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Comparative selective pressure potential of antibiotics in the environment☆

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ABSTRACT

To guide both environmental and public health policy, it is important to assess the degree of antibiotic resistance selection pressure under measured environmental concentrations (MECs), and to compare the efficacy of different mitigation strategies to minimize the spread of resistance. To this end, the resistance selection and enrichment potential due to antibiotic emissions into the environment must be analysed from a life cycle perspective, for a wide range of antibiotics, and considering variations in the underlying fitness costs between different resistance mutations and genes. The aim of this study is to consistently derive fitness cost-dependent minimum selective concentrations (MSCs) from readily available bacterial inhibition data and to build MSC-based species sensitivity distributions (SSDs). These are then used to determine antibiotic-specific resistance selection concentrations predicted to promote resistance in 5% of exposed bacterial species (RSC_5). Using a previously developed competition model, we provide estimated MSC_{10} endpoints for 2,984 antibiotic and bacterial species combinations; the largest set of modelled MSCs available to date. Based on constructed SSDs, we derive RSC_5 for 128 antibiotics with four orders of magnitude difference in their 'selective pressure potential' in the environment. By comparing our RSC_5 to MECs, we highlight specific environmental compartments (e.g. hospital and wastewater effluents, lakes and rivers), as well as several antibiotics (e.g. ciprofloxacin, norfloxacin, enrofloxacin, and tetracycline), to be scrutinized for their potential role in resistance selection and dissemination. In addition to enabling comparative risk screening of the selective pressure potential of multiple antibiotics, our SSD-derived RSC_5 provide the point of departure for calculating new life cycle-based characterization factors for antibiotics to compare mitigation strategies, thereby contributing towards a 'One-Health' approach to tackling the global antibiotic resistance crisis.

1. Introduction

Antibiotic-resistant bacterial infections have exceeded both malaria and HIV/Aids as a leading cause of death worldwide, directly responsible for 1.27 million deaths in 2019 (Murray et al., 2022). Bacteria develop resistance through spontaneous mutations, or acquire antibiotic resistance genes (ARGs) via horizontal gene transfer (Aminov, 2011; Davies, 1996). A large body of experimental and epidemiological evidence finds the acquisition of resistance to be associated with a small, yet significant, *fitness cost* (Bengtsson-Palme et al., 2021; Hughes & Andersson, 2017; Luangtongkum et al., 2012; Melnyk et al., 2015; Millan et al., 2015; Smani et al., 2012), expressed in terms of e.g., reduced reproductive ability relative to a susceptible ancestor (Martinez & Baquero, 2000). This cost of resistance and the strength of *selection pressure* – partly in function of antibiotic exposure – are believed to be the most important parameters shaping the evolution, persistence and dissemination of ARGs, both in host populations as well as natural environments (Bengtsson-Palme et al., 2021; Hughes & Andersson, 2017).

Antibiotic pollution in aquatic and terrestrial environments has repeatedly been shown to exert sufficient selection pressure to enrich resistance determinants (Bengtsson-Palme & Larsson, 2016; Darlica, 2003; Gullberg et al., 2011, 2014; Liu et al., 2011; Tello et al., 2012).

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Table 1
Default values used in the estimation of the ratio of minimum selective concentration (MSC) to minimum inhibitory concentration (MIC) for the sensitive population based on the competition model developed by Greenfield et al. (2018) (see **Eq. 3**).

Parameter	Description	Default value (95% CI)
$N_{int,r}/-N_{min}$	Ratio of the intrinsic net growth rate of the resistant/sensitive strain, in the absence of the antibiotic, to the minimum possible growth rate after accounting for the growth-limiting activity of the antibiotic	0.38 (0.01–10.2)
$N_{int,s}/-N_{min}$		0.4 (0.01–10.6)
sc	Relative selection coefficient	0.04 (0.008–0.2)
κ	Hill-coefficient of the antibiotic dose-response curve in the sensitive strain	1.8 (0.5–6.6)

Recognizing the environment as the single largest reservoir of mobilizable ARGs (Finley et al., 2013; Gaze et al., 2013) thus places it at the heart of tackling clinical drug resistance and associated human health morbidity and mortality (Surette & Wright, 2017). To guide both environmental and public health policy it is therefore important to assess the degree of selection pressure under current antibiotic pollution, and to develop and compare the efficacy of different mitigation strategies targeted at taming the risk of resistance selection, enrichment and transmission from the environment to humans (Finley et al., 2013; Gaze et al., 2013; Le Page et al., 2017).

Life cycle assessment (LCA) is an increasingly used standardized methodology that allows quantifying the potential environmental impacts of product life cycles, as well as comparing between e.g., product alternatives and intervention strategies. Nyberg et al. (2021) recently developed a characterization model to express the potential resistance enrichment in the environment due to life cycle emissions of antibiotics. The authors derive *minimum selective concentrations* (MSCs), defined as the lowest concentration of antibiotic at which a resistant strain begins having a survival advantage over its sensitive ancestor, from minimum inhibitory concentrations (MICs) using a fixed assessment factor of 10, representing approximately the median MIC/MSC ratio reported by Gullberg et al. (2011). Based on constructed *species sensitivity distributions* (SSDs), Nyberg et al. (2021) then derive the antibiotic concentration that will promote resistance in 50% of exposed bacteria, the hazardous concentration or HC_{50}. Combining their HC_{50} estimates with the fate of antibiotics emitted into water compartments, and subsequent exposure of the bacterial community, the authors determine 'antibiotic resistance enrichment potentials' or so-called 'characterization factors' (CFs) for 14 antibiotics. The authors' approach to extrapolate MSCs from MICs is commonly used to predict MSCs (Bengtsson-Palme & Larsson, 2016; Rico et al., 2017) and was shown for specific antibiotics to lie close to empirically determined MSCs (Lundström et al., 2016; Stanton et al., 2020). Nevertheless, this generic assessment factor fails to account for mutation/ARG-specific differences in underlying fitness costs and in relative fitness levels of resistant vs. sensitive strains, which can significantly affect MSC-to-MIC ratios (Greenfield et al., 2018; Gullberg et al., 2011; Liu et al., 2011). Furthermore, Nyberg et al. (2021) rely on the concentration below the lowest MIC of an antibiotic-species combination (resembling a 'no-observed effect concentration', NOEC) to extrapolate towards MSCs, which is often argued to be less suitable for comparative assessments of ecological effects in the context of e.g., comparative risk screening or life cycle impact assessment (LCIA) (Fantke et al., 2018; Owsianiak et al., 2023). This is because (1) NOECs are likely more affected by the tested concentrations than the shape of the curve, and (2) they are 'no-effect' oriented, which is more applicable in deriving protective thresholds than species sensitivities that can later be linked to damage at the endpoint level (Kosnik et al., 2022; Carney Almroth et al., 2022; Oginah et al., 2023).

To address these research gaps, the present study aims to enable a more consistent and comprehensive assessment of potential resistance selection in the environment, allowing comparison between the selective pressure potential of different antibiotics, as well as between the contribution of whole product life cycles to resistance selection and enrichment. To achieve this goal, we specifically aim to (1) develop and apply an integrated methodological framework to consistently derive fitness cost-dependent MSCs and construct MSC-based bacterial selection sensitivities from bacterial inhibition data, (2) determine for a variety of antibiotics multi-species, antibiotic-specific resistance selection concentrations that are predicted to promote resistance in 5% of species types in an exposed bacterial community (RSC_5), and (3) quantify the uncertainty around all relevant calculated endpoints (MIC, MSC and RSC) to enable more accurate predictions of potential resistance selection under ambient antibiotic concentrations and related confidence intervals.

