Modeling the dynamics of bacterial colonisation in a murine model of Urinary Tract Infections

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Abstract

TODO Change Abstract

1 Introduction

- Importance, motivation Why?
- What is know about it?
- What is the purpose of this work?
- Migration dynamics depends on the bacteria strain.
- Urinary tract infections (UTI) are the second most common infection worldwide with high recurrence rates, with E. coli the most prevalent cause of infection [6].
- Understanding the survival mechanisms and prevalence of these pathogens plays an important role in avoiding resistant bacteria's appearance and proliferation and tolerance.
- How to explain that non-dividing bacteria are the ones that survives?

2 Methods

2.1 Data Description

A total of 203 healthy 8-week-old female CBA/J mice, in an ascending pyelonephritis UTI mouse model [5], were bacteria infection were administered into the bladder through a urethral catheter after general anesthesia on day 0 with approximately 10^8 colony-forming units (CFU) of PAS, NILS69, UTI, or CFT bacterial strains without any antibiotic treatment. Of these, 29 mice received ciprofloxacin at doses of either 2.5 mg/kg or 10 mg/kg over a 48-hour period.

The bacterial density (CFU/g or CFU/ml) in each organ was determined, after sacrifice by culturing the bacteria overnight in Luria-Bertani (LB) broth. Additional details can be found in [2]. CFU counts in each organ were calculated by scaling the CFU/g values obtained from the kidney and bladder based on the measurement of organ's weight after sacrifice. For urine we assumed a standard volume of 1 ml as a scaling factor. Mice without antibiotic treatment were sacrificed on days 1, 2, 4, 6, 10, 16, and 22, while those receiving antibiotic treatment were sacrificed on day 4 only. For more detailed information table, please refer to Table 5 in the supplementary materials.

2.2 Statistical model

Nonlinear mixed effects modeling approach was used to analyze the different observations over time. A nonlinear mixed effects model for multiple responses is defined as follows. Let $y_i \equiv (y_{i,K}, y_{i,B}, y_{i,U})$ denote the vector of the number of CFU for kidney $y_{i,K}$, bladder $y_{i,B}$, and urine $y_{i,U}$ at time t_i for individual i. Let f denote the global structural model characterizing all responses, based on a system of ordinary differential equations, similar for all individuals. Then one can define $y_{i,l} = f_l(\theta_i, \xi_{i,l}) + \epsilon_{i,l}$ where f_l is the component of the global model f describing the lth response ($l \in \{K, B, U\}$), θ_i is the vector of individual parameters, $\xi_{i,l}$ is the vector of the $n_{i,l}$ sampling times and $\epsilon_{i,l}$ is the vector of residual errors for the response l in individual i. Each individual parameter θ_i can be decomposed as a fixed effect μ, which represents the mean value of the parameter in the population, and a random effect $b_i \sim \mathcal{N}(0, \Omega)$ where Ω accounts for the inter-individual variability. Assuming an exponential random effect model, the individual parameters are modeled as : $\theta_i = \mu \exp(b)$. Lastly we assumed that $\epsilon_{i,l} \sim \mathcal{N}(0, \Sigma_{i,l})$ where $\Sigma_{i,l}$ is a $n_{i,l} \times n_{i,l}$-diagonal matrix with lth elements equal to $(\sigma_{inter,l} + \sigma_{slope,l} \times f_l(\theta_i, \xi_{i,l}))^2$ with $\sigma_{inter,l}$ being the paremeter for the additive part and $\sigma_{slope,l}$ the parameter for the proportional part of the variance error model. Constant ($\sigma_{inter,l} \neq 0$, $\sigma_{slope,l} = 0$), proportional ($\sigma_{inter,q} = 0$, $\sigma_{inter,l} \neq 0$,) or combined ($\sigma_{inter,l} \neq 0$, $\sigma_{slope,l} \neq 0$) variance error models were tested for each response l.

2.3 Modeling Strategy

2.3.1 Modeling PAS strain

To investigate bacterial dynamics in a mouse model of urinary tract infection, we tested two structural models: a logistic growth model [1], shown in Eq. (1), and an effective net rate model in Eq. (2). Evaluating both models across each of the three defined compartments: kidney (K), bladder (B), and urine (U). The logistic growth model combines linear growth with a non-linear elimination rate, for saturation effects. In contrast, the net rate model considers the overall linear growth and elimination effects, simplifying the dynamics by focusing on net changes of proliferation and elimination in each compartment.

The logistic growth model for a compartment X is defined as

$$\frac{d}{dt}N_X = \alpha_X \left(1 - \frac{N_X}{N_X^{ss}}\right) N_X + \sum_{Y \neq X}^{\mathcal{O}} (-q_{X,Y} N_X + q_{Y,X} N_Y) \tag{1}$$

where N_X is the amount of CFU in compartment X, $\mathcal{O} \equiv \{K, B, U\}$ is the set of all compartments, α_X the bacterial growth rate, N_X^{ss} the carrying capacity which defines the saturation point of bacteria in each compartment, and $q_{X,Y}$ the migration or exchange rates from compartment X to compartment Y where $X, Y \in \mathcal{O}$.

