the behavior of its constituent individuals. It consists of:

***i*-state equations** $\frac{dx}{dt} = g(E, x) \quad t > 0, x \in \Omega$

***p*-state equations** $\frac{\partial n(t, x)}{\partial t} + \text{div}(gn)(t, x) = b(E, t, x) - \mu(E, x)n(t, x) \quad t > 0, x \in \Omega$

***e*-state equations** $\frac{dE}{dt} = f(t, E, \Psi(n(t, \cdot))) \quad t > 0$

Boundary conditions $v(x) \cdot g(t, x)n(t, x) = \alpha(t, x) \quad t > 0, x \in \partial\Omega$

Initial conditions $E(0) = E_0 \quad n(0, x) = n_0(x) \quad x \in \Omega$

The distribution of the structure x in the population is described by the density function $n(t, x)$ which by definition yields the number of subjects in the population:

$$N(t) = \int_{\Omega} n(t, x) dx$$

Target Binding Structure

- Since number of free and bound receptors expressed on a cell are the individual cell characteristics, r and b are physiological structures.
- Under simplifying assumptions r and b are determined by r_0 (initial free receptors per cell) and K_D (equilibrium disassociation constant). Therefore r_0 and K_D can be considered as individual characteristics of a cell.
- Heterogeneity assumption:** Free receptors and binding affinity can vary from cell to cell.
 $n(t, r_0, K_D)$ = density distribution of r_0 and K_D among cells in the cell population.

Polymixin Mechanism of Action [2]

- Lipopolysaccharide (LPS) is the major surface component of gram-negative bacteria.
- Lipid A is the bioactive component of LPS that comprises the outer leaflet of the gram-negative bacterial outer membrane. Phosphate groups on lipid A and LPS core components result in the bacterial surface having a net negative charge.
- This charge plays a significant role in the electrostatic interaction of cationic polymyxin B with the bacterial surface.
- Gram-negative organisms can synthesize LPS with specific lipid A and core modifications in response to environmental conditions.
- Lipid A modifications result in a less negatively charged bacterial surface, which reduces binding and promotes resistance to the cationic polymyxin B.

Assumptions:

- Negative charges on the outer membrane of gram-negative bacterium are targets for polymyxins.
- Polymyxin displaces Ca^{2+} and Mg^{2+} from their binding sites on the outer membrane.
- Binding polymyxin disrupts outer membrane and leads to bacterium death.
- Expression of negative charges on the outer membrane varies between bacteria is negligible.
- Binding affinity of polymyxin to negative charge varies between bacteria.**

$$n(t, r_0, K_D) \equiv n(t, K_D)$$

Methods

Data

- In vitro* time-kill studies were performed [3] to describe the pharmacodynamic effect of PB (0, 1, 2, and 4 mg/L) against an initial inoculum of 10^6 CFU (colony forming units)/mL of *Klebsiella pneumoniae* strain, BAA1705.
- Serial cultures obtained at 0, 1, 2, 4, 6, 8, 24, 28, 32, and 48 h were quantified.

Target Binding Structured Population Model for Polymixin

***i*-state equations** $\frac{dK_D}{dt} = 0$

***e*-state equations** $\frac{dC}{dt} = 0$

PB concentration in the media C is the environment for the population of bacteria.

***p*-state equations** $\frac{\partial n(t, K_D)}{\partial t} = \frac{VG_{\max} n(t, K_D)}{N_m + N(t)} - \mu(b)n(t, K_D)$

Initial conditions $n_0(K_D) = \frac{b}{a} \left(\frac{K_D}{a} \right)^{b-1} \exp\left(-\left(K_D/a\right)^b\right)$

Weibull distribution with shape parameter b and scale a .

Mortality rate

$$\mu(b) = \mu_0 \left(1 + \frac{E_{\max} b^\gamma}{Eb_{50}^\gamma + b^\gamma} \right) \quad b = \frac{r_{\text{tot}} C}{K_D + C}$$