Our predicted RSC_5 can firstly be used in combination with measured environmental concentrations (MECs) to estimate the potential of selection under current antibiotic pollution and prioritize environmental compartments with likely high selection pressure (Ashbolt et al., 2013; Lundström et al., 2016). More importantly, they can be used in the context of LCIA as points of departure to define new CFs for antibiotics reflective of their selective pressure potential in the environment. This will enable a variety of stakeholders (e.g., pharmaceutical companies, farmers, policymakers) to assess the resistance selection potential associated with their activities, as well as compare the efficacy of life cycle-based solutions to the resistance problem (Fantke & Illner, 2019; Persson et al., 2022). The developed RSC_5 can thus contribute towards a 'One-Health' approach to tackling the global antibiotic resistance crisis (Larsson and Flach, 2022; Puyvelde et al., 2018).

2. Methods

Fig. 1 summarizes the proposed methodological framework developed in the present study to link bacterial inhibition data (MIC distributions) on a variety of antibiotic-species combinations (called hereinafter 'combinations') to environmental RSC_5, and provides information on the number of antibiotics, species or combinations thereof included at each step of the analysis.

Using a previously developed model of competition between sensitive and resistant bacterial strains, called hereinafter 'competition model', that accounts for the key factors favouring growth of resistant strains at concentrations far below those used in clinical settings (Greenfield et al., 2018), we calculate combination-specific MSC-to-MIC ratios for 2, 984 antibiotic-species combinations present in the database of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2020). To determine the ratio of MIC for resistant isolates (MIC_r) to that for sensitive isolates (MIC_s) of a given combination or the 'fold-increase in MIC' required within the applied competition model, we derived fitted MIC distributions for both sensitive and resistant bacterial isolates of a given species from the EUCAST MIC distribution data. We then determined the corresponding $MIC_{10,r}/MIC_{10,s}$ ratio, representing the 10th percentile of the relation between the resistant strains' inhibition level and that of the sensitive strains across a large number of resistant isolates with different underlying resistance mechanisms against a respective antibiotic. Using the competition model's results, we constructed MSC_{10}-based SSDs for a total of 128 antibiotics and derived antibiotic-specific RSC_5, reflecting their comparative selective pressure potential. .

All methodological decisions and assumptions made at the different stages of the proposed framework (Fig. 1) are discussed in more detail in the appendix, Table A1 (appendix, Section A-1).

For estimated key parameters, we additionally quantified uncertainty and calculated the associated 95% confidence interval (CI) limits. A detailed step-by-step guide of the uncertainty analysis is presented in the appendix, Section A-3.

All analyses were carried out using R, the software environment for statistical computing (R version 4.1.0) (R Core Team, 2021). all

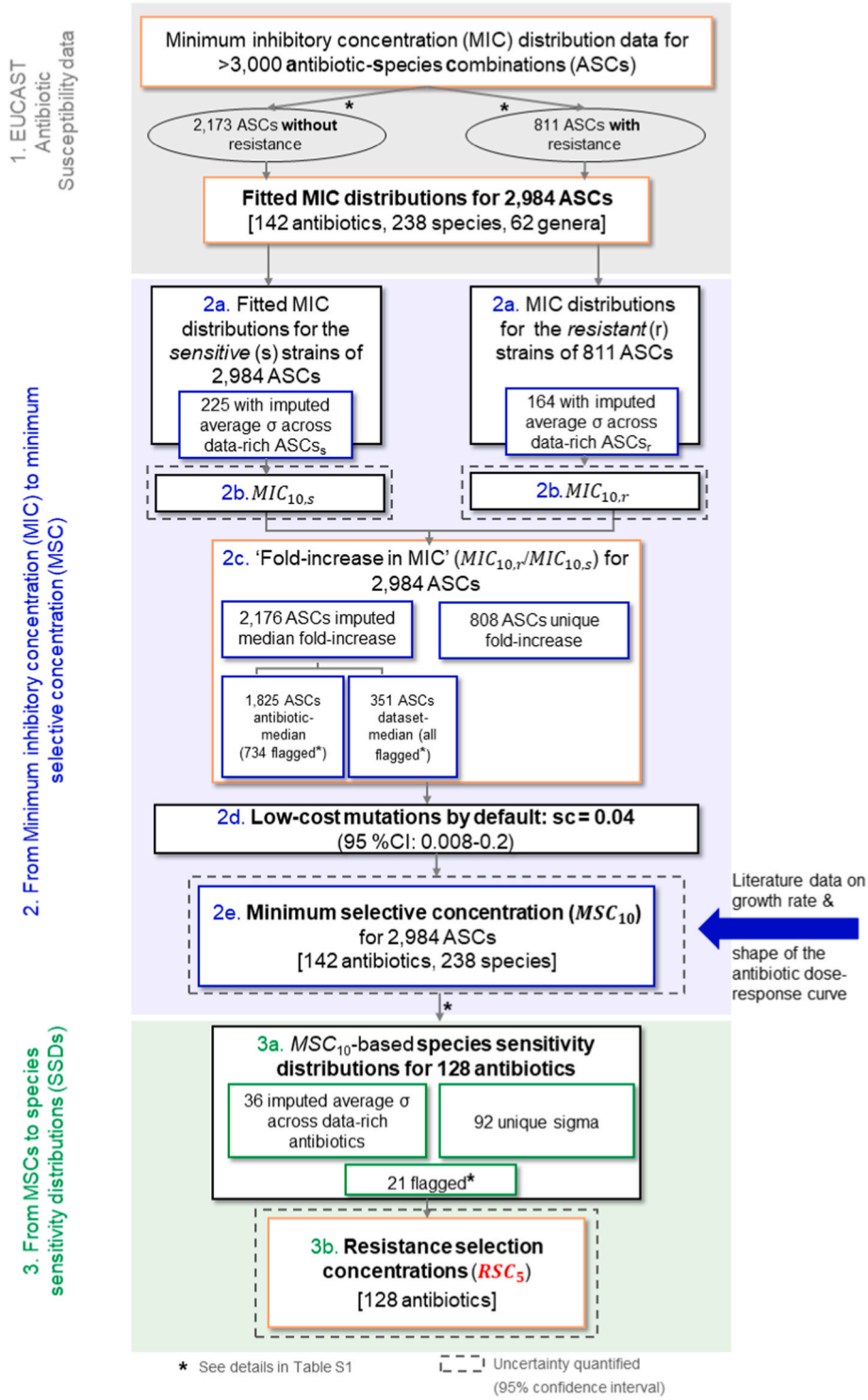

Fig. 1. Proposed methodological framework to link bacterial growth inhibition data (MIC distributions) to antibiotic-species combination-specific minimum selective concentrations (MSCs), and further to antibiotic-specific resistance selection concentrations (RSC_5), at which resistance is promoted in 5% of bacterial species in the environment.

subsequent figures were generated using ggplot2 version 3.3.6 (Wickham, 2016).