The net rate model, for all compartments is defined as

$$\frac{d}{dt}N_X = \delta_X N_X + \sum_{Y \neq X}^{\mathcal{O}} (-q_{X,Y} N_X + q_{Y,X} N_Y) \tag{2}$$

where δ_X is the effective net rates(proliferation if positive and elimination if negative) which is the overall result of both linear proliferation and elimination rates that cannot be distinguished from the data.

We studied the natural progression of infection and bacterial spread without external intervention considering only mice without antibiotic treatment and infected with PAS strain. We selected the optimal structural model by testing all possible combinations of logistic growth and net rate models across the compartments (kidney, bladder, urine), without accounting for inter-individual variability. For example, one combination could involve logistic growth in the kidney while applying net rate models to the bladder and urine. This allowed us to determine which structural combination best describes bacterial dynamics across the different compartments. After selecting the optimal structural model, we evaluated exchange rates between compartments to further simplify the model. We then introduced inter-individual variability sequentially to each parameter, repeating this process until no further model improvement was observed.

2.3.2 Modeling strain effect

Finally, to investigate strain-specific differences in the dynamics, we used the PAS strain as a reference and employed a forward covariate selection procedure on all model parameters one at the time until no model improvement.

2.3.3 Modeling PAS strain under antibiotic treatment

In the absence of antibiotic concentration data, we modeled the antibiotic's effect assuming no direct influence of the antibiotic on the exchange rates between compartments and solely through its impact on the net bacterial growth rates (δ_X, where $X \in \{K, B, U\}$ for the kidney, bladder, and urine compartments).

The net rate under an antibiotic administration is given by:

$$\delta_X^{Ab} = \left(1 + \mathcal{A}_X(D^{Ab})\right) \delta_X \tag{3}$$

where $\mathcal{A}_X(D^{Ab})$ is the functional dependency of corresponding administrated dosage D^{AB}, and δ_X denotes the net rate in compartment X without the antibiotic effect, where $X \in \{K, B, U\}$.

We evaluated three different structural forms of the antibiotic effect: no effect Eq. (4), a linear effect Eq. (5), and a non-linear effect Eq. (6) that is not proportional to dosage

$$\mathcal{A}_X(D^{Ab}) = 0, \qquad \text{no effect} \tag{4}$$

$$\mathcal{A}_X(D^{Ab}) = \epsilon_X D^{Ab}, \qquad \text{linear} \tag{5}$$

$$\mathcal{A}_X(D^{Ab}) = \mathbb{I}_{D^{Ab},D_1}\Phi_{D_1}^X + \mathbb{I}_{D^{Ab},D_2}\Phi_{D_2}^X, \qquad \text{non-linear} \tag{6}$$

where ϵ_X is the constant rate of the impact of the antibiotic, D_l is the dosage l ($l = 1, 2$ for dosage 1 and 2) of the antibiotic Ab, $\mathbb{I}_{D^{Ab},D_l}$ is 1 if $D^{Ab} = D_l$ and 0 otherwise, $\Phi_{D_l}^X$ is the parameter that characterize the effect of the dosage l in compartment X.

Given the limited data available for mice with antibiotic treatment, we investigated the influence of antibiotics on the PAS strain by maintaining the model parameters unchanged from the non-antibiotic scenario. To assess the antibiotic effect, we explored various possibilities: no effect Eq. (4), linear Eq. (5) and nonlinear Eq. (6) dependencies on dosage across all compartments. Once the optimal model capturing the antibiotic effect was identified, we simplified the model by examining the impact of antibiotics on each compartment individually.

Based on PAS model, we studied the antibiotic effects for the mice treated with two different ciprofloxacin doses $D_1 = 2.5mg/kg$ and $D_2 = 10mg/kg$ by fixing the parameters of the model without antibiotic and selected the best model considering all possible combinations of the effect in each compartment.

Given the absence of measured drug concentrations, we assumed that the antibiotic primarily influences the effective growth rates of the bacteria. To explore this assumption, we tested three different models: a model with no effect of the antibiotic, a linear model with dose dependence, and a non-linear model to evaluate the impact of the antibiotic on bacterial dynamics.

2.4 Model selection and evaluation

Estimation of population parameters was performed using the stochastic approximation expectation maximization algorithm (SAEM) [4], implemented in Monolix 2021R2 (Lixoft, Orsay, France, www.lixoft.eu), a software devoted to parameter estimation by maximum likelihood in nonlinear mixed effect models. Data below the lower limit of quantification were treated as left-censored data. Their contribution to the likelihood was computed as the probability that these data are indeed below the lower limit of quantification[7].

Model selection was guided by comparing BICc with threshold of 2 points difference and improvement of parameters uncertainty with $RSE < 100\%$. The final model was selected based on the best fit to the data.

Model evaluation was carried out using several goodness-of-fit plots, particularly individual fits, normalized prediction distribution errors (NPDEs) vs. time and NPDE vs. predictions, and visual predictive checks (VPCs) generated with monolix.

2.5 Simulations

We performed computational simulations to study bacterial infection dynamics and clearance in a population of 1,000 virtual mice, both with and without antibiotic treatment. These simulations were carried out using Simulx 2021R2 and modeled bacterial growth, migration between organs, and the effects of antibiotics.

