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► To cite this version:

Paul Henriot, Elena Buelow, Fabienne Petit, Marie-Cécile Ploy, Christophe Dagot, et al.. Modeling the impact of urban and hospital eco-exposomes on antibiotic-resistance dynamics in wastewaters. *Science of the Total Environment*, 2024, 924, pp.171643. 10.1016/j.scitotenv.2024.171643 . hal-04519887

HAL Id: hal-04519887

<https://hal.science/hal-04519887v1>

Submitted on 27 Mar 2024

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Modeling the impact of urban and hospital eco-exposomes on antibiotic-resistance dynamics in wastewaters

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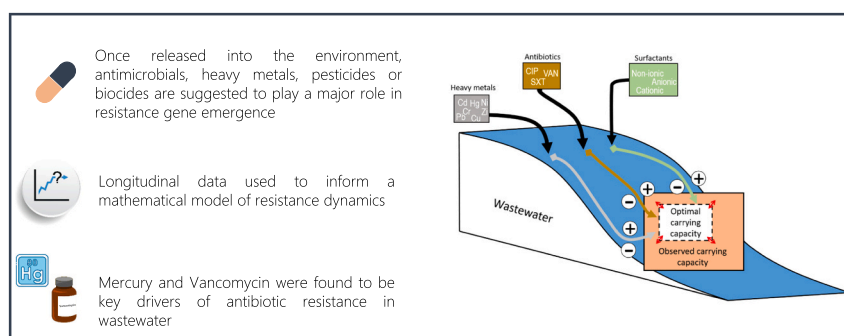
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HIGHLIGHTS

- We analyzed wastewater resistance associated genetic and environmental longitudinal data.
- We developed a simple hypothesis-driven model to formalize resistance selection.
- Model analysis of data identify mercury and vancomycin as key drivers of antibiotic resistance in wastewater

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:

Mathematical modeling
Antibiotic resistance
Effluents
Dynamics
Eco-exposome

ABSTRACT

The emergence and selection of antibiotic resistance is a major public health problem worldwide. The presence of antibiotic-resistant bacteria (ARBs) in natural and anthropogenic environments threatens the sustainability of efforts to reduce resistance in human and animal populations. Here, we use mathematical modeling of the selective effect of antibiotics and contaminants on the dynamics of bacterial resistance in water to analyze longitudinal spatio-temporal data collected in hospital and urban wastewater between 2012 and 2015. Samples were collected monthly during the study period at four different sites in Haute-Savoie, France: hospital and urban wastewater, before and after water treatment plants. Three different categories of exposure variables were collected simultaneously: 1) heavy metals, 2) antibiotics and 3) surfactants for a total of 13 drugs/molecules; in parallel to the normalized abundance of 88 individual genes and mobile genetic elements, mostly conferring resistance to antibiotics. A simple hypothesis-driven model describing weekly antibiotic resistance gene (ARG) dynamics was proposed to fit the available data, assuming that normalized gene abundance is proportional to

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<https://doi.org/10.1016/j.scitotenv.2024.171643>

Received 21 April 2023; Received in revised form 10 January 2024; Accepted 9 March 2024

Available online 11 March 2024

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antibiotic resistant bacteria (ARB) populations in water. The detected compounds were found to influence the dynamics of 17 genes found at multiple sites. While mercury and vancomycin were associated with increased ARG and affected the dynamics of 10 and 12 identified genes respectively, surfactants antagonistically affected the dynamics of three genes. The models proposed here make it possible to analyze the relationship between the persistence of resistance genes in the aquatic environment and specific compounds associated with human activities from longitudinal data. Our analysis of French data over 2012–2015 identified mercury and vancomycin as co-selectors for some ARGs.

1. Introduction

Over the past few decades, the emergence and selection of antibiotic resistance worldwide has become a major public health concern. The increasing detection of clinically relevant antibiotic resistance in environmental reservoirs increases the threat of antimicrobial resistance and the risk of new emergence of antibiotic resistant pathogens that can colonise humans and animals (Cantas et al., 2013). The use of antimicrobials in humans, animals and agriculture is likely to influence the emergence and selection of antibiotic resistance in the different reservoirs (Davies and Davies, 2010; Manyi-Loh et al., 2018; Hernando-Amado et al., 2019). However, once released into the environment, antimicrobials, heavy metals, pesticides or biocides are thought to play an important role in the emergence and (co-)selection of resistance genes in bacterial communities on a more global scale (Singer et al., 2016).

Reflecting human demographics and associated anthropogenic activities, hospital and urban wastewater are major sources of antibiotic resistant bacteria (ARBs), antibiotic resistance genes (ARGs) and chemical pollutants that contaminate the aquatic environment despite wastewater treatment (Pärnänen et al., 2019). In such an environment, selection for resistant bacteria could be favoured by the co-presence of bacteria and antibiotics, but could also be enhanced by co-selection phenomena, which are reinforced by the presence of other chemicals (heavy metals, surfactants, pesticides) through the mechanisms of co-and cross-selection: the presence of these chemicals is either co-selected (e.g. via the presence of mobile genetic elements carrying multiple ARGs such as plasmids and integrons) or cross selection, e.g. where efflux pumps that transport metals can also transport antibiotics out of the cells (cross selection by cationic agents or heavy metals) (Baker-Austin et al., 2006; Seiler and Berendonk, 2012; Devarajan et al., 2015; Henriques et al., 2016; Li et al., 2017; Dickinson et al., 2019; Søgaard Jørgensen et al., 2020; Buelow et al., 2021). To date, current understanding of the mechanisms underlying resistance emergence and spread is mainly supported by laboratory experiments (Baharoglu and Mazel, 2011; Gullberg et al., 2014). Identifying the cumulative effects of multiple exposures to contaminants, and assessing the meaningful associations between them and bacteria, on the emergence of resistance in natural environments remain real challenges.