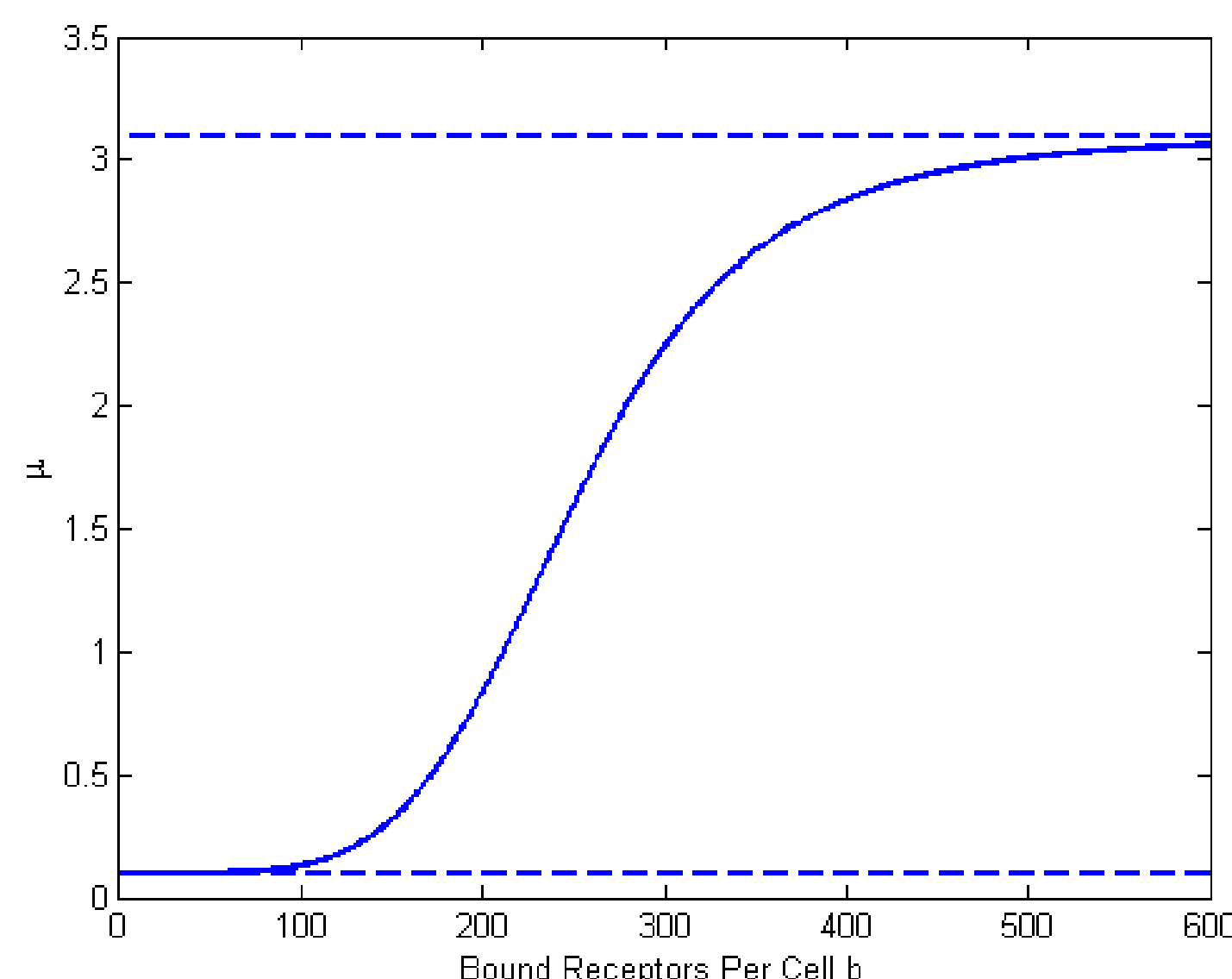


Fig. 1. Binding of drug to the target increases the hazard of killing the cell

Software

Nonlinear regression of the data were performed using the nlinfit function in MATLAB R2013a.