To account for individual variability, parameter values for bacterial growth, elimination rates, and migration were drawn from corresponding distributions. Additionally, covariates for different bacterial strains were included to evaluate the impact of strain-specific characteristics on infection outcomes. Bacterial clearance probabilities were calculated at each time step throughout the simulation.

In the model, infection was considered cleared in each organ when 95% of the virtual mice had bacterial levels below 1 CFU in that organ.

3 Results

3.1 Data

Of the total of 203 healthy 8-week-old female CBA/J mice 179 were divided in your groups with the , 76 (PAS), 27(NILS69), 31(UTI), 40 (CFT) (see Table 5) were analyzed in three groups: First, only mouse with no antibiotic treatment and infected with PAS to determine the natural progression of the infection. Then the

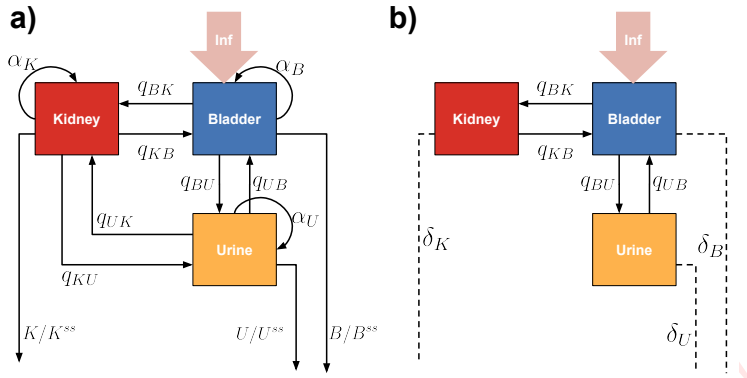

Figure 1: a) Logistic growth initial model b) net rate of change final model without antibiotic treatment.

3.2 Model Selection

To characterize the dynamics of bacterial infection in kidney, bladder, and urine compartments in untreated mice, we evaluated logistic growth and net rate models across each compartment. A series of candidate models with various combinations of logistic growth and net rate dynamics were compared using Bayesian Information Criterion (BIC) values, as shown in Table 3.3.1.

3.3 Final Models and Estimated Parameters

Here we describe the final models and their respective estimated parameters for analyzed case

3.3.1 Model for PAS strain

For the group with PAS only the model was best described by net rates model with migration exchanges on bladder to kidney, kidney and urine as show in figure 1b, details on BICc selection performance are provided in supplemetary material.

- TODO Add monolix structural model in appendix
- TODO Think in a way to encapsulate all the BICc for each model in a single table with the covariates BICc, and maybe move it to supp.

Final selected model defined by Eq. (7-9) is composed using only net rate model for all compartments, represented in Fig. 1b, and with estimated parameter in table 3.3.1.

Logistic model	Net rate model	BICc	Model nickname
K, B, U		803.117	General
K, B	U	709.026	NOSS U
B, U	K	717.472	NOSS R
K, U	B	709.307	NOSS V
K	B, U	713.777	NOSS VU
U	K, B	714.093	NOSS RV
B	K, U	714.535	NOSS RU
	K, B, U	702.108	NOSS RVU
	K, B, U wo UR	**688.156**	NOSS RVU without exchanges between U and R

Table 1: Model selection process for a structural model, where logistic and net rate models are applied to different compartments. The Logistic model and Net rate model columns indicate which model is used in each compartment. The BICc column shows the Bayesian Information Criterion.

$$\frac{d}{dt}K = \delta_K K \ -q_{K,B}K + q_{B,K}B \tag{7}$$

$$\frac{d}{dt}B = \delta_B B \ -q_{B,K}B + q_{K,B}K$$

$$-q_{B,U}B + q_{U,B}U \tag{8}$$

$$\frac{d}{dt}U = -\delta_U U \ -q_{U,B}U + q_{B,U}B \tag{9}$$

The estimated parameters for the model for strain PAS. without antibiotic treatment capture the rates of bacterial net proliferation or clearance and transfer between different compartments, as well as associated variabilities. Specifically, δ_K and δ_B represent a similar net proliferation in kidney and bladder around 12 CFU/day. Meanwhile, in the urine compartment a net elimination represented in $\delta_U = 9.75$ CFU/day.

The parameters $q_{BK} = 0.0021$ CFU/day describe a slow bacteria transfer from bladder to kidney been the parameter with highest uncertainty(92.3%) which contrast with the high bacteria transfer from kidney to bladder $q_{KB} = 12.3$ CFU/day that has been precise estimated (1.06%). This suggest that as infection start in bladder, even with a slow migration rate the high proliferation rate rapidly increase the amount of bacteria in kidney. Now the fast migration rate from kidney to bladder q_{KB} and from bladder to urine $q_{BU} = 59.2$ CFU/day indicates that the effective elimination path is the done in urine.