Addressing those combined effects requires the acquisition of longitudinal data collected at representative sites of anthropized environments that enable an analysis through dynamic and mechanistic approaches. In this context, mathematical models formalizing the dynamics of resistant bacteria or genes and mobile genetic elements (MGEs) under exposition to different selective compounds can be developed and used to help quantify and predict selection dynamics in aquatic environments: from wastewater treatment plant (WWTP) effluents to rivers. To date very few dynamic models were proposed to assess driving parameters for the origin of resistance selection in effluents. Existing studies mostly describe either complex models, considering multiple biological or physical mechanisms, but with no or limited application to field data (Novozhilov, 2005; Ibargüen-Mondragón et al., 2014) or data-driven models focusing only on a single compound (Hellweger et al., 2011; Gothwal and Thatikonda, 2018).

Herein, we used a large spatiotemporal study investigating the presence of chemicals and genetic elements in effluent and affluent

hospital and urban wastewaters to develop a simple hypothesis-driven model of antibiotic-resistance selection in anthropized water systems and evaluate resistance-compound associations.

2. Materials and methods

2.1. Data collection and presentation

2.1.1. Study sites

Between 2012 and 2015, data on hospital and urban wastewaters, before and after water treatment, were monthly collected from a national study site in the French Alps (Haute-Savoie). The site, located in Scientrier, includes a wastewater treatment plant with a capacity of 32,000 population equivalents (classical biological system). One biological basin (5400 m³) is dedicated to the biological treatment of effluents from the Alpes Leman Hospital Center, which has 450 beds, 8 operating rooms and various medical departments.

Samples were collected in four distinct locations, upstream and downstream (i.e., untreated and treated) of each WWTP (Fig. 1) at monthly intervals by flow-proportional sampling. A database with >50,000 entries houses all analytical data (Bertrand-Krajewski et al., 2021). The four sample sites yielded 30, 28, 31 and 19 available time points corresponding to, respectively: untreated and treated hospital wastewaters, and untreated and treated urban wastewaters.

2.1.2. Chemicals

Three different exposure variables were collected simultaneously: (1) heavy metals (ng/L): zinc, nickel, mercury, lead, copper, chromium and cadmium, measured with inductively coupled plasma and atomic emission spectroscopy; (2) antibiotics (ng/L): vancomycin, sulfamethoxazole and ciprofloxacin, measured by solid-phase extraction and liquid chromatography with a tandem mass spectrometry (LC-MS/MS); (3) surfactants (ng/L):

anionic, non-ionic and cationic, measured as described by Chonova et al. (2018).

2.1.3. Microbiology

Microorganisms were recovered from filters after water filtration and DNA was isolated using the Power Water DNA Extraction Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). Nanoliter-scale quantitative polymerase chain reactions (qPCRs) quantified levels of 88 individually targeted resistance genes and MGEs conferring resistance to antibiotics, surfactants and/or heavy metals, as described previously (Buelow et al., 2018). qPCR-determined 16S rRNA gene-copy numbers served as a surrogate marker of bacterial biomass, as described previously by Stalder et al. (2014).

For each sampling time, normalized abundance of 88 individual genes and MGEs, most of them conferring resistance to antibiotics and some conferring resistance to heavy metals or ammonium compounds, were determined (Fig. 1(b)). ARGs, surfactants, heavy metal resistance genes and MGEs were detected by high-throughput qPCRs, as described above. Individual gene abundance was normalized to the 16S rRNA gene. In our analysis, all values below the limit of quantification (LoQ) were replaced by 0.

More information on sampling, study design, genetic characterization and chemical analysis are available in Buelow et al. (2020), site

descriptions are available in [Chonova et al. \(2018\)](#).

2.2. Model choice and hypothesis

2.2.1. Model

We developed a dynamic model to formalize the persistence of resistance genes and MGEs in effluents and the factors affecting their temporal dynamics. We assumed that resistance genes and MGEs were directly associated with the presence of bacteria and, to simplify formalization, modeled the population dynamics of these microbes carrying these genetic elements (Fig. 2). In addition, MGEs were considered to be drivers of antibiotic resistance.

2.2.2. Bacterial population dynamics

We assumed that the dynamic system reached saturated equilibrium every week. We also assumed that the input of resistant bacteria was exogenous, and that the available substrate was limited compared to the bacterial biomass, so that almost no bacterial growth occurred and the modeling of bacterial division could be neglected. In addition, we hypothesized that the proportion of free DNA within the sites was negligible and thus did not interfere with the model. Therefore, every week the resistant bacterial population was converted to a stationary state (i. e., $dR/dt = 0$, corresponding to the fixed-point $R(t) = K(t)$, with $K(t)$ corresponding to the carrying capacity at time t and $R(t)$ the number of resistant bacteria at time t). Hence, the carrying capacity of resistant organisms on a given week t was defined as follows:

$$K(t) = R(t) = K_{opt} \times \beta(t) \tag{1}$$

where $\beta(t)$ corresponds to a rate of interaction with the environment at time t and K_{opt} is the optimal carrying capacity in absence of heavy metals, antibiotics and surfactants.

2.2.3. Modeling the impact of compound exposure

Because compound concentrations were measured at different day each week, we assumed that their concentrations were constant for each week or underwent only small variations that had no impact on the population's equilibrium level.