Results

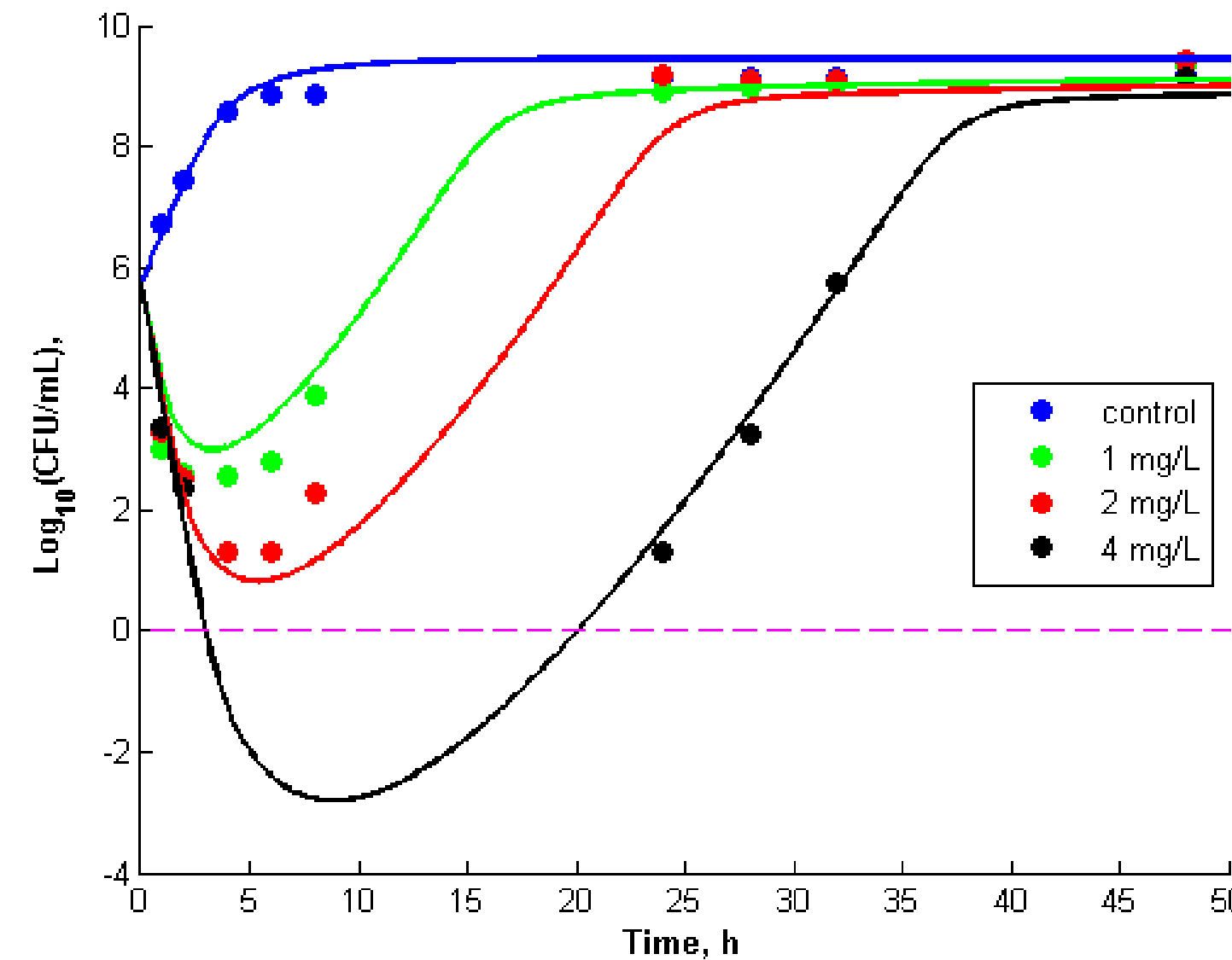


Fig. 2. Time kill data (symbols) of bacteria exposed to various concentration of PB. Lines represent PSP model fits with the bacteria number $N(t)$:

$$N(t) = \int_0^\infty n(t, K_D) dK_D$$

Parameter	Estimate	CV%
$E_{\max}$	115.3	11.0
Eb_{50}/r_{tot}	2.28	12.3
γ	1.69	15.6
a , nM	54.8	24.1
b	0.717	1.3
$t_{1/2kg}$, h	0.307	7.0
$\log_{10}(N_0)$	9.45	1.8
$\log_{10}(N_{ss})$	5.74	3.1
μ_0 , h^{-1}	0.3	FIXED