3.3.2 Model with different strains without antibiotic

Here we present the model considering the four different strains in Table 3.3.2, as a main difference with the case with only PAS here we have the same elimination path in urine compartment. The proliferation rate in kidney gain a substantial

Parameter	Estimates(r.s.e.)	Units
δ_K	12.2(1.09%)	$(days)^{-1}$
δ_B	12.7(11.2%)	$(days)^{-1}$
δ_U	9.75(30.6%)	$(days)^{-1}$
q_{BK}	0.0021(92.3%)	$(days)^{-1}$
q_{KB}	12.3(1.06%)	$(days)^{-1}$
q_{BU}	59.2(35.5%)	$(days)^{-1}$
q_{UB}	4.24(16.6%)	$(days)^{-1}$
ω_{δ_U}	3.36(36.9%)	$(days)^{-1}$
$\omega_{q_{BU}}$	0.231(25.3%)	$(days)^{-1}$

Table 2: Estimates of model without antibiotic treatment for PAS straind, with variabilities in δ_U and q_{BU} with normal and log-normal distribution respectively.

increase, that is compensated by the substantial increase in transfer rate from kidney to bladder, as shown in Table 3.3.2 and 3.3.1. At the main effect for CFT and UTI is slower elimination rate in urine and a faster elimination in urine for NILS69. However, we should notice that NILS69 is the strain with data only at days 2 and 4. Also notice that we get a reduction in the relative stantard error (r.s.e.) for the migration from bladder to kidney q_{BK}

3.4 Simulations strains without antibiotic treatment

We performed 1000 individual simulations, sampled from the model, for the different bacteria strains considering to evaluate the clearance different for the different strains. We defined that a mouse cleared the bacteria if the CFU are bellow 1 and the time to clearance when at least 80% of the sampled individuals reach the limit of 1 CFU, and the results of this- are shown in Fig. 3.

For the kidney compartment, strain NILS69 clears the fastest at 34.5 days, while UTI takes the longest at 55.5 days, indicating strain-dependent differences in clearance efficiency. Similarly, in the bladder, NILS69 again shows the shortest clearance time at 33.5 days, while UTI exhibits the longest time at 72.5 days, suggesting that UTI may be more resilient to clearance in this compartment.

In the urine compartment, NILS69 clears markedly faster than the other strains, achieving clearance in just 26 days. In contrast, UTI requires 94 days for clearance, underscoring a substantial difference in clearance rates for this strain compared to others. These results highlight NILS69 as the strain with the most rapid clearance across all compartments, while UTI consistently shows the slowest clearance, particularly in the bladder and urine. The findings suggest both compartment-specific and strain-specific factors influence the time required for bacterial clearance.

3.4.1 Model with antibiotic PAS strain

We investigated the effect of the antibiotic on mice infected with the PAS strain, focusing on groups that received no treatment as well as those treated with two

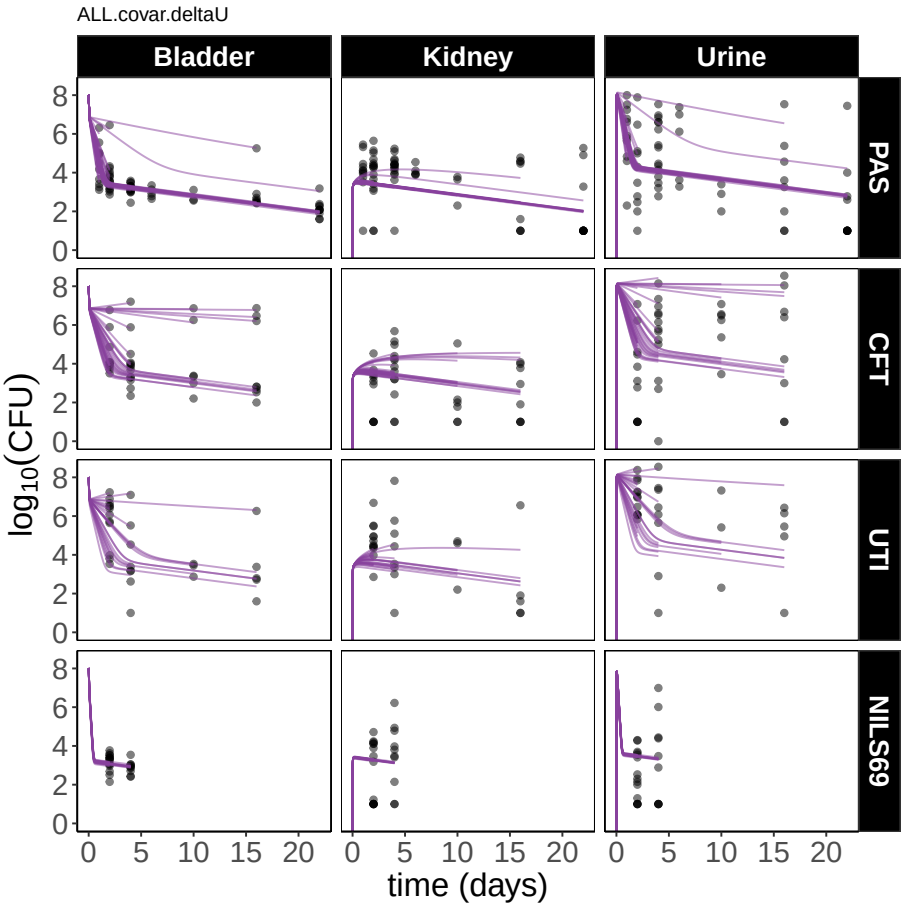

Figure 2: Time evolution of CFU for each organ compartment, open circles experimental data, individual fits gray line, purple curve population parameters for different strains.

