Emerging Contaminants 9 (2023) 100257

Contents lists available at ScienceDirect

Emerging Contaminants

journal homepage: www.keaipublishing.com/en/journals/emerging-contaminants

Effects of selected emerging contaminants found in wastewater on antimicrobial resistance and horizontal gene transfer

Sophie van Hamelsveld [a], Farideh Jamali-Behnam [a], Izzie Alderton [a], Brigitta Kurenbach [b], Andrew W. McCabe [b], Barry R. Palmer [c], Maria J. Gutiérrez-Ginés [a], Louise Weaver [a], Jacqui Horswell [c], Louis A. Tremblay [d, e, **], Jack A. Heinemann [b, *]

[a] Institute of Environmental Science & Research (ESR), New Zealand
[b] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
[c] School of Health Sciences, Massey University, Wellington, New Zealand
[d] Cawthron Institute, Nelson, New Zealand
[e] School of Biological Sciences, University of Auckland, Auckland, New Zealand

ARTICLE INFO

Article history:
Received 7 June 2023
Received in revised form
22 August 2023
Accepted 22 August 2023
Available online 22 August 2023

Keywords:
Pharmaceuticals
Biocides
Fluoxetine
Carbamazepine
Dimethyl sulfoxide

ABSTRACT

The widespread use of emerging contaminants (ECs) may be compounding the problem of antibiotic resistance. Various non-antibiotic pollutants have been shown to alter bacterial responses to antibiotics and increase horizontal transfer of antimicrobial resistance (AMR) genes. ECs include components of medicines, foods, disinfectants, personal care products and agrichemicals. ECs concentrate in some environments such as in wastewater, where the pollutants and pathogenic microorganisms mix. We investigated the effects on antibiotic resistance and gene transfer of nine ECs and one commercial product formulation (Roundup). We used the bacterium *Salmonella enterica* serovar Typhimurium and the antibiotics ampicillin and gentamicin as indicators of the effects of antibiotic-EC co-exposures. We measured intra- (*Escherichia coli*) and interspecies (*E. coli* x *S. enterica)* conjugation frequencies during exposure to ECs. Interestingly, the observed effect could change at different antibiotic concentrations. Exposures to increasing concentrations of ECs was associated with increased conjugative transmission within species, but rarely increased interspecies transmission. We report the first test ever of clotrimazole on AMR and horizontal gene transfer and a newly described effect of dimethyl sulfoxide (DMSO), often used as a solvent for organic compounds.

1. Introduction

The rise of antimicrobial resistance (AMR) is a public health hazard, reducing the ability to prevent and treat commonplace infections [1,2]. The contribution of antibiotics to the development and spread of AMR has been extensively studied [1,3]. However, other chemical compounds have been linked to AMR [3–8], and are under investigation.

Emerging contaminants (ECs) are a diverse group of chemicals of natural or synthetic origins, such as pesticides, pharmaceuticals, components of personal care and household products [6,7]. Many are recalcitrant [9,10], and bio-accumulative [11,12]. ECs have been detected in terrestrial and aquatic environments [9,10,13,14] and are also found as residues in foods, such as honey and shellfish [10,15]. There is evidence for toxicity of various ECs to both prokaryotes and eukaryotes [9,11,12,16]. The contribution of ECs to the AMR crisis may be overlooked as recently reported [3].

Classes of ECs that have been shown to modulate AMR include, pesticides [16–19], pharmaceutical agents [7,8,20,21], preservatives [6,18], and disinfectants [5]. Exposures to ECs could result in increased tolerance or susceptibility to antibiotics [16,18,19]. Although the effects on antibiotic tolerance were in different directions depending on antibiotic and EC, both an increase in resistance or an increase in susceptibility accelerated the

* Corresponding author.
** Corresponding author. Cawthron Institute, Nelson, New Zealand.
E-mail addresses: louis.tremblay@cawthron.org.nz (L.A. Tremblay), jack.heinemann@canterbury.ac.nz (J.A. Heinemann).
Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.emcon.2023.100257

rate of resistance evolution [16]. In addition, ECs have been shown to increase the frequency of horizontal gene transfer (HGT) of AMR genes, particularly conjugation, a plasmid-mediated HGT [5–7,21,22].