We assumed that compounds could have either a selective effect on resistance, thereby increasing the carrying capacity of resistant bacteria,

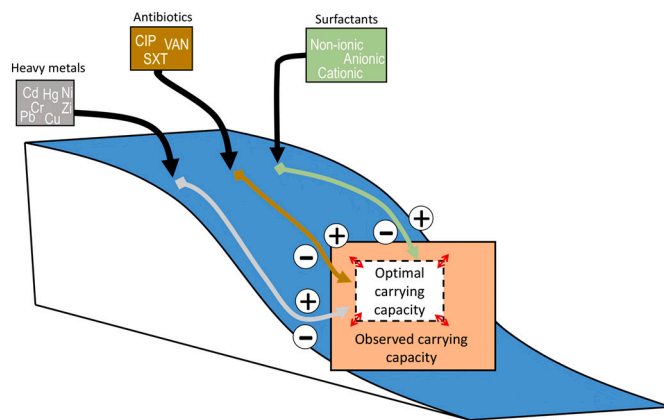


Fig. 2. Schematic view of the mechanisms considered in the model. Each compound, varying over time, may have either a selective effect (+) or a reductive effect (-) on resistance status. They directly impact the optimal carrying capacity (K_{opt}), in the absence of these compounds.

or a reductive effect, thereby reducing the carrying capacity of resistant bacteria.

According to the first hypothesis (resistance selection), the effect of a given compound, Cpd , on $K(t)$ is written as follows:

$$\beta_{+,cpd}(t) = \left(\frac{Conc_{cpd}(t) + F_{cpd}}{F_{cpd}} \right) \tag{2}$$

where $Conc_{cpd}(t)$ is a measured compound concentration at time t and F_{cpd} is a constant selection-threshold concentration value associated with that same agent. When F_{cpd} is low compared to $Conc_{cpd}(t)$, the selective pressure is high.

According to the second hypothesis (resistance reduction), the effect of the compound on $K(t)$ is written as follows:

$$\beta_{-,cpd}(t) = \left(\frac{MIC_{cpd}}{Conc_{cpd}(t) + MIC_{cpd}} \right) \tag{3}$$

where MIC_{cpd} is a constant resistance-reduction threshold concentration

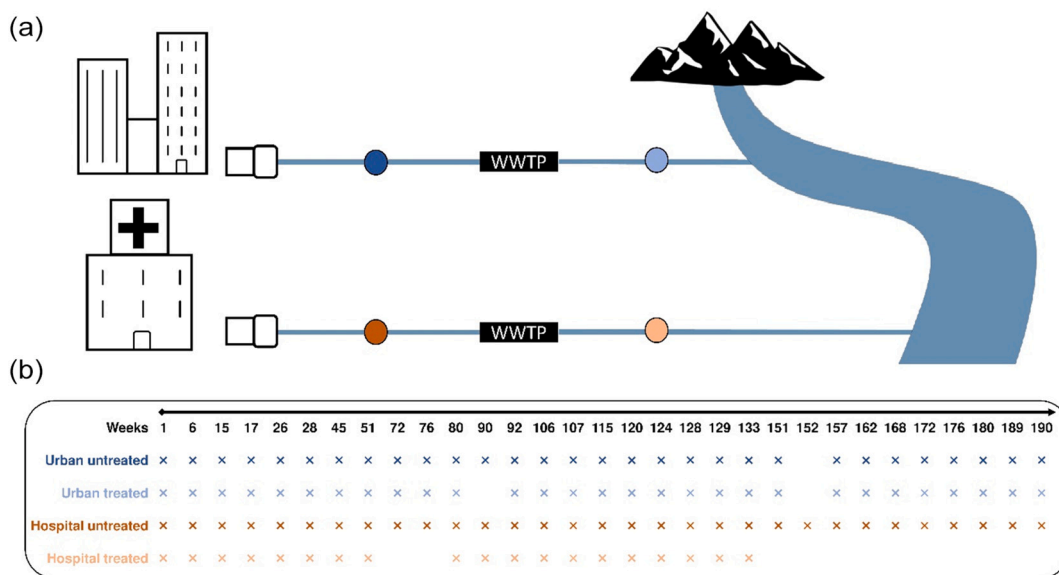


Fig. 1. (a) Location of sampling sites (colored circles): the data used were collected between March 2012 and November 2015. This figure is adapted from [Buelow et al. \(2020\)](#). (b) Site-dependent data (88 individual genes and mobile genetic elements, most of them conferring resistance to antibiotics, and some conferring resistance to heavy metals or ammonium compounds) available weekly throughout the sampling period; any missing point corresponds to no available data for that week. WWTP: Wastewater treatment plant.

value (i.e., minimum inhibitory concentration (MIC)) associated with that same agent. When MIC_{cpd} is low compared to $Conc_{cpd}(t)$, resistance inhibition is high.

Because very little is known about the effect of these different compounds on resistance, we assumed that both processes were possible for all types of agents: antibiotics, heavy metals and/or surfactants. In the model, including all mechanisms, henceforth called “full”, $\beta(t)$, including all the possible compounds and their effects in one direction or the other, is expressed as follows:

$$\beta(t) = \prod_{hm=1}^{N_{hm}} \left(\frac{MIC_{hm}}{HM_{hm}(t) + MIC_{hm}} \right) \times \prod_{a=1}^{N_a} \left(\frac{MIC_a}{A_a(t) + MIC_a} \right) \times \prod_{s=1}^{N_s} \left(\frac{MIC_s}{S_s(t) + MIC_s} \right) \times \prod_{hm=1}^{N_{hm}} \left(\frac{HM_{hm}(t) + F_{hm}}{F_{hm}} \right) \times \prod_{a=1}^{N_a} \left(\frac{A_a(t) + F_a}{F_a} \right) \times \prod_{s=1}^{N_s} \left(\frac{S_s(t) + F_s}{F_s} \right) \quad (4)$$

where resistance-reduction constants are MIC_{hm} (MIC associated with heavy metal hm), MIC_a (MIC associated with antibiotic a), and MIC_s (MIC associated with surfactants s); selection constants are: F_{hm} (selection-facilitation constant associated with metal hm), F_a (selection-facilitation constant associated with antibiotic a), and F_s (selection-facilitation constant associated with surfactant s); $A_a(t)$, $HM_{hm}(t)$ and $S_s(t)$ correspond, respectively, to model input variables: antibiotic a concentration, heavy metal hm exposure concentration and surfactant s concentration in the studied environment during week t .