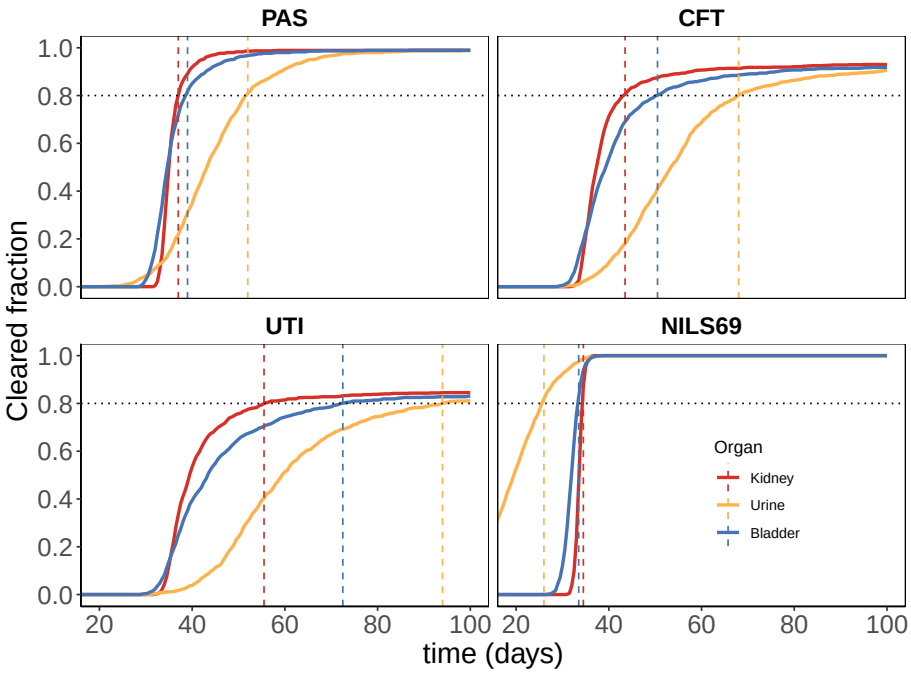

Figure 3: Fraction of bacteria cleared over time by strain for each organ, based on results from 1,000 simulated mice per strain. The figure includes four panels, each corresponding to a different bacterial strain (PAS, CFT, UTI, and NILS69). The dotted horizontal line indicates the 80% clearance threshold, and dashed vertical lines mark the specific time points at which each organ individually reaches this threshold

Parameter	Estimates(r.s.e.)	Units
δ_K	40(0.74%)	$(days)^{-1}$
δ_B	13(19.26%)	$(days)^{-1}$
δ_U	12(33.15%)	$(days)^{-1}$
q_{BK}	0.0016(34.95%)	$(days)^{-1}$
q_{KB}	40(0.74%)	$(days)^{-1}$
q_{BU}	66(10.84%)	$(days)^{-1}$
q_{UB}	2(6.86%)	$(days)^{-1}$
ω_{δ_U}	1.2(15.28%)	$(days)^{-1}$
$\omega_{q_{BU}}$	0.11(40.13%)	$(days)^{-1}$
$\beta_{\delta_U,CFT}$	-1.1(30.03%)	$(days)^{-1}$
$\beta_{\delta_U,UTI}$	-1.6(23.54%)	$(days)^{-1}$
$\beta_{\delta_U,NILS69}$	3.8(25.51%)	$(days)^{-1}$

Table 3: Estimates of the model parameters without antibiotic treatment for strains:PAS, CFT, UTI, NILS69, showing variabilities in δ_U and q_{BU} with normal and log-normal distributions, respectively. β_{δ_U} represents the covariate effect on δ_U for each strain, with PAS as the reference strain.

Parameter	Estimates(r.s.e.)	Units
ϵ_K	0.0057(17.4%)	$g/(days.kg)$
ϵ_U	464.37(30.8%)	$g/(days.kg)$

Table 4: Parameter estimates for model with antibiotic treatment.

doses: 2.5 mg/kg and 10 mg/kg of ciprofloxacin.

The best model we could found for the antibiotic treatment was the linear effect Eq. 5 acting on the $delta_U$ and $delta_K$ as

$$\begin{aligned} \delta_K^{Cipro} &= \left(1 - \epsilon_K D^{Cipro}\right) \delta_K \\ \delta_U^{Cipro} &= \left(1 + \epsilon_U D^{Cipro}\right) \delta_U \end{aligned} \tag{10}$$

4 Discussions

- Only one point per mice, is this consider longitudinal data?

5 Conclusions

TODO Fix it The competition of bacteria elimination and proliferation are different in the three compartments. The relationship among bladder, kidney and urine exchange rates indicates that the urine compartment is the main path to elimination and the large proliferation in the kidney positions it as the primary source of a long lasting bacteria. The persistence of bacteria in the bladder

despite the administration of ciprofloxacin, even at higher concentrations has been reported before.[3] In this study we presented a reduced comprehensive mathematical model of the colonisation in UTI organs for PAS E. coli under the administration of two doses of ciprofloxacin which is the starting point to develop better models with other antibiotics with different mechanism of action and different strains.

Tab. 5

6 Bibliography

References

[1] Rosalind J Allen and Bartlomiej Waclaw. Bacterial growth: a statistical physicist's guide. *Reports on Progress in Physics*, 82(1):016601, 2018.