2.1. Deriving minimum selective concentrations

Greenfield et al. (2018) provide an analytical solution to the MSC, defining the MSC-to-MIC ratio as:

$$MSC_x \,/\, MIC_{x,s} = \left[\frac{sc}{1 + \frac{N_{int,r}}{-N_{min}} - \frac{(1-sc)\left(1+\frac{N_{int,s}}{-N_{min}}\right)}{\left(\frac{MIC_r}{MIC_s}\right)^{\kappa}}} \right]^{1/\kappa} \quad [1]$$

where $MSC_x/MIC_{x,s}$ is the ratio of the minimum selective concentration for x % of exposed bacteria to the minimum inhibitory concentration in the sensitive (s) ancestor [dimensionless], sc is the relative selection coefficient, describing fitness differences between the sensitive and resistant strain [dimensionless] and obtained by reversing the sign of the reported absolute selection coefficient (σ) and dividing this value by the net growth rate of the sensitive strain, $N_{int,r}$ and $N_{int,s}$ are the intrinsic net growth rates of the resistant and sensitive strain, respectively, in the absence of the antibiotic [per hour], calculated as the difference between the intrinsic growth rate and the intrinsic loss due to mortality (or, in continuous cultures, dilution), N_{min} is the minimum possible growth rate after accounting for the growth-limiting activity of the antibiotic, $\frac{MIC_r}{MIC_s}$ is the ratio of the inhibitory concentration of the resistant mutant to that of its sensitive counterpart (or the fold-increase in MIC), and κ describes the shape ('sigmoidicity') of the antibiotic dose-response curve in the sensitive ancestor.

The presented competition model was developed for conspecific bacteria and aims to enable, where data is available, a mutation-/ARG-specific, fitness cost-dependent estimate of the MSC-to-MIC ratio, while assuming that κ and N_{min} are the same for sensitive and resistant strains (for more details, see the full derivation of the model in Greenfield et al. (2018)). However, since mutation-/ARG-specific data (e.g. on $N_{int,s}$, $N_{int,r}$, sc) are only available for a limited number of combinations, we apply this model to literature-derived distributions of the model's parameters (see details below) in order to reflect possible variations in the combinations present in the EUCAST database, as well as calculate combination-specific distributions of the fold-increase in MIC. Details for deriving the fold-increase in MIC or $MIC_{10,r}/MIC_{10,s}$ are given in the appendix, Section A-2.

With regard to the fitness costs, the distribution of the sc was derived based on a meta-analysis of 77 competition experiments (Vogwill & Maclean, 2015) with a 95% confidence interval between 0.008 and 0.2 and a geometric mean of 0.04, selected as the lower end of the average costs of plasmid-borne mutations (see appendix, Table A1). The choice to model low-cost, (plasmid-borne) mutations by default was informed by (1) the knowledge that the most likely origin of resistance in pathogens today is low-cost resistance present on mobile genetic elements (MGE) in human-associated or environmental bacteria (Bengtsson--Palme et al., 2021), (2) the fact that only MGE-borne resistance can move *between* species and is, thus, more likely to spread and be transmitted to human pathogens, and (3) the repeatedly evidenced negative correlation between measured fitness costs and resistance prevalence in clinical settings (Dunai et al., 2019; Hughes & Andersson, 2017). Low-cost, MGE-borne resistance thus represents a more relevant scenario in terms of resistance dissemination and human health risks.

As for the other model parameters, the ratio of $N_{int,r}$ or $N_{int,s}$ to $-N_{min}$ and κ, we determined their distributions based on literature data from *in vitro* time-kill curve experiments and pharmacokinetic-pharmacodynamic modelling of the growth and killing kinetics of bacteria exposed to antibiotics. The variability analysis of the four parameters in 34 collected studies (list in appendix, Section A-4) enabled us to set their geometric mean and 95% confidence interval (see appendix, Table A1 and Section A-3).

Using the distributions of all model parameters, summarized in Table 1, we predict based on the $MSC_{10}/MIC_{10,s}$ endpoint, lying at the lower end (10th percentile) of the distribution of MSC-to-MIC_s ratios across various mutations/ARGs in a given antibiotic-species combination. Based on the estimated $MSC_{10}/MIC_{10,s}$ ratios, we ultimately derive a distribution of MSC_{10} values, representing the range of antibiotic concentrations that is likely to select for resistance among low-cost ('potent') resistance mutations. By modelling this specific and realistic 'cost scenario', we are able to make early useful predictions of the potential for human health-relevant resistance selection in the environment.

2.2. Deriving SSDs and resistance selection concentrations (RSCs)

Following the same mathematical approach as for the fitted MIC distributions, we derived an SSD for each antibiotic by fitting a log-normal model to its pooled MSC_{10} thresholds. We included all species on which an antibiotic had been tested into its respective SSD (Rico et al., 2017) as opposed to focusing on a pre-selection of pathogenic bacteria with evidence of their growth in the natural environment (see appendix, Table A1). The SSD curve, defined via its fitted distribution parameters μ and σ, expresses the selection sensitivity of exposed bacteria in potentially affected fraction of bacterial species ($PAF_{species}$) at gradient exposure concentrations of an antibiotic. In this context, 'exposed bacteria' describes the diversity of species within the environmental microbiome without including their respective abundance. Based on a minimum of 10 species, we construct the MSC-based SSDs to represent the bacterial community as a whole. How far data on pathogenic bacteria from the EUCAST data can be used for modelling the community's response is discussed in **Section 4.2**. For data-poor antibiotics ($n_{MSC} < 10$), we imputed a default σ equal to the average σ across all 'data-rich' antibiotics (Posthuma et al., 2019) (see appendix, Table A1 and Section A-5).

Based on the fitted SSDs, we finally determined the antibiotic 'resistance selection concentration' (RSC_5), at which 5% of bacterial species are exposed above their MSC, leading to potential positive selection of resistance and enrichment in the environment. We quantified the uncertainty around the RSC_5 estimates, while transparently attributing it to the underlying inter- and intra-species variability. This helps differentiate between the uncertainty in the SSD's shape parameter (σ), as well as in the underlying effect data (here: MSC_{10}) and the resulting location parameter (μ), and allows future reduction and re-evaluation of the uncertainty when data quality is improved (Section A-3, appendix). The choice of RSC_5 as working point on the SSD maintains the balance between predicting a threshold with relatively low uncertainty using a minimum of ten data points per SSD and an effect level signalling potentially dangerous and irreversible resistance selection in the environment.

2.3. Comparing RSCs with measured environmental concentrations (MECs)

To determine whether a potential for resistance selection is currently present in the environment and assess the role of different compartments in fuelling resistance, we compared our RSC_5 estimates with MECs extracted from the German Environment Agency's 'Pharmaceuticals in the Environment database' (PiE) (Beek et al., 2016; UBA, 2021). We only included MECs from the PiE database that were measured in μg/L to allow comparison with our RSC_5 (measured in mg/L). This comprised aquatic samples taken from surface water (river/stream), surface water (lake), surface water (sea or ocean), groundwater, drinking water, wastewater treatment plant (WWTP) effluent, and treated hospital sewage. Comparison to MECs in other matrices (soils and sediments) – while relevant for the environmental dimension of the resistance problem – would require extrapolation of our RSC_5 to corresponding selection concentrations in such matrices. Due to the lack of a coherent & statistically robust method to extrapolate towards corresponding RSC_5 in terrestrial environments (Fantke et al., 2018; Golsteijn et al., 2013; Owsianiak et al., 2023), we have focused on deriving RSC_5 for aquatic

environments. As for manure and sludge, their composition, specific bacterial communities and antibiotic pollution levels make these matrices less representative of natural environments, and our RSC_5 results more difficult to directly extrapolate towards such settings. Other data curation steps to filter for relevant records in the PiE (e.g. filtering out entries 'below detection') are described in the appendix, Section A-6.