$$k_g = \frac{\ln 2}{t_{1/2kg}}$$

$$N_m = \frac{N_{ss}}{k_g / \mu_0 - 1}$$

$$VG_{\max} = k_g CFU_m$$

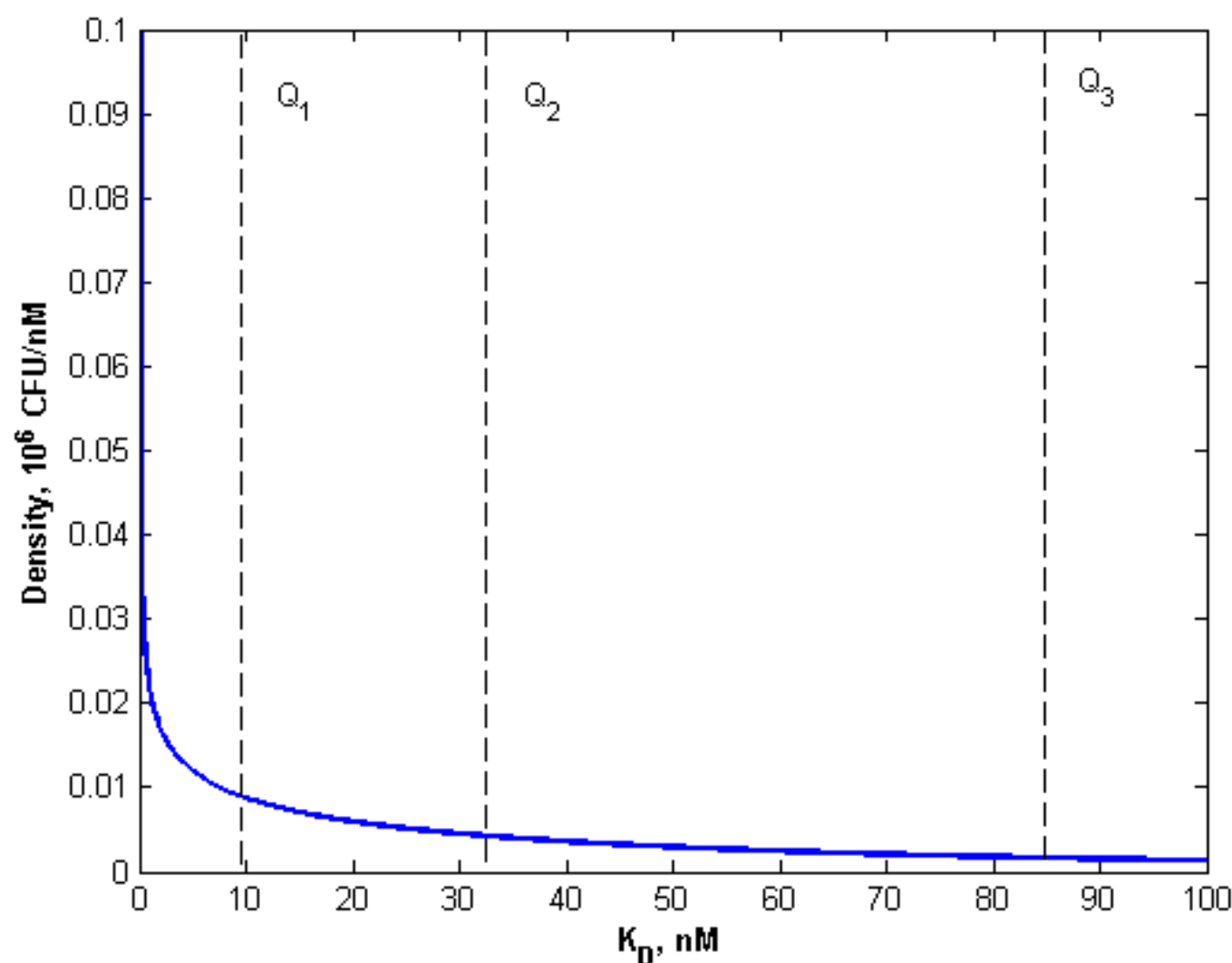


Fig. 3. Density distribution of K_D among bacteria at time $t = 0$. Q_1 , Q_2 , and Q_3 denote the quartiles of the distribution