Because the full model is complex, with 27 covariables, two sub-models, each incorporating only one of the two mechanisms, were defined, and assessed.

Sub-model 1 (i.e., resistance selection), which includes only the amplification effect for all compounds, is written as follows:

$$K(t) = K_{opt} \times \prod_{hm=1}^{N_{hm}} \left(\frac{HM_{hm}(t) + F_{hm}}{F_{hm}} \right) \times \prod_{a=1}^{N_a} \left(\frac{A_a(t) + F_a}{F_a} \right) \times \prod_{s=1}^{N_s} \left(\frac{S_s(t) + F_s}{F_s} \right) \quad (5)$$

Sub-model 2 (i.e., resistance reduction), which includes only the reductive effect for all compounds, is written as follows:

$$K(t) = K_{opt} \times \prod_{hm=1}^{N_{hm}} \left(\frac{MIC_{hm}}{HM_{hm}(t) + MIC_{hm}} \right) \times \prod_{a=1}^{N_a} \left(\frac{MIC_a}{A_a(t) + MIC_a} \right) \times \prod_{s=1}^{N_s} \left(\frac{MIC_s}{S_s(t) + MIC_s} \right) \quad (6)$$

2.3. Statistical analyses

The model was used to analyze the longitudinal data collected in the four sampling sites. Compound—antibiotic, heavy metal, and surfactant—quantities, listed in Table S1, were considered model inputs. The quantity of resistant bacteria in the environment at each time, was represented by normalized abundance of genes and MGEs that we considered proportional to the number of resistant bacteria in the environment.

The general model and two sub-models were independently fitted to the gene- and MGE-abundance data using a Monte-Carlo Markov Chain approach with an adaptive Gibbs sampler. The analysis was run with JAGS software using the R (ver. 4.0.0) interface, “rjags”. For each estimation, 300,000 iterations were obtained (including 150,000 iterations for the adaptive phase). Chain convergence was assessed through calculation of the potential scale-reduction factor (PSRF; Brooks and Gelman, 1998). The PSRF, noted \hat{R} , estimates the potential decrease of between-chain variability with respect to the within-chain variability. Convergence was admitted when \hat{R} was ≤ 1.2 . For all parameters, non-informative, uniform prior distributions were assumed (see details in

Table A1), because of lacking a priori information on possible parameter values.

In the first step, each of the three models was independently fitted to each of the four site datasets for each gene and MGE. All the model parameters were then estimated for each gene and MGE and each site separately, i.e., 1056 (88 genes \times 4 locations \times 3 models) independent analyses.

2.3.1. Individual fits

When fitting the model to a specific gene's dynamics at a given site, it was assessed based on a Gaussian likelihood. Total parameter numbers were 27 for the full model, 14 for sub-models 1 and 2. The likelihood can be written as:

$$L(\theta, d) = \prod_{t=1}^T \frac{1}{\sigma \times 2\sqrt{\pi}} \times e^{-\frac{(d(t)-K(t))^2}{2\sigma^2}} \quad (7)$$

where θ corresponds to the set of parameters, $d(t)$ is the resistant-bacteria concentration observed at time t , $K(t)$ is the simulated carrying capacity at time t (output of the model) and σ is the standard deviation, unknown and also estimated.

2.3.2. Model comparisons

For each individual gene, the three models of varying complexity were fitted individually to each site. Models were compared based on a quantitative criterion, the deviance information criterion (DIC), which was calculated for each model. The DIC enables discriminating the best model by favoring models with good likelihoods, while penalizing the most complex ones (i.e., with more parameters). The DIC is calculated as follows:

$$DIC = \overline{D(\theta)} + p_D \quad (8)$$

where $D(\theta) = -2 \times \log(p(y|\theta))$ is the deviance and p_D the effective number of parameters.

The model with the lowest DIC is preferred. When two DIC values were close (i.e., separated by <5 points), the model with the lower number of parameters was preferred.

2.3.3. Model gene selection

The model fitting was applied to each of the 88 genes and MGEs coding for resistance to the different types of compounds (heavy metals, antibiotics, surfactants) considered.

For all 1056 individual fits, the following procedure was used to identify the genes with relevant results and discarded all others. For each individual gene fit, the result was considered significant when at least two parameters (the optimal carrying capacity, K_{opt} , plus at least one additional compound-associated constant) were identified. Estimation procedure for each estimated parameter was based on a prior-posterior distribution overlap assessment (Gimenez et al., 2009), a method in which we compute a similarity index between the prior distribution of the MCMC (defining our a priori knowledge of parameter distribution - set to non-informative uniform distributions as detailed in Table A1) and the MCMC resulting posterior distribution (the updated parameter distribution obtained from MCMC through the analysis of data). We considered enough information was available in the data to identify a parameter and estimate its value when the distributions overlap was $<50\%$.

In addition, for a given gene, if $>30\%$ of data points were below the limit of quantification (i.e., equal to 0) the corresponding model was excluded.

2.3.4. Assessment of compounds effect

Because a direct comparison of compounds effect across genes within sites is made complicated by differences in chemicals concentration levels, we proposed an indicator measuring the compound's maximum effect on resistance gene abundance over the study period. This

indicator allows the effect of different compounds on genes abundance to be compared across genes. For each compound found to have a significant positive effect on resistant-bacteria persistence (i.e., leading to resistance selection), we defined the maximum effect of positive interaction from the 95th percentile value of the compound's concentration distribution over time, $Cpd_{95\%}$, and the estimated value of the positive effect, F_{cpd} .

$$\beta_{+,cpd,95\%} = \frac{Conc_{cpd,95\%} + F_{cpd}}{F_{cpd}} = \frac{Conc_{cpd,95\%}}{F_{cpd}} + 1 \tag{9}$$

The values of $\beta_{+,cpd,95\%} \in [1, +\infty[$. A value close to 1 indicates low positive effect of a given compound on a given gene abundance, while a value far from 1 indicates high positive effect.