[2] Ariane Amoura, Claire Pistien, Camille Chaligné, Sara Dion, Mélanie Magnan, Antoine Bridier-Nahmias, Alexandra Baron, Françoise Chau, Emmanuel Bourgogne, Minh Le, et al. Variability in cell division among anatomical sites shapes escherichia coli antibiotic survival in a urinary tract infection mouse model. *Cell Host & Microbe*, 2024.

[3] Lotte Jakobsen, Carina Vingsbro Lundberg, and Niels Frimodt-Møller. Ciprofloxacin pharmacokinetics/pharmacodynamics against susceptible and low-level resistant escherichia coli isolates in an experimental ascending urinary tract infection model in mice. *Antimicrobial Agents and Chemotherapy*, 65(1):10–1128, 2020.

[4] Estelle Kuhn and Marc Lavielle. Maximum likelihood estimation in nonlinear mixed effects models. *Computational statistics & data analysis*, 49(4):1020–1038, 2005.

[5] Francoise Labat, Olivier Pradillon, Louis Garry, Michel Peuchmaur, Bruno Fantin, and Erick Denamur. Mutator phenotype confers advantage in escherichia coli chronic urinary tract infection pathogenesis. *FEMS Immunology & Medical Microbiology*, 44(3):317–321, 2005.

[6] David A Rosen, Thomas M Hooton, Walter E Stamm, Peter A Humphrey, and Scott J Hultgren. Detection of intracellular bacterial communities in human urinary tract infection. *PLoS medicine*, 4(12):e329, 2007.

[7] Adeline Samson, Marc Lavielle, and France Mentré. Extension of the saem algorithm to left-censored data in nonlinear mixed-effects model: Application to hiv dynamics model. *Computational Statistics & Data Analysis*, 51(3):1562–1574, 2006.

A Data

TODO Table Supp.

Antibiotic Group	Strain	Days								Total
		1	2	4	6	10	14	16	22	
Control	PAS	13	17	18	5	3		9	11	76
	NILS69		15	12						27
	UTI		12	11		3		5		31
	CFT		11	13		8		8		40
Ciprofloxacin 2.5 mg/kg	PAS			17		5				22
	UTI			7						7
	CFT			11						11
Ciprofloxacin 10 mg/kg	PAS			7						7
	NILS69			7						7
	UTI			7						7
	CFT			6						6
Cefotaxime 100 mg/kg	PAS			14		6				20
Fosfomycin 100 mg/kg	PAS			8						8
Delafloxacin 10 mg/kg	PAS			7						7
		13	55	145	5	14	11	22	11	276

Table 5: Mice sampling distribution with antibiotic treatments on bacterial strains in time.

TODO Change or remove sampling figure, maybe better to add numbers with the symbols or just text description.

- TODO Add table with mice organ distribution CFU counts? or write directly med [min;max]
- TODO LacZ too? Let's see that later

A.1 Model evaluation

TODO VPC, residuals?

A.1.1 Ethics Statement

TODO The study was conducted in accordance with the ethical standards of ... and received approval from the ...

TODO Define order strain or antibiotic effect first? I think is better strain, because antibiotic has less data perhaps? TODO Make a better figure 4 and include the strains

TODO Should I include the logistic growth equations? I imaging that for completeness I could write it but maybe in the appendix. Or maybe a general equation for one compartment but probably will not be easy to read.

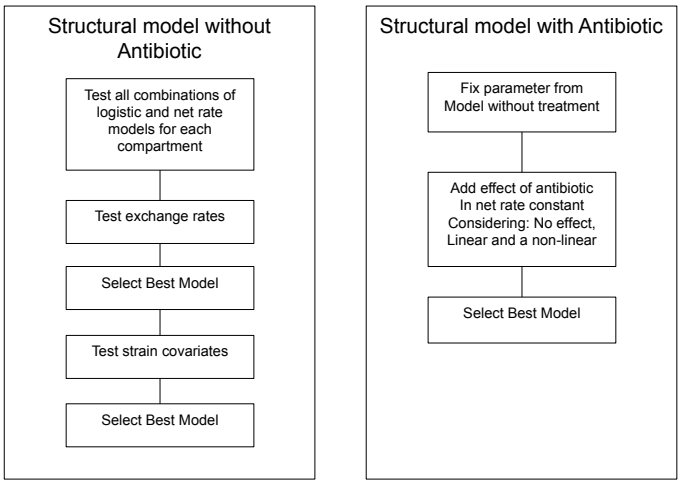

Figure 4: Model diagram for PAS strain without antibiotic treatment.

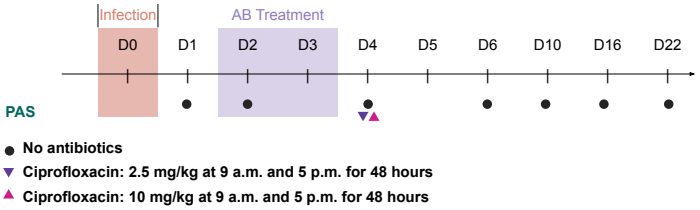

Figure 5: Data description.