3. Results

3.1. Minimum selective concentrations

Maximum-likelihood estimates of the distribution parameters, μ and σ, of the fitted MIC distributions and corresponding MIC_{10} thresholds were determined for 2, 984 sensitive and 811 resistant bacterial isolates in the EUCAST database (see Table A4). Distribution plots for the fitted μ and σ of the MIC distributions are presented in Figure A9. The log-normal distribution model was retained for all raw MIC distributions, after comparing four different distributions ('log-normal', 'normal', 'Weibull' and 'gamma') and finding that for a majority of selected sensitive and resistant strain distributions (71.5% and 69.5%, respectively), the log-normal model was associated with the lowest AIC score (i.e. providing the best fit). A more detailed discussion on the 'goodness-of-fit' test is in the appendix, Section A-8.

The $MIC_{10,r}$/ $MIC_{10,s}$ ratios varied across all antibiotic-species com-

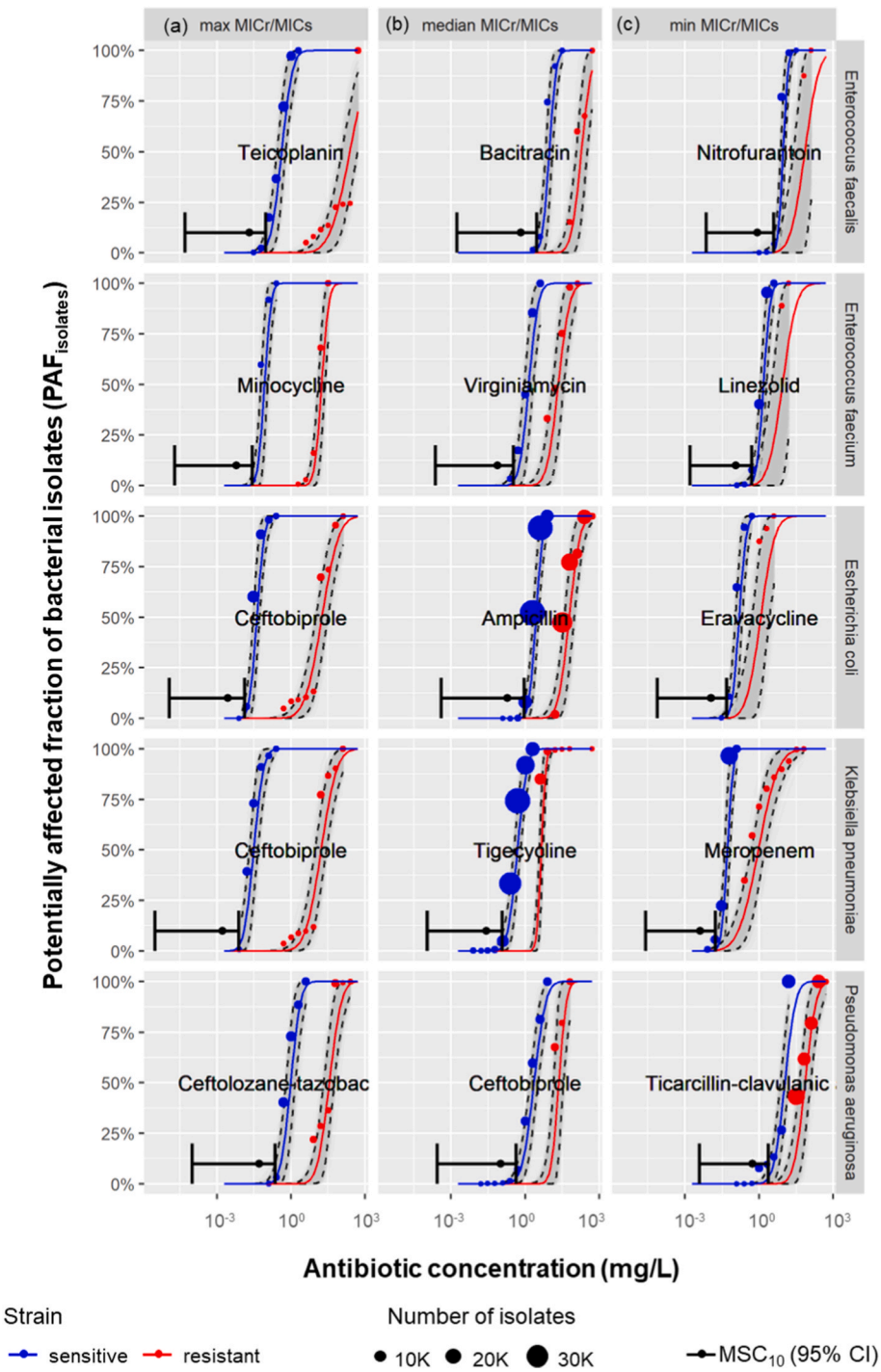

Fig. 2. Original (points) and fitted (curves) minimum inhibitory concentration (MIC) distributions for a selected subset of environmentally- and clinically-relevant bacterial species in the EUCAST database and the antibiotics to which they developed a (a) high, (b) median and (c) low fold-increase in MIC. The size of the points reflects the aggregate number of clinical isolates, whose growth had been inhibited at each concentration. 95% confidence intervals (CI) around the fitted curves have been determined via parametric bootstrapping. The estimated minimum selective concentrations for each antibiotic-species combination, at which 10% of resistant isolates are preferentially selected for (MSC_{10}), along with their 95% CI, are also plotted.

binations between 1.1 and >3800 (see Figure A13). The ten antibiotic-species combinations with the lowest estimated fold-increase in MIC are presented in Table A2, along with eight combinations with an estimated $MIC_{10,r}/\ MIC_{10,s} > 1,000$. Combination-specific $MSC_{10}/MIC_{10,s}$ ratios and, finally, MSC_{10} endpoints (with respective 95% CI) were also determined for a total of 2,984 antibiotic-species combinations. The $MSC_{10}/MIC_{10,s}$ ratios varied between 0.14 - 0.19 across the 2,984 combinations included in our analysis. This means that – assuming low fitness costs by default – MSCs can be one order of magnitude below the MIC in the sensitive wild-type. The central tendency of our results is consistent with the previously suggested assessment factor of 10 (MIC-to-MSC) (Bengtsson-Palme & Larsson, 2016; Nyberg et al., 2021; Rico et al., 2017), while providing a consistent rationale for and quantifying the distribution of this factor. How sensitive the *MSC*-to-MIC_s ratio is to changes in fitness costs or the *sc*, as well as to other model parameters, is presented in more detail in the appendix, Section A-11.

Fig. 2 presents for a selected subset of antibiotic-species combinations the raw MIC distribution data as extracted from the EUCAST website, the fitted MIC distributions, and finally, the derived MSC_{10} endpoints. The five presented bacterial species had been suggested as bacterial indicators for assessing the status of human-relevant antibiotic resistance under environmental settings (Berendonk et al., 2015). We include those antibiotic agents to which these species had developed high (Fig. 2a), median (Fig. 2b) and low (Fig. 2**c**) $MIC_{10,r}/\ MIC_{10,s}$.