Similarly, if a variable was found to have a negative effect on resistant-bacteria persistence (i.e., resistance reduction) the maximum negative-interaction rate was calculated as:

$$\beta_{-,cpd,95\%} = \frac{MIC_{cpd}}{Conc_{cpd,95\%} + MIC_{cpd}} \tag{10}$$

where MIC_{cpd} is the value of the negative effect of a given compound, and $Cpd_{95\%}$ is the 95th percentile of the pollutant-concentration distribution over time. The values of $\beta_{-,cpd,95\%} \in [0, 1]$. A value close to 0 indicates high negative effect, while a value close to 1 indicates low negative effect.

3. Results

3.1. Model selection

The model was used to analyze the observed dynamics for each gene and MGE at each site independently. Resulting DICs for each fit and

model comparison are summarized in Table S2 that includes only those genes for which a relevant association was found: only 17 site-specific genes, among a total of 352 analyzed (88 unique genes \times 4 sites). Sub-model 1 was selected for 11 of the 17 site-specific genes. The full model was retained for only 5 of the 17 genes. Notably, sub-model 2, which assumes a negative effect of compounds on resistance (i.e., resistance-reduction) was only retained for *tetQ* gene in untreated urban wastewater

Estimated compound effects are provided in Table 1. Among all variables entered into our model, mercury was significantly associated with 10 out of 17 genes and MGEs; suggesting a selection-facilitation effect on the bacterial-resistance level, mostly in Hospital treated water; vancomycin was the most frequently relevant antibiotic, selected for 13 out of 17 genes and MGEs; and anionic surfactants were the most impacting among surfactants (significant for 3 out of 17 genes). Our modeling results suggested that mercury and vancomycin acted as resistance-selection facilitators, while anionic surfactant reduced resistance selection.

Illustration of predicted abundance of the selected genes for the *bacA1* gene in hospital treated wastewater, the *aac(3)-II* gene in urban untreated wastewater and the *aadA* gene in hospital untreated wastewater is depicted in Fig. 3. Predicted fits obtained for each of the other site-specific genes are available in SI (Figs. A1-A3). Absolute effects of mercury, vancomycin and anionic surfactants for the different genes and sites are summarized in Fig. 4 through the distributions of their maximum effects on the abundance of resistance genes.

3.2. Mercury

For the *bacA1*-gene, the estimated median maximum effect for mercury was 8.7 (Fig. 4A), meaning that when mercury reaches its 95 %

Table 1
Estimated effects of compound-associated parameters for each selected gene with prior-posterior overlap <50 % (colored rectangles).

Gene	H or U site	Resistance	Antibiotic			Heavy metal							Surfactant					
			CIP	SXT	VAN	Cd	Cr	Cu	Pb	Hg	Ni	Zn	Anionic	Cationic	Norionic			
<i>aac(3)I</i>	U untreated	Aminoglycosides		Red														
<i>bacA 1</i>	U untreated	Bacitracin					Blue			Red								Blue
<i>tetQ</i>	U untreated	Tetracycline																Blue
<i>aadA</i>	H untreated	Aminoglycosides			Red													
<i>aph(3)Ja,-Ic</i>	H untreated	Aminoglycosides			Red					Red								
<i>blaTEM</i>	H untreated	β -lactams			Red		Blue											
<i>vanB</i>	H untreated	Vancomycin	Red		Red													
<i>int12</i>	H untreated	Multiple	Red		Red													
<i>aadB-like gene</i>	H treated	Aminoglycosides			Red					Red								
<i>aph(3)II</i>	H treated	Aminoglycosides			Red					Red								
<i>bacA 1</i>	H treated	Bacitracin			Red					Red								
<i>cbIA</i>	H treated	β -lactams			Red					Red								
<i>dfrF</i>	H treated	Trimethoprim			Red					Red								
<i>mfsA</i>	H treated	Multiple			Red					Red								
<i>tetO</i>	H treated	Tetracycline			Red					Red								
<i>tetQ</i>	H treated	Tetracycline			Red					Red								
<i>tetW</i>	H treated	Tetracycline			Red					Red								

Blue, resistance-elimination effect; red, resistance-selection effect. If overlap exceeded 50 %, the parameter was considered to be weakly identifiable regarding the available data (grey rectangles). H, hospital; U, urban, CIP, ciprofloxacin; SXT, sulfamethoxazole; VAN, vancomycin; Cd, cadmium; Cr, chromium; Cu copper; Pb, lead, Hg, mercury, Ni, nickel; Zn, zinc.