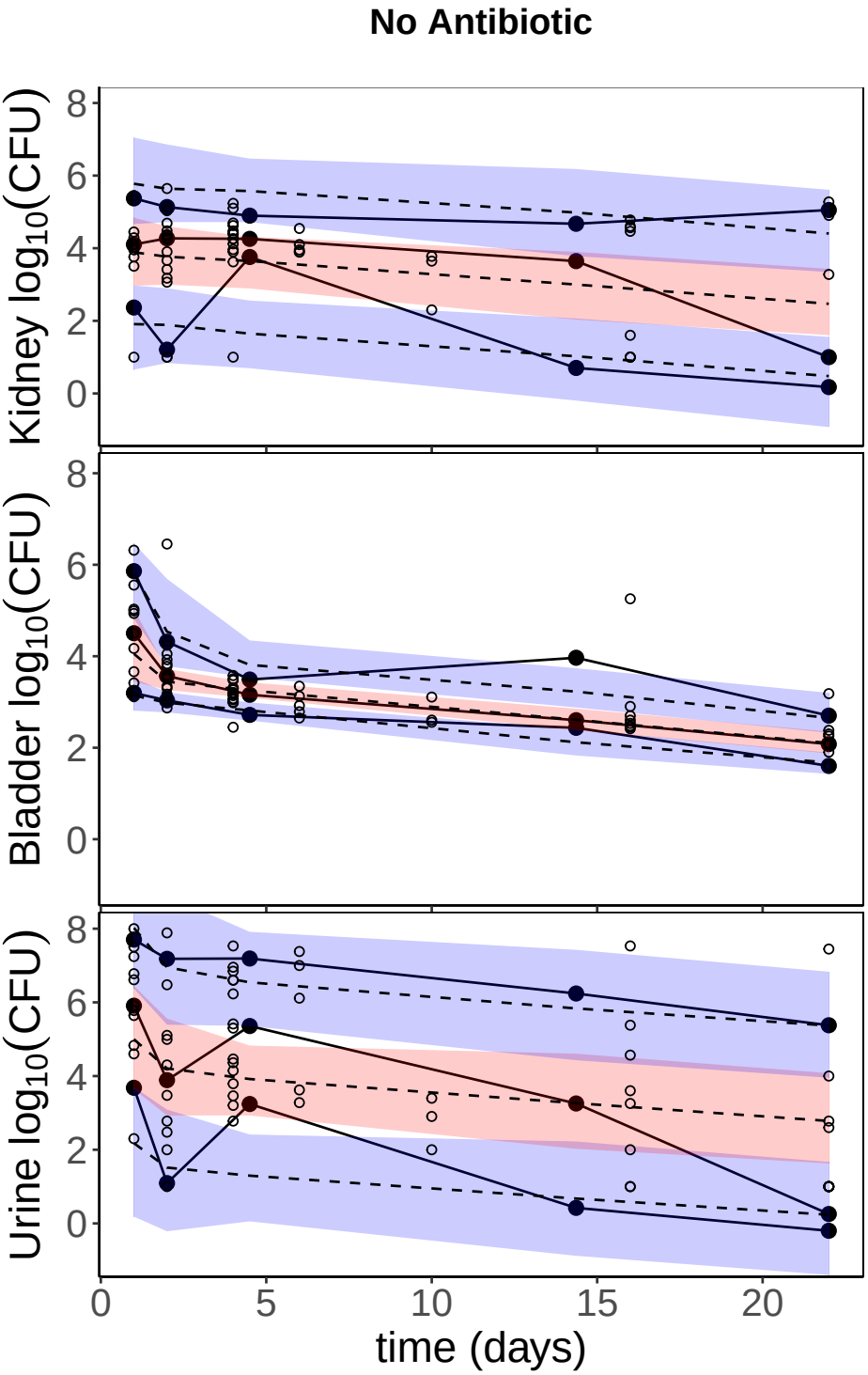

Figure 6: Visual Predicte Check for model with antibiotic, for each compartment.