The toxicity of any particular EC, such as an antibiotic, may be countered by a dedicated resistance mechanism. The outcome is usually high level resistance. The focus of our work is on changes to the effectiveness of an antibiotic arising from other chemical exposures and which generally have a much smaller effect on resistance. Those changes commonly arise from induction of the adaptive resistance response [23].

The adaptive response is an innate form of resistance that is conditionally expressed [16,24]. Molecules of very different chemical properties are capable of inducing the response, even if the inducer is itself of low or negligible toxicity to the bacterium. Once induced, however, the bacteria can respond to many different molecular species leading to an incremental change in susceptibility to toxins including clinical antibiotics.

A change in efflux or influx due to changes in membrane permeability are examples of the adaptive response [19]. Membrane pumps have broad specificities. Therefore, once a particular pump is expressed, the bacterium can display cross-resistance (or heightened susceptibility) to a variety of different agents. Exposure to low toxicity ECs can raise or lower the concentration of an antimicrobial agent that causes a change in fitness [16].

Wastewater treatment plants are places where high concentrations of human pathogenic organisms and ECs can be found. Following treatment, the residues, such as sludge, may be concentrated in disposal sites or used as fertilizer. We focused on ECs found in wastewater and wastewater impacted environments for this study. The ECs we chose have all been detected in aquatic environments, including those found in Aotearoa New Zealand [9,10,14,25,26]. Our first aim was to investigate whether these ECs can induce AMR in a human pathogen also found in wastewater: *Salmonella enterica* serovar Typhimurium. Our second aim was to investigate whether the presence of these ECs could accelerate the rate at which bacteria may gain AMR genes through conjugation.

Although antibiotics themselves fit the description of ECs, here we are not evaluating their effects on antibiotic resistance. We made a distinction between chemicals used for purposes other than treating bacterial diseases (what we and others refer to as ECs) and the antibiotics themselves. One rationale for this approach is that while antibiotic use could be reduced in many cases, the need for antibiotics in human and animal medicine is indispensable for effective treatment at present [1]. However, the use of ECs, many of which are not essential, could be compounding the problem of AMR [3], and is therefore worthy of separate consideration and study.

We tested altered responses of *S. enterica* to ampicillin and gentamicin. These two drugs were chosen because of their chemical dissimilarity with distinct mechanisms of action and different (dedicated) resistance mechanisms but not because of their present clinical utility for specific bacteria. We evaluated nine ECs and one formulation of concern for effects on antibiotic resistance and HGT – carbamazepine, clotrimazole, diclofenac, fluoxetine, ibuprofen, methyl paraben, Roundup, sodium benzoate, triclocarban and triclosan.

2. Materials and methods

2.1. Media, chemicals, and strains

E. coli and *S. enterica* strains (Table 1) were routinely cultured in Luria–Bertani (LB) broth (Invitrogen, MA, USA) and LB agar (Oxoid, Nepean, Canada). In liquid medium, strains were grown with aeration provided by an orbital shaker set at 180 rpm, unless otherwise stated. Antibiotics were purchased from Becton Dickinson (Franklin Lakes, NJ, USA), Sigma-Aldrich (St. Louis, MO, USA), Oxoid or Duchefa (Haarlem, Netherlands). Stock solutions were prepared according to the manufacturers' instructions. Phosphate buffered saline (PBS) (Oxoid) was used for making dilutions of bacterial cultures. Carbamazepine, clotrimazole, diclofenac, fluoxetine, ibuprofen, methyl paraben, sodium benzoate, triclocarban and triclosan were purchased from ThermoFisher (Waltham, MA, USA) or Sigma-Aldrich. Stock solutions were prepared in either dimethyl sulfoxide (DMSO; Sigma-Aldrich) or sterilised distilled water. Solvent controls were included where applicable. The commercial glyphosate formulation Roundup (Kiwicare, Christchurch, New Zealand) was used as a reference toxicant.

Details of the bacterial strains and plasmids used are provided in Table 1.