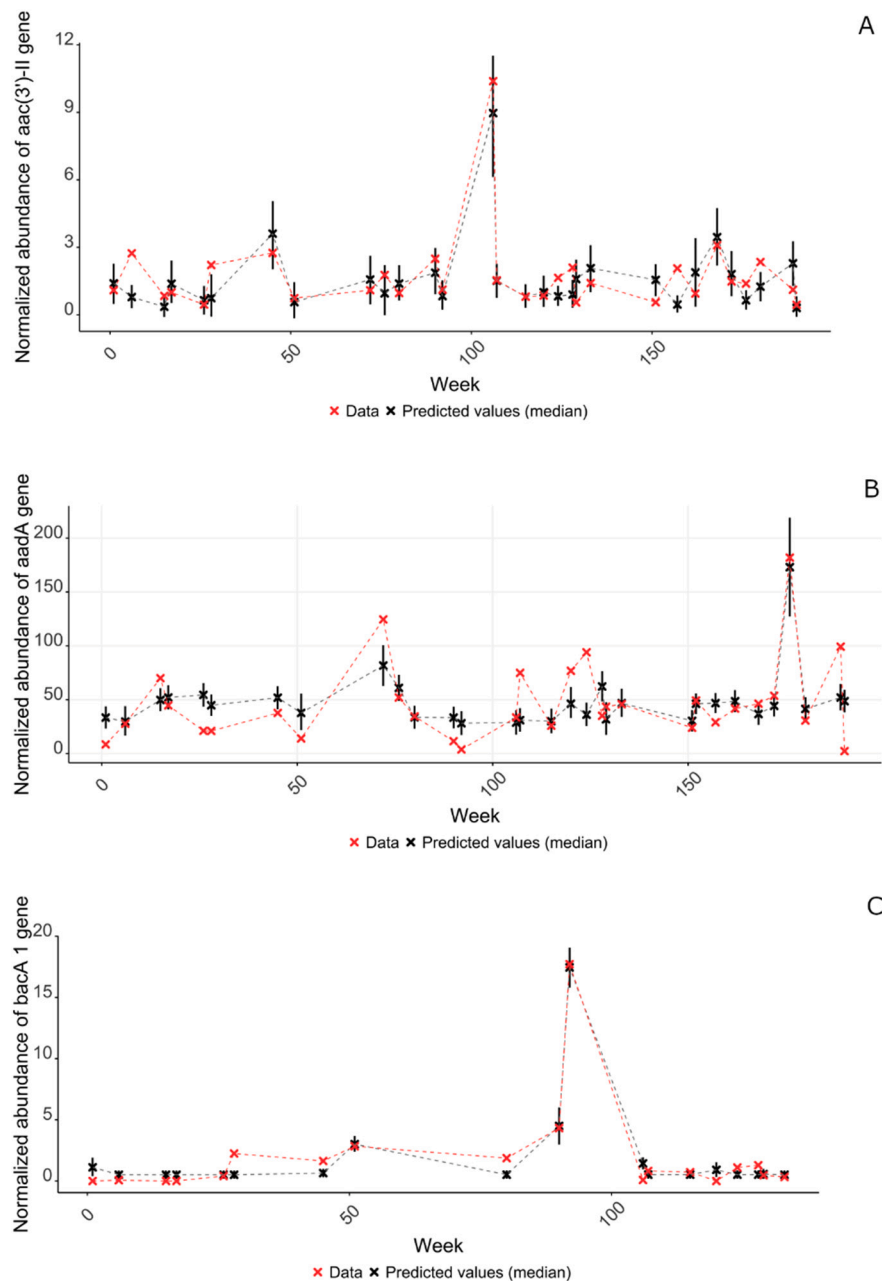


Fig. 3. Illustration of model fits. Predicted fits for the (A) *aac(3)-II* gene in urban untreated wastewater, (B) *aadA* gene in hospital untreated wastewater and (C) *bacA1* gene in the hospital treated wastewater using best selected models (full model for fit A, and sub-model 1 for fits B and C). Medians of predictions are given by black squares and supported by 95 % prediction intervals. Red dots represent collected data on each available week.

highest measured concentration of 28.2 ng/L, the population size of bacteria harboring the *bacA1*-gene was effectively increased by a factor of 8.7 (in median). For the other selected genes, the median maximum effect varied widely, from 6.9 (*aph(3)-III* gene) to 20.1 (*tetW* gene) in treated hospital water. Some effect was also detected for *bacA1* gene (median 4.6) in untreated urban wastewater.

3.3. Vancomycin

This antibiotic had a meaningful effect on the dynamics of *blaTEM*, a β -lactam resistance gene, based on data collected in untreated hospital wastewater (Fig. 4B). An estimated vancomycin concentration of 2.4 ng/L generates a 4.5-fold increase of the carrying capacity of bacteria harboring *blaTEM*. The median maximum vancomycin effect ranged from 2.5 (*aadA* gene) to 4.5 (*blaTEM* gene) in untreated hospital water,

where relevant effects were also detected for the following genes and MGEs: *aph(3)-Ia -Ic*, *vanB* and *IntI2*. In the hospital treated wastewater relevant effects of vancomycin was detected for *bacA1*, *tetQ*, *aph(3)-III*, *tetO*, *dfrF*, *mfsA* genes, and an *aadE*-like gene, with median maximum effects ranging from 2.2 to 6.2.

3.4. Anionic surfactants

Finally, anionic surfactants inhibited the dynamics of three genes in untreated urban wastewater (Fig. 4C). At a concentration of 3855 ng/L, an 80 % reduction (median 0.2) of the carrying capacity was observed for bacteria harboring the *aac(3)-II(acde)* gene. For all selected genes, the median estimated maximum effect ranged from 0.2 (*aac(3)-II(acde)* gene) to 0.4 (*tetQ* gene).