The highest fold-increase in MIC among the presented examples ($MIC_{10,r}/\ MIC_{10,s} = 308$) was observed for ceftobiprole-resistant *Klebsiella pneumoniae* with an $MSC/\ MIC_s = 0.14$ and an $MSC_{10} \approx 0.002$ mg/L (95%*CI*: 3×10^{-6} to 0.007 mg/L) vs. a $MIC_s \approx 0.012$ mg/L (95%*CI*: 0.0119 to 0.013 mg/L). In contrast, the lowest fold-increase in MIC of approximately 3, which was found for linezolid-resistant *Enterococcus faecium*, was associated with an $MSC/\ MIC_s = 0.15$ at an $MSC_{10} \approx 0.11$ μg/mL (95% *CI*: 0.002 to 0.5 mg/L) vs. a $MIC_s \approx 0.77$ mg/L (95%*CI*: 0.75 to 0.79 mg/L). Uncertainties around the estimated MSC_{10} level are larger at the lower end, where smaller differences in fitness between the sensitive and resistant strain lead to higher variability in the estimation of the MSC (Murray et al., 2018) (see sensitivity analysis in Section A-11). Data underlying Fig. 2 are available in the appendix (Table A3).

The two described examples from Fig. 2 highlight how, based on the way we build the present framework, a low fold-increase in MIC does not necessarily translate into low predicted MSC_{10}. This model design deliberately decouples the 'degree of resistance' from fitness costs, which is often the case when e.g., compensatory mutations ameliorate costs while maintaining resistance levels (Dunai et al., 2019), or when ARGs are co-selected (Hernando-Amado et al., 2017). Assuming default low costs (*sc*), the MSC_x level in our model is additionally determined predominantly by (a) the shape of the antibiotic-dose response curve in the susceptible strain (κ), and (b) the MIC_s (see appendix, Section A-11). This specifically places the focus on *competition* between the sensitive and resistant strain as a determinant in the trajectory of resistance, as opposed to characteristics of the resistant mutant alone. Thus, high-cost mutations and ARGs may also be selected for and enriched under environmental antibiotic pressure, if for example the respective susceptible strain is already inhibited at very low antibiotic concentrations, giving way to the resistant strain to flourish.

Results of the MIC_{10} thresholds, the fold-increase in MIC and the final MSC_{10} values (and 95% CI) for the 2,984 modelled combinations are provided in Table A5.

3.2. Species sensitivity distributions (SSDs)

We derived maximum-likelihood estimates of the distribution parameters, μ and σ, for the SSDs of 128 antibiotics. Histograms of the distribution parameters across the dataset are available in Figure A16. Fig. 3 illustrates the MSC_{10}-derived SSDs for four selected antibiotics with the highest and lowest σ in the dataset (Fig. 3a and Fig. 3b), as well as the highest and lowest predicted RSC_5 (Fig. 3c and Fig. 3d). The steeper the SSD (e.g. Fig. 3b), the more sensitively exposed bacteria respond to marginal increases in antibiotic exposure concentrations around the RSC_5.

Full results at the SSD-level are reported in Table A6, along with the predicted RSC_5 values for 128 antibiotics and their 95% CI limits.

3.3. Resistance selection concentrations and comparison with measured environmental concentrations

Based on the fitted μ and fitted or imputed σ describing each SSD, we finally derived respective RSC_5 values for the 128 antibiotics included in our analysis. We derived 1,000 RSC_5 values per antibiotic from a log-normal distribution, where the median (50th percentile) estimate of the 5th percentile is consistently below the 'deterministic' RSC_5 (i.e. the point value determined based on the single fit to predicted MSC_{10} per antibiotic) (see Figs. 3 and 4a). This is the result of accounting for the 'intra-species' variability, where the uncertainty and therefore the frequency of sampled MSC_{10} values on the lower end is higher than on the upper end (see Section A-3). Both the deterministic RSC_5, as well as the median RSC_5, are reported in Table A6. For use in LCIA or comparative risk screening, we recommend use of the median RSC_5, as it better captures the underlying uncertainty in each predicted MSC. Therefore,

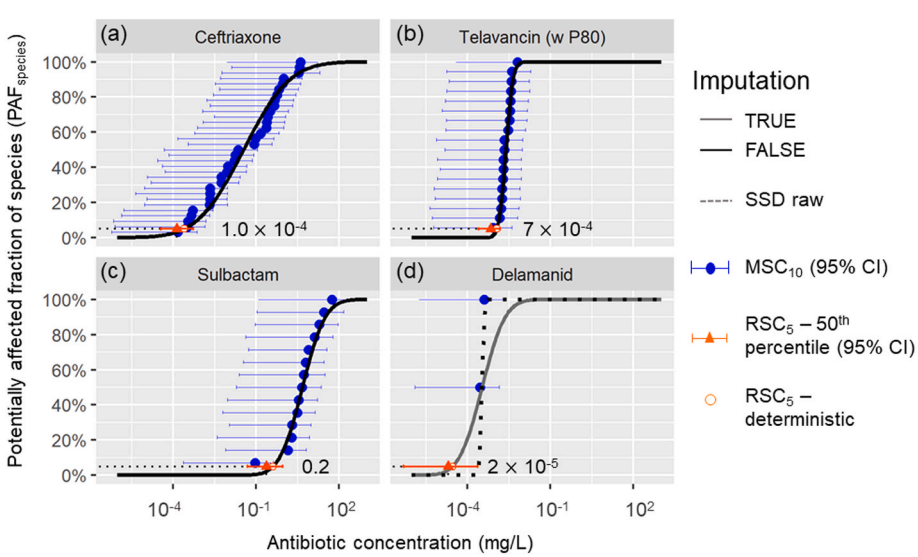

Fig. 3. Species sensitivity distributions for four selected antibiotics built from their pooled minimum selective concentrations (MSC_{10}) for each species that they had been tested on in the EUCAST data. The SSDs with (a) the highest and (b) lowest σ are plotted, as well as the two antibiotics with the (c) highest and (d) lowest predicted resistance selection concentration, at which resistance is promoted in 5% of environmental bacteria (RSC_5). For antibiotics tested on less than ten species, the standard deviation of their SSD curve (σ) was imputed by a default σ equal to the average σ across all 'data-rich' antibiotics (Imputation = TRUE).