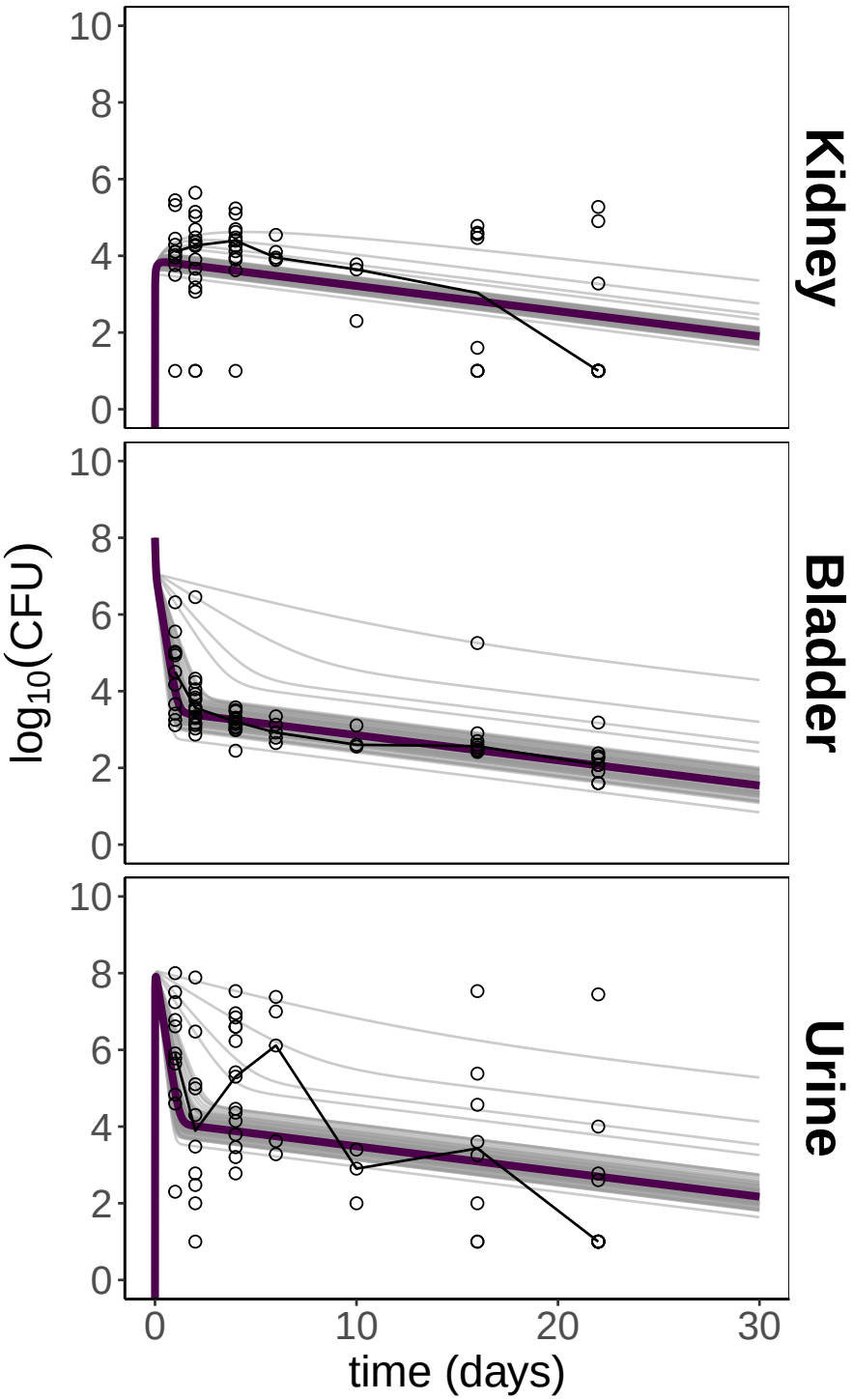

Figure 7: Time evolution of CFU for each organ compartment, open circles experimental data, individual fits gray line, purple curve population parameters, black solid line medianvalue at each sampling day

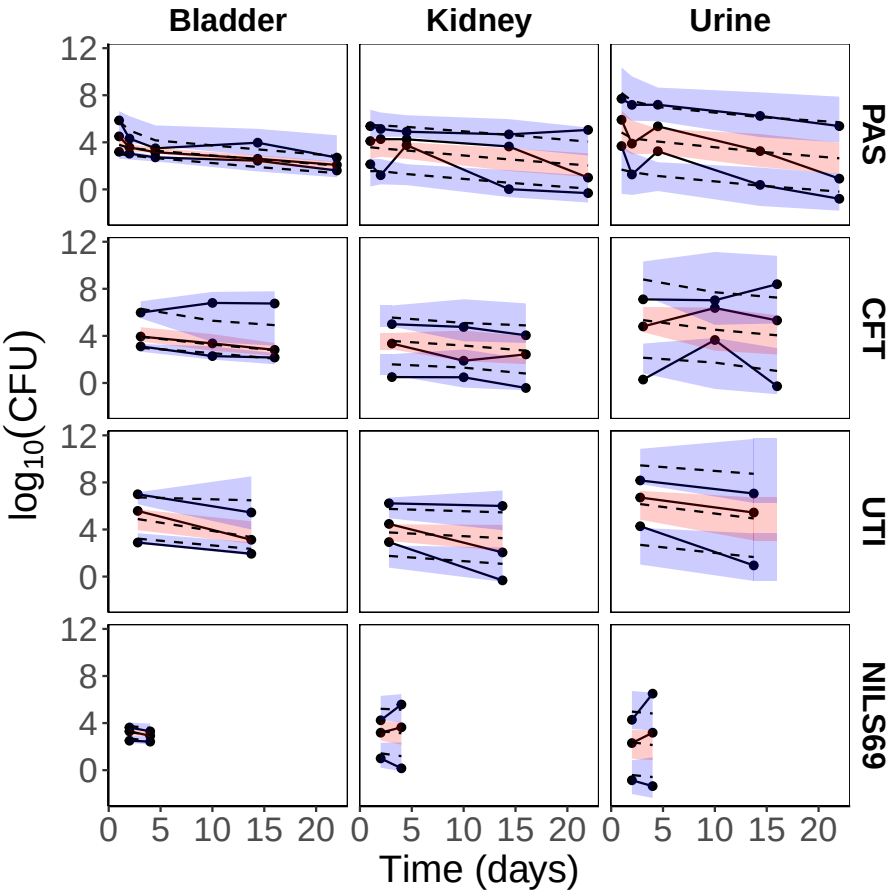

Figure 8: Visual Predicte Check for different strains and compartments.