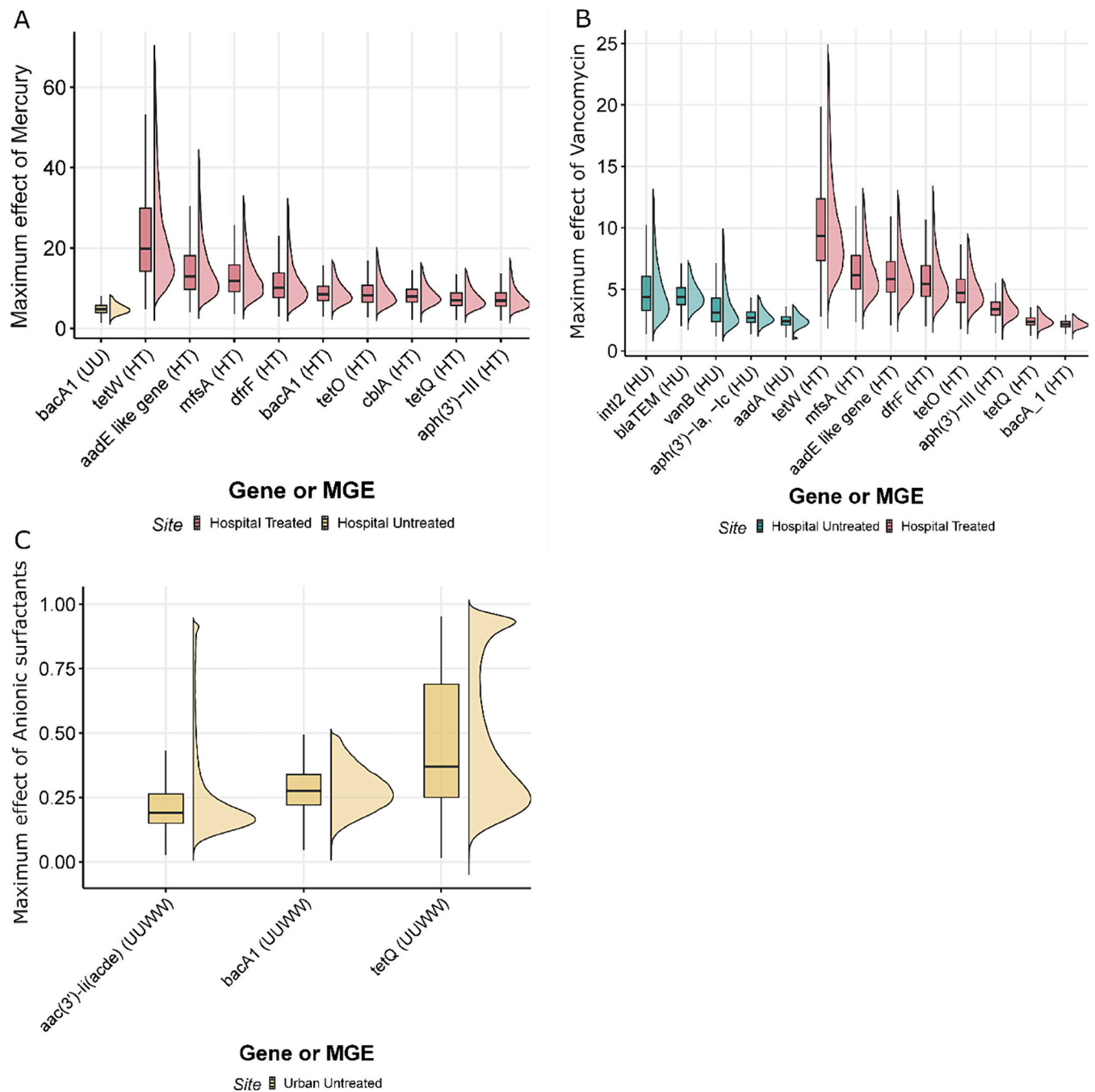


Fig. 4. Distributions of the maximum effects of selected compounds on genes abundance as a reflection of the estimated strength associated with a given agent for all genes and sites: (A) mercury, (B) vancomycin and (C) anionic surfactant. The box plots report the median (internal horizontal line), 25th and 75th percentiles (lower and upper limits) in addition to the distribution of the estimated effects.

4. Discussion

4.1. Main findings

We propose here a modeling framework that formalizes the relationships between the persistence of bacterial antibiotic-resistance genes in wastewater and the detection of specific compounds inherent in human activities. Using longitudinal data collected within a large temporal analytical study and bringing together determinations of antibiotic-resistance gene presence in different types of wastewaters, and characterizations of environmental exposomes, we estimated the strengths of selective or reductive effects of various compounds on the 88 genes and MGEs evaluated.

First, our results demonstrate that a relatively simple mathematical representation is able to identify compound-interaction signals for 17 site-specific genes and MGEs out of 88 assessed at four sites and 13 chemical agents. Hypothesizing that chemicals present in wastewater could be key drivers of resistance in bacterial population dynamics in such aquatic systems, we were able to identify, from our modeling results, an association between some players of the exposome and the evolutions of resistance-gene abundance.

We found that mercury and vancomycin had a positive effect on the abundance of several genes and MGEs (Table 1). The identification of such drivers can support the future possible design of interventions aimed at reducing co-selecting molecules concentrations in aquatic environments to limit resistance gene selection.

4.2. Heavy metals

According to our models, mercury was positively associated with the increase in abundance of several antibiotic resistance genes, for example for several genes encoding resistance to tetracycline (tetW, tetQ and tetO in treated hospital wastewater). This finding is consistent with previous studies reporting effects of mercury on antibiotic resistance selection (Skurnik et al., 2010). Lloyd et al. (2018) showed that mercury-exposed fish microbiomes in the environment were more likely to contain antibiotic multidrug resistant bacteria compared to unexposed controls. Resistance associated with mercury is not a new phenomenon, as demonstrated by Wardwell et al. (2009). Their study showed that indigenous bacterial strains exposed to mercury in 2000-year-old sphagnum core samples exhibited a multidrug resistance phenotype.

Our modeling analysis did not show a significant effect of mercury on the normalized abundance of the merA gene, a mercury resistance gene. However, the hypothesis of co-selection of antibiotic resistance genes by mercury remains likely if other mercury resistance genes, not investigated here, were located on the same plasmids as other genes encoding antibiotic resistance genes. Cross-selection, such as selection for efflux pumps that can export multiple substrates, could also be a possible mechanism of selection for AMR by mercury.

Interestingly, chromium and cadmium, antagonistically affected the *bacA1*-(in untreated urban wastewater) and *blaTEM*-gene (in untreated hospital wastewater) dynamics respectively.

These findings are consistent with previous research highlighting the selective effect of heavy metals on resistance. Seiler and Berendonk (2012) conducted a risk assessment and found that mercury, zinc, copper and cadmium might be significant factors influencing co-selection for antibiotic resistance. Another modeling study by Hellweger et al. (2011) suggested that metals could co-select for tetracycline resistance. In particular, their study identified signals for zinc and copper, which were not confirmed herein.