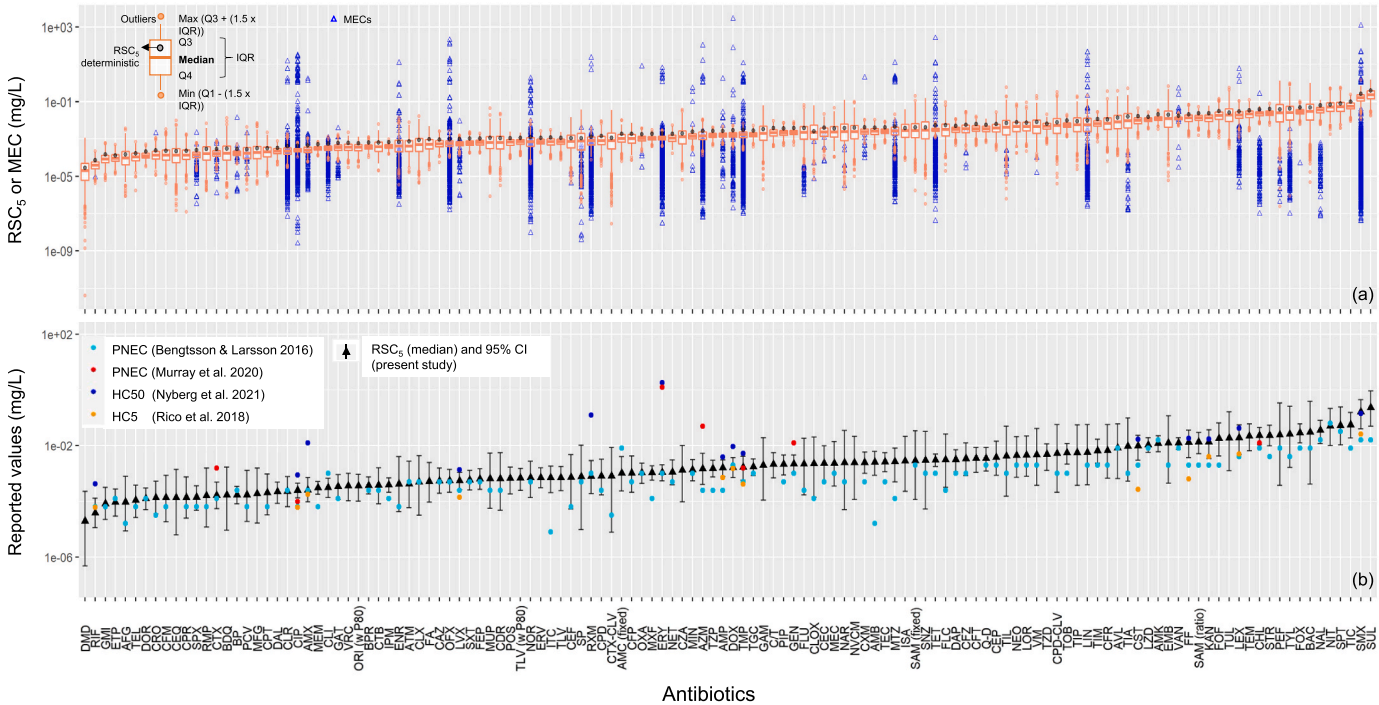

Fig. 4. Comparison of the resistance selection concentrations (RSC_5) for 128 antibiotics calculated in this study to (a) measured environmental concentrations (MECs) in eight different environmental matrices (surface water (river/stream), surface water (lake), surface water (sea or ocean), groundwater, drinking water, wastewater treatment plant effluent and treated hospital sewage), extracted from the German Environment's Agency (UBA) "Pharmaceuticals in the Environment" database (UBA, 2021), and (b) predicted no-effect concentrations (PNECs) for resistance selection in the environment, and hazardous concentrations (HC_x), promoting resistance in 5% or 50% of bacterial genera, reported in other risk assessment/LCIA studies. In (a) and (b) the uncertainty around our RSC_5 estimates is also presented. The full list of antibiotic names is available in the appendix (Section A-18, Table A7).

we only discuss results of the median RSC_5 in the following sections.

The five lowest predicted RSC_5, indicating the antibiotics with the highest 'selective pressure potential', were estimated for delamanid (2×10^{-5} mg/L; 95% CI: 4.8×10^{-7} - 0.00023 mg/L), rifampicin (3.8×10^{-5} μg/mL; 95% CI: 1×10^{-5} - 0.00013 mg/L), gemifloxacin (8.3×10^{-5} μg/mL; 95% CI: 2.210^{-5} - 0.00031 mg/L), ertapenem (9.3×10^{-5} mg/L; 95% CI: 3×10^{-5} - 00027 mg/L), anidulafungin (9.5×10^{-5} mg/L; 95% CI: 8.6×10^{-6} - 0.0008 mg/L). An analysis of estimated RSC_5 per antibiotic class (see Figure A15 in the appendix) highlights some classes that comprise predominantly antibiotics with relatively higher RSC_5 estimates (i.e. lower selective pressure potential), such as aminoglycosides and combinations of beta lactam - beta lactamase inhibitors, while other classes appear to predominantly compromise antibiotics with relatively higher selective pressure potential (e.g. carbapenems, cephalosporins, and fluoroquinolones). Nevertheless, variation in RSC_5 levels within single antibiotic classes is quite high (e.g up to 3 orders of magnitude for penicillins). The relatively small number of antibiotics available per class renders a differentiated comparison between the selective abilities of different classes difficult. As more RSC_5 estimates are derived in the future, we might be able to better understand inter- and intra-class differences in selection ability.

Whether potential resistance selection and enrichment is actually present in the environment can only be determined in relation to MECs of these antibiotics. In Fig. 4a, we present the resulting RSC_5 for 128 antibiotics (deterministic, median and the range of 1,000 RSC_5 values per antibiotic), and, where available ($n = 45$), respective MECs extracted from the PiE database (UBA, 2021).

For 11 (out of 45) antibiotics, *all* MECs are below the predicted RSC_5 (median). However, considering the uncertainty around the RSC_5, represented as boxplots with outliers, as well as the uncertainty around reported MECs, for which no information is given in the PiE, it is not possible to entirely exclude the potential for resistance selection and dissemination even for some of the antibiotics with $MEC <$ median RSC_5. For some other notable examples, e.g., ciprofloxacin, ofloxacin, norfloxacin, and tetracycline, a considerable number of MECs exceeds the estimated RSC_5, highlighting the role these antibiotics play in both medicine, as well as in potentially fuelling antibiotic resistance in the environment.

A refined evaluation of potential resistance selection in the environment, where MECs are broken down by environmental compartment, allows us to compare the degree to which different compartments may contribute to the increased prevalence of resistance. In Fig. 5, MECs per compartment are compared to the estimated RSC_5 (median) per antibiotic. Each tile in the heat map represents the fraction of MECs in a given compartment above the respective antibiotic's RSC_5, as well as the number of underlying MECs. Fig. 5 enables a comparison of the selection pressure potential in different environmental compartments, and allows prioritizing them for intervention strategies. Highest selection pressures (i.e. MECs exceed RSC_5 in >50% of samples) are observed in treated hospital sewage, followed by wastewater effluent and lakes, and finally rivers. The degree to which certain environmental matrices retain higher antibiotic concentrations is affected by a variety of factors including photolysis, temperature, pH and dilution factors (Liu et al., 2021). The observed differences in antibiotic concentrations between environmental compartments, and in the resulting selection potential, further highlights the importance of examining synergies between antibiotics in different compartments and quantifying their potential combined selection pressure, along with co-selection effects by other contaminants (Ye et al., 2017).

Most data on antibiotic pollution in the PiE database are available for WWTP effluent and surface water (river/stream) and, hence, these compartments paint the most diverse picture in terms of the degree of resistance selection and enrichment under current antibiotic pressure. In WWTP effluents, six antibiotics, such as clarithromycin, ciprofloxacin, and amoxicillin, are detected in 20% - 50% of the samples at concen-

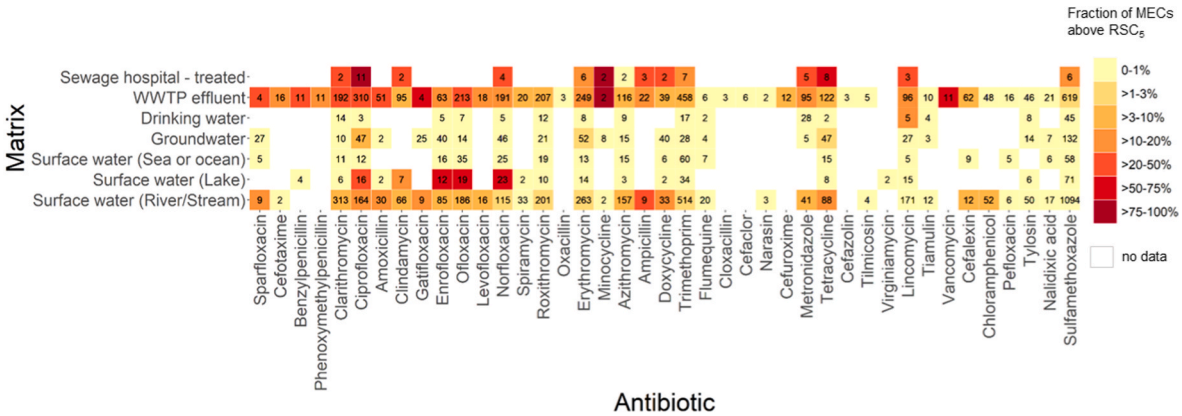

Fig. 5. Heat map indicating the fraction of measured environmental concentrations (MECs) of a given antibiotic in different environmental compartments above its predicted resistance selection concentration (RSC_5 – median). The underlying number of MECs is written inside the tiles. MECs were extracted from the 'Pharmaceuticals in the Environment' database of the German Environment's Agency (UBA, 2021). Cases with 1 MEC to compare against the RSC_5 have been excluded from the plot.