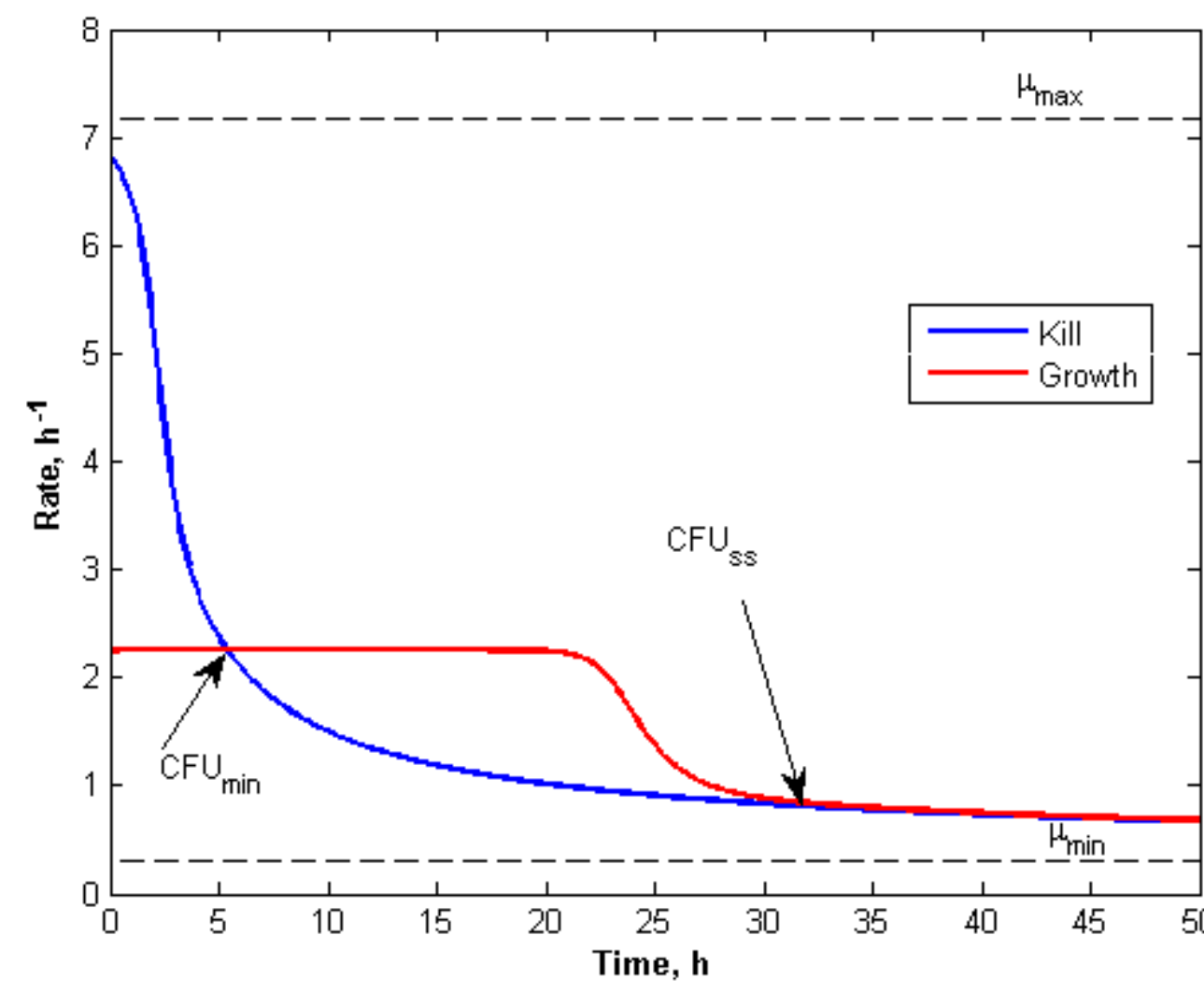


Fig. 4. Simulated time courses of the growth and kill rates for the bacterium population.

$$\text{Growth Rate} = \frac{VG_{\max}}{N_n + N(t)} \quad \text{Kill Rate} = \frac{\int_0^\infty \mu(b)n(t, K_D) dK_D}{N(t)}$$