4.3. Antibiotics

Based on our models, of the three antibiotic concentrations monitored in this study, vancomycin was identified as having the most significant influence on antibiotic resistance. In particular, vancomycin interacted with the temporal dynamics of 14 different site-specific genetic elements. These elements were not exclusively associated with vancomycin resistance but were observed in both untreated and treated hospital wastewater. These findings are consistent with previous studies demonstrating robust selection for vancomycin resistance with prolonged exposure to vancomycin (Cetinkaya et al., 2000; Hiramatsu, 2001). Furthermore, it is plausible that this antibiotic acts as a co-selector for other resistance genes, given the potential inclusion of some vancomycin resistance genes within large, transferable genetic elements (Poyart et al., 1997; Ahmed and Baptiste, 2018; Bender et al., 2016).

Our modeling unexpectedly showed that vancomycin acts as a facilitator for non-vancomycin-resistance genes, likely from simultaneous use of other, undetected antibiotics in hospitals. This suggests vancomycin serves as a proxy for overall antibiotic contamination in the analyzed hospital wastewater.

4.4. Surfactants

The model results revealed an intriguing association between surfactants and a reduction in resistance for certain genes. Specifically, anionic surfactants were found to decrease the abundance of specific resistance genes, such as *bacA1* in untreated urban wastewater, while non-ionic surfactants had a restrictive effect on *tetQ* in untreated urban wastewater. These genes are primarily carried by Gram-negative bacteria, like *tetQ* in *Bacteroides fragilis* (Jasemi et al., 2021) and *acc3* in *Acinetobacter baumannii* (Nie et al., 2014). This suggests that surfactants

may induce cell death in these Gram-negative bacteria, reflecting in the observed reduction of resistance genes.

4.5. Concurrent compounds effect

We observed simultaneous effects of several compounds, including vancomycin and mercury, in treated hospital wastewater. This suggests that two different mechanisms are likely to work together to influence or alter the persistence of resistance genes over time. First, as mentioned above, our modeling results indicate that vancomycin is associated with an increase in the abundance of tetracycline resistance genes, suggesting that vancomycin may co-select for resistance to other antibiotics such as tetracycline. Second, for the same genes, mercury is shown to facilitate resistance, possibly by promoting the transfer of mobile genetic elements carrying mercury resistance genes in addition to tetracycline resistance genes. The same effects were observed for other resistance genes such as *bacA1* (conferring resistance to bacitracin) and *cblA* (conferring resistance to β -lactams). The results suggest that these two hypothetical resistance pathways are likely to contribute to gene persistence in water.

4.6. Limitations

Several modeling assumptions and limitations of our study deserve discussion. First, the model developed here is deliberately simple, tailored to the available data, and thus has inherent limitations due to its simplicity. Bacterial abundance was considered at equilibrium and growth rate wasn't included in our model for simplicity. In fact, we did not have samples representing bacterial growth, but only information on the total copy number of 16S rRNA genes, representing bacterial biomass. Nevertheless, the copy numbers were stable, suggesting stable bacterial populations over time and per sampling site. Bacterial growth, assumed to be negligible over large temporal scales, could be refined in the future by considering transient variations in population size and their association with the eco-exposome, but this would require more detailed data.

Secondly, despite the assessment of integrase encoding and transposase genes associated with gene transfer and antibiotic resistance, the lack of detailed bacterial information (genus and species) hinders the consideration of specific horizontal gene transfer mechanisms such as conjugation or transposition.

Additionally, we analyzed genes independently, assuming each gene represents a distinct carrying organism. Multiple genes might be carried by the same organism, potentially explaining why the same compounds often influenced the persistence of different genes. This assumption was necessary due to the absence of detailed bacterial identification and quantification in our dataset.

Furthermore, for simplicity, our analysis considered only a limited number of pollutants and omitted variables such as temperature, water flow or local epidemiology. Future studies should include more variables to avoid potential confounding effects.

Finally, in model fitting, we used a rather conservative 50 % threshold to select significant effects using the prior-posterior overlap method, which was somewhat arbitrary. As a sensitivity analysis, a 35 % threshold was also evaluated (as proposed in Gimenez et al., 2009), which yielded consistent results.

Our results highlight the site-specific dependence of water composition, with compounds and taxa showing strong specificity. No genetic element emerged as a universal indicator of antibiotic resistance in the anthropised effluents analyzed, even when hospital and urban effluents were considered separately.

5. Conclusion

In this study, we present a novel mechanistic modeling framework to improve our understanding of the role of different compounds in the

selection of antibiotic resistance in bacteria in aquatic environments. Our results indicate co-selection for antibiotic resistance driven by the eco-exposome, particularly for mercury and vancomycin. In the future, the effect of water indices such as temperature or water flow could be incorporated into our model to better understand the dynamics of resistance in wastewater.

CRedit authorship contribution statement

Paul Henriot: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Elena Buelow:** Methodology, Validation, Writing – review & editing. **Fabienne Petit:** Writing – review & editing. **Marie-Cécile Ploy:** Writing – review & editing. **Christophe Dagot:** Data curation, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. **Lulla Opatowski:** Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank the SIPIBEL consortium for displaying data.

Funding

This study was funded by Agence Nationale Sécurité Sanitaire Alimentaire Nationale (Pandore Project, EST 2017/3 ABR/28). It was also supported directly by internal resources from the French National Institute for Health and Medical Research, the Institut Pasteur, and the University of Versailles–Saint-Quentin-en-Yvelines/University of Paris-Saclay. This study received funding from the French Government's 'Investissement d'Avenir' program, Laboratoire d'Excellence 'Integrative Biology of Emerging Infectious Diseases' (Grant ANR-10-LABX-62-IBRID).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.171643>.

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