trations high enough to select for resistance. Gatifloxacin and vancomycin, as well as minocycline, indicate higher potential of resistance selection (50% - 75% and 75%, respectively), albeit based on fewer data points. Notably, sulfamethoxazole was measured in 619 effluent samples, of which only 1% - 3% exceed its estimated RSC_5. This is owed to sulfamethoxazole's relatively high RSC_5 of 0.16 mg/L (95% CI: 0.04 - 0.45), marking it as a 'safe' antibiotic in terms of its selective pressure potential in the environment.

While antibiotic concentrations in rivers are expectedly less likely to exert selection pressure beyond the RSC_5, for several antibiotics (e.g., ciprofloxacin, amoxicillin, doxycycline, tetracycline), 10% - 20% of samples still indicate a potential for selection. Despite lower data availability, 50% - 75% of samples taken from lake water indicate concentrations for norfloxacin and enrofloxacin that surpass critical exposure levels for selection. These results direct the focus of resistance selection, transmission and mitigation strategies on surface waters alongside the often-discussed wastewater/hospital effluents. Especially notable is the indication of selection pressure in groundwater, here detected for ciprofloxacin in 3% - 10% of samples, and for erythromycin and tetracycline in 1% - 3% of samples.

Aside from identifying 'priority environmental compartments' to address the spread of resistance, these results underline several antibiotics that, either because of their high predicted selective potential or their high concentrations in the environment (or both), seem to be driving resistance selection in several environmental compartments (e.g., ciprofloxacin, norfloxacin, enrofloxacin, tetracycline). Nevertheless, the lack of available MECs data on many antibiotics makes it hard to determine the current selection pressure potential in the environment due to the presence of these antibiotics, and limits a full assessment of the potential resistance selection and enrichment.

4. Discussion

4.1. Comparison with previous studies

We compared our SSD-derived RSC_5 to different previously published environmental selection thresholds (e.g. HC_x and predicted no-effect concentrations, PNECs). In the majority of cases, PNECs lie below or within the lower 95% CI of our predicted RSC_5 concentrations, underlining the different interpretation and application of each endpoint. *PNECs* are to be understood as protective selection thresholds intended for use in e.g., risk assessment, while our RSC_5 estimates are intended to express a potential ecological effect and to enable comparisons between the effect pressure (here: selection) of different antibiotics, as well as between the resistance enrichment potential of whole product systems when applied in the context of LCIA. Despite the expected relation of $RSC_5 > PNEC$ holding in most cases, there are a few antibiotics where both values are similar or where the *PNEC* is higher (e.g., benzylpenicillin and clindamycin). These differences may arise due to differences in the applied approach such as the choice of MIC_s threshold at the start of the analysis ($MIC_{1\%}$ in Bengtsson-Palme & Larsson (2016) vs. $MIC_{10\%}$ in the present study). Most importantly, though, our choice of default fitness costs with an $sc = 0.04$ led to MSC-to-MIC_s ratios that are very similar to the assessment factor of 10 used by Bengtsson-Palme & Larsson (2016). By modelling this specific cost scenario, the herein predicted RSC_5 values are expected to be statistically very similar to *PNECs*. These RSC_5 results can be further tailored when mutation-/ARG-specific data is used within the competition model to build SSDs from predicted, cost-dependent MSC thresholds. Lower fitness costs would thus drive our RSC_5 closer to the *PNECs* and vice versa, if everything else in **Eq. (1)** was kept constant (see sensitivity analysis in Section A-11).

In comparison to the HC_x estimates by Rico et al. (2018) or Nyberg et al. (2021), results divert in some cases from the expected relation to our RSC_5, i.e. $RSC_5 \approx HC_5$ and $RSC_5 < HC_{50}$.This is, again, most likely the result of using varying MIC_s thresholds as a starting point. Both Rico et al. (2017) and Nyberg et al. (2021) build their SSDs from MSC_{NOEC} as opposed to an MSC_{10} as in the present study. With regard to Murray et al. (2020), the authors derive *PNECs* based on experimentally measured lowest observed concentrations, at which growth of the entire bacterial community is significantly reduced. These differences in the underlying approach to derive selection thresholds may explain the variations seen in Figure 4b.

Nevertheless, the relatively good overall agreement between our RSC_5 and different selection thresholds in other studies supports the preliminary use of default parameter values within the proposed approach to model a relevant and representative scenario, namely low-cost resistance mutations/ARGs, based on which predictions of selection potentials and targeted mitigation strategies can already be assessed and compared. Experimental studies, especially those that are more closely resembling environmental settings (e.g. using species assemblages), will be required to (1) empirically evaluate the performance of the proposed 'simpler model' (i.e. using default values), (2) understand how MSC levels relate to selection at the community level and which ecological interactions are likely to affect outcomes (Durão et al., 2018), and (3) validate the different selection thresholds proposed in different studies and determine the degree to which they are more or less conservative.

4.2. Limitations of the proposed appraoch

Our proposed methodological framework to link bacterial growth inhibition data to fitness cost-dependent MSCs, and further to antibiotic-specific RSC_5 is limited by a number of factors. Some are embedded in our model assumptions and some are related to the use of experimental, *in vitro* bacterial inhibition data on pathogenic bacteria to extrapolate towards selection potential in environmental settings and in complex bacterial communities. The latter limitations have been discussed in detail in previous risk assessment studies of environmental resistance selection also relying on MIC distribution data from the EUCAST database as a starting point for their assessment (Bengtsson-Palme & Larsson, 2016; Nyberg et al., 2021; Rico et al., 2017; Tello et al., 2012). The most important of these limitations are:

1. The different exposure timeframes between MIC tests (acute) and chronic selective pressures in the environment (Kümmerer, 2009). This is closely related to the question of how long selection pressures must be sustained for resistance to be 'significantly' enriched.
2. The use of exclusively clinical data on pathogenic bacteria to model selection sensitivities of the environmental bacterial community as a whole. For lack of better data, use of widely abundant clinical data is accepted to represent the environmental microbiome, because (1) several ARGs have their origins in non-infectious environmental bacteria (Finley et al., 2013), and (2) several pathogens are closely related to environmental bacteria. To reduce uncertainties, a minimum number of 10 species was used in the present study to construct respective SSDs. However, the phenotypic and genetic diversity of the environmental microbiome remains poorly captured by clinical data, and several resistance mechanisms in the environment may not yet be detected in clinical settings.
3. Exclusion of bioavailability of antibiotics in the environment, which were found to *e.g.,* sorb to particles, influencing their bioactivity (Chander et al., 2005; Córdova-Kreylos & Scow, 2007)
4. Exclusion of synergistic interactions between antibiotic mixtures (Gullberg et al., 2014)
5. Exclusion of additional effects taking place at sub-inhibitory antibiotic concentrations that will likely have an impact on overall prevalence and persistence of resistance. These include increased mutation rates (Cortes et al., 2008; Gutierrez et al., 2013), increased rates of horizontal gene transfer between often phylogenetically-distant bacteria and between non-pathogenic bacteria and pathogens (Johnson et al., 2015; Lerminiaux & Cameron, 2019; Prudhomme et al., 2006), co-selection by *e.g.,* heavy metals and cross-selection (Melnyk et al., 2015; Seiler & Berendonk, 2012), and finally biofilm formation that can protect bacteria from the impacts of antibiotic pressure (Balaji et al., 2013; Serwecí, 2020).