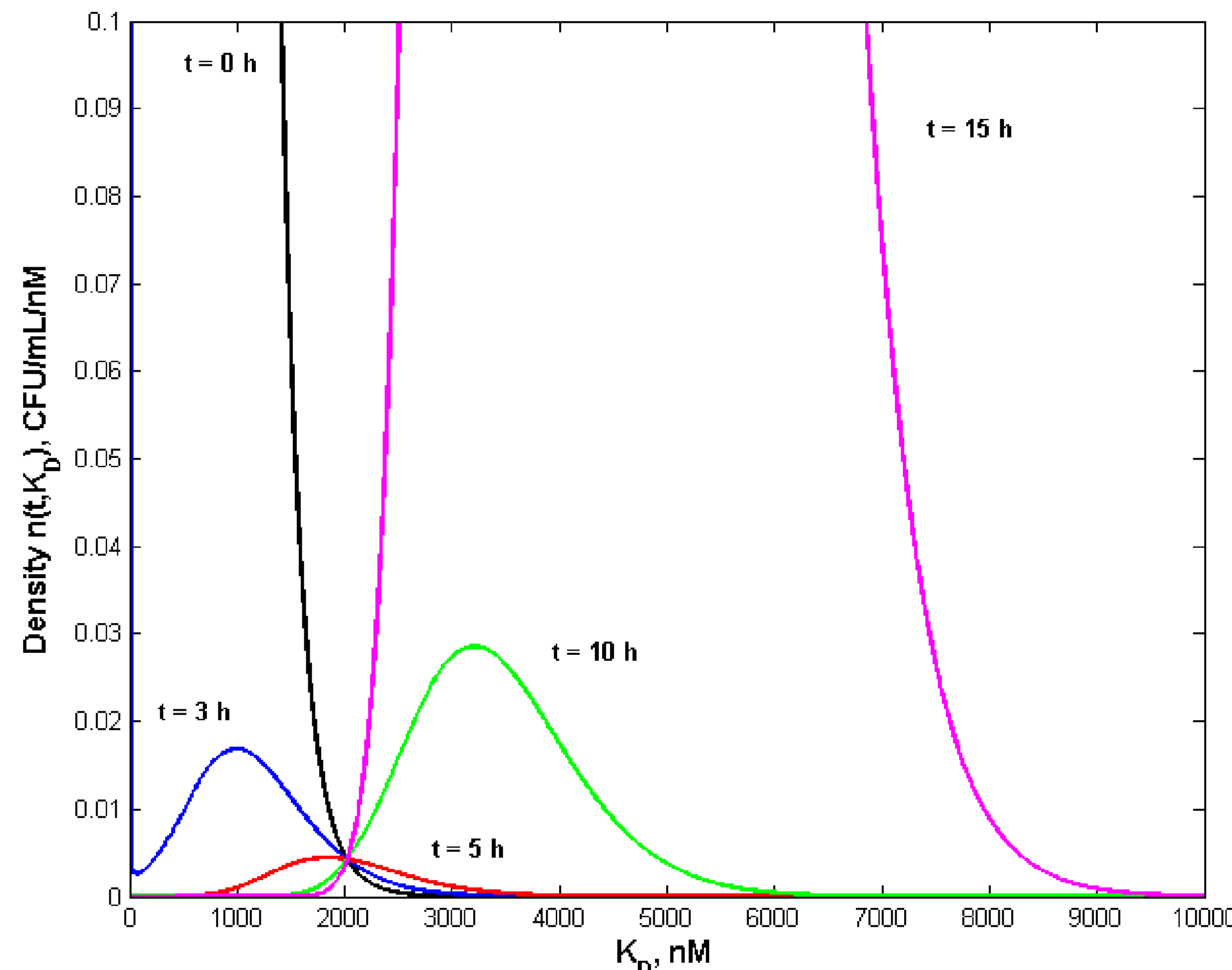


Fig. 5. Density distributions of K_D at various times for PB concentration of $C = 2$ mg/mL. Simulations imply that bacteria of $K_D > 2000$ nM continuously grow whereas cells with $K_D < 2000$ nM are eradicated. The former can be considered to be resistant to PB.

Conclusions

- Cell resistance can be explained by difference in drug target binding among cells.
- The hazard of cell removal depends on the number of bound receptors.
- Selection of the resistant cells is determined by their affinity to the drug. The resistant sub-population are simply cells that weakly bind the drug.
- Similar resistance mechanism can be proposed due to heterogeneity of target expression.
- Polymyxin B mechanism of action justifies heterogeneous binding to negative charge on outer membrane of bacteria as a mechanism of resistance.

References

- [1] de Roos, A gentle introduction to physiologically structured population models structured-population models in marine, terrestrial, and freshwater systems. In Tuljapurkar S, Caswell H (Eds.) Structured-population Models in Marine, Terrestrial, and Fresh Water Systems. (1997) Chapman and Hall, New York.
- [2] Gunn et al. Infection and Immunity, 68:6139-6146 (2000)
- [3] Begic D, von Eiff C, Tsuji BT. Daptomycin pharmacodynamics against Staphylococcus aureus hemB mutants displaying the small colony variant phenotype. J. Antimicrob. Chemother. 63: 977-981 (2009)..