Due to these limitations, there are high uncertainties associated with using environmental resistance selection concentrations derived from bacterial inhibition data on pathogenic bacteria, which must be taken into consideration when using the predicted RSC_5 estimates to assess the resistance selection and enrichment potential in the environment.

With regards to our specific methodological assumptions (Table A1), it is important to highlight several aspects increasing the uncertainty of our results. First, due to the lack of comprehensive mutation-/ARG-specific data on e.g., the intrinsic net growth rates of sensitive and resistant strains ($N_{int,s}$ or $N_{int,s}$), the minimum possible growth rate (N_{min}), κ, and most importantly, fitness costs or the *sc*, we relied on relatively large distributions of the parameter values in the competition model to predict MSCs (**Eq. (1)**) for all antibiotics-species combinations. At current model assumptions, we may thus be under- or overestimating MSC levels for a variety of antibiotic-species combinations. Nevertheless, we have attempted to capture the possible variability of each parameter as found in the literature within the uncertainty analysis, which led to relatively large 95% CI around the predicted MSC_{10} thresholds representing the range of possible MSC values for a combination.

Finally, an important limitation is the use of the EUCAST data for sensitive and resistant isolates of *different* bacterial strains across various mutations/ARGs to determine one representative MIC_r/ MIC_s , and the choice of a single endpoint (MSC_{10}) along the *MSC* distribution of a given antibiotic-species combination. This pools inhibition data on all thus far detected mutation mechanisms (chromosomal or MGE-borne) in a given species into one group instead of predicting mutation/ARG-specific *MSC* s for which the competition model was originally developed. Given that it is relatively expensive and time-intensive to generate mutation/ARG-specific data and given the abundance of data on clinical isolates, modelling one representative MSC for each antibiotic-species combination across a variety of mutations/ARGs (here MSC_{10}) represents a realistic and consistent way forward to predict MSCs and make use of currently available clinical inhibition data. If mutation-/ARG-specific data becomes available in the future, our approach will enable practitioners to model a 'full' distribution of MSC-to-MIC ratios to represent the various mutations and ARGs within the same species against a given antibiotic; a complex task if comprehensiveness is desired.

4.3. Applicability of our results and future research needs

Despite the above-mentioned limitations, our RSC_5 can guide researchers and decision-makers in assessing the potential for resistance selection and enrichment in the environment with possible implications for human health. Additionally, RSC_5 may be used by antibiotic-producing companies or farmers, seeking to assess the potential for resistance enrichment in the environment due to their respective economic activities (e.g., effluent discharge, use of manure on agricultural soils). Finally, our RSC_5 estimates provide the point of departure for deriving new characterization factors for antibiotic emissions to be used in LCIA. Our RSC_5 (measured in mg/L) indicate a selection potential in aquatic environments only. We propose new effect factors (EFs) for resistance selection to then be calculated as $EF = 0.05/ RSC_5$, and to be combined with fate and exposure factors from USEtox (Fantke et al., 2021; Owsianiak et al., 2023) to express comparative 'resistance selection potentials' (RSPs) in potentially affected fractions of bacterial species, integrated over the exposed water volume (m^3) and time (d), $PAF_{bacteria}$ m^3 d per kg emitted into freshwater. The resulting RSPs will enable evaluating and comparing e.g., wastewater treatment technologies with regard to their potential contribution to selection and spread of resistance, and help identify effective life-cycle based mitigation strategies. Due to the importance of other environmental compartments (e.g. soils and sediments) in the selection and transmission of resistance, consistent methods to extrapolate towards corresponding RSC_5 in these matrices will need to be developed to further enable evaluation of e.g. different manure and sludge treatment technologies in mitigating the resistance problem.

Future research will be needed to understand and model the link between resistance selection in the environment and human health impacts. This would require generating a variety of data on e.g. transfer rates of resistance from environmental to new hosts, including pathogenic bacteria, and exposure of humans to resistant bacteria/ARGs in the environment, as well as the infective dose (Martinez, 2009; Martínez, 2012; Vikesland et al., 2017). Based on such a framework, we can begin to understand the environmental dimension of clinical resistance.

Additionally, future research would greatly benefit from (a) better data availability to refine the methodological framework proposed in the present study (especially by replacing default values for κ and the sc), and (b) development of models that include aspects of the 'real' circumstances in the environment (e.g. co-selection, horizontal gene transfer, direct flows of resistant bacteria/ARGs into the environment, antibiotic bioavailability, and species assemblages).

5. Conclusions

In the present study, we proposed a novel methodological framework to consistently derive MSCs from readily available bacterial inhibition data (MIC distributions). MSCs provide a relevant effect endpoint to evaluate the extent to which selection for resistance may occur under environmental antibiotic pressure. Based on our proposed framework, we provide estimated MSC_{10} endpoints for 2,984 antibiotic-species combinations; the largest set of modelled MSCs available to date. Our modelled MSCs can be used next to experimentally determined MSCs to drive early predictions of environmental selection pressure, as competition essays continue to be expensive and complex to perform, thereby limiting data availability. The utility of our methodological framework lies in the following: (1) it establishes a semi-mechanistic framework to predict MSCs, providing an understanding of the factors affecting the MSC and differentiating between antibiotics; (2) this framework can later be used in predicting more accurate mutation/ARG-specific and cost-dependent *MSCs* as more data becomes available; and (3) it highlights the most important knowledge gaps, where data are most needed (especially fitness costs and κ) to help understand and better model selection and dissemination of resistance in the environment.

We further derived MSC_{10}-based species sensitivity distributions and predict for the largest number of antibiotics to date ($n = 128$) antibiotic-specific resistance selection concentrations (RSC_5) that are predicted to promote resistance in 5% of environmental bacterial species. By comparing our predicted RSC_5 values to measured environmental concentrations, we highlight specific environmental compartments (e.g. wastewater effluents, lakes and rivers), as well as several antibiotics (e.g. ciprofloxacin, norfloxacin, enrofloxacin, and tetracycline), to be further scrutinized for their role in resistance selection and dissemination. Even though a long list of antibiotics were associated with a stronger selective pressure potential (i.e. lower RSC_5) than those above, the lack of MECs for these antibiotics limits our ability to evaluate the degree to which they are responsible for resistance selection and enrichment in the environment. Monitoring programs should be extended to include those antibiotics with low RSC_5 so that we can begin to understand the role of the environment and other vectors, such as microplastics (Zhu et al., 2022), in the spread of resistance. Our comprehensive quantification of the relatively large uncertainty around all relevant endpoints for addressing resistance selection and potential dissemination in the environment (MIC_{10}, MSC_{10} and RSC_5) reflects the variability in the 'real-world', enables a more accurate assessment of current selection pressure, and underlines the continued need for research to improve our predictions on the environmental dimension of resistance as well as evaluate the efficacy of our mitigation actions.

Author statement

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120873.

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