2.2. Efficiency of plating assay

Efficiency of plating (EoP) assays [16,18] were used to compare EC/antibiotic co-exposure effects to antibiotic exposure effects. The antibiotics used were ampicillin, at concentrations of 0, 0.25, 1.0, 1.5 and 2.0 μg mL^{-1}, and gentamicin, at concentrations of 0, 0.25, 1.0, 2.0 and 4.0 μg mL^{-1}. The EC concentrations were: 0.25 μg mL^{-1} for triclocarban, methyl paraben, sodium benzoate, ibuprofen, diclofenac, carbamazepine, and fluoxetine, 0.01 μg mL^{-1} for triclosan, and 5 mM for glyphosate, delivered as part of a Roundup formulation.

Solid medium was used for EoP assays. The medium was supplemented with antibiotics ± an EC. A DMSO control without EC was added when this solvent was used to ensure that the effect we report is independent of the solvent used.

The EoP assay was thereafter as per Kurenbach et al. [18]. Briefly, strain NZRM4470 (Table 1) was grown to an OD_{600} of approximately 0.05 in LB broth at 37 °C with aeration. Serial dilutions of the three replicate cultures were prepared in PBS and plated on plates prepared with and without EC/DMSO. EoP was determined by dividing the cfu mL^{-1} measured on treatment plates by the cfu mL^{-1} of the same culture grown without antibiotic/EC (except for DMSO in the case of the DMSO control series). An EoP greater than 1 therefore indicated increased survival (greater tolerance to the antibiotic), while an EoP of less than 1 indicated decreased survival. If the EoP of the plate supplemented with both antibiotic and EC was higher than the EoP of the plate supplemented only with antibiotic, the effect of the EC was to increase the bacteria's tolerance to the antibiotic, and vice versa. All EoP experiments were performed independently three times.

2.3. Conjugation experiments

Plasmid RP4 transmission frequencies were measured in either a liquid or on a solid medium. The media on which the bacteria mated was supplemented, or not, with either or both the EC and solvent at concentrations specified in Table 2.

An isogenic pair of *E. coli* strains, differing only in their resistance to streptomycin or rifampicin (Table 1) [27] was used for intra-species conjugations. Separate cultures of CMB44_Str^R_RP4 and CMB44_Rif^R were cultured with aeration to an OD_{600} of approximately 0.05 in LB broth at 37 °C. They were mixed at a recipient:donor 10:1 ratio in 10 mL LB broth with exposures as described. DMSO without EC was used as a control for solvent effects. Roundup and sodium benzoate were diluted with sterile water.

Mating mixes were incubated in liquid medium for 3 h at 37 °C without aeration. Serial dilutions were prepared in PBS and

Table 1
Strains and plasmids.

Strain name	Species	Genotype	Reference
NZRM4470	*Salmonella enterica* s. Typhimurium	Wild type strain isolated from bovine faeces in New Zealand	This study, NZ isolate ERL09/2121[a]
CMB44		Wild type environmental isolate	van Hamelsveld et al. [27]
CMB44_Str^R	*Escherichia coli*	Spontaneous Str^R (spontaneous 100 μg mL^{-1}) derivative of CMB44 (spontaneous 100 μg mL^{-1})	van Hamelsveld et al. [27]
CMB44_Str^R_RP4	*Escherichia coli*	CMB44_Str^R + RP4$^+$ (Kan^R, Tet^R, Amp^R)	van Hamelsveld et al. [27]
CMB44_Rif^R	*Escherichia coli*	Spontaneous Rif^R (spontaneous 100 μg mL^{-1}) derivative of CMB44.	van Hamelsveld et al. [27]
SL1344_RP4	*Salmonella enterica* s. Typhimurium	*rps*L, *his*G46, Nal^R, RP4$^+$ (Kan^R, Tet^R, Amp^R)	Ferguson et al. [28]

[a] Isolate taken from the Institute of Environmental Science and Research's New Zealand Reference Culture Collection. Footnote: Str: streptomycin, Kan: kanamycin, Tet: tetracycline, Amp: ampicillin, Rif: rifampicin, Nal: nalidixic acid.^{R}: Denotes resistance.

Table 2
List of emerging contaminants. E number: Europe number. CAS ID: Chemical Abstracts Service Identification number.

Chemical name	Trade name(s) or E number	Application	CAS ID	Concentration(s) found in the environment[a]	Reference[b]	Concentrations used for conjugation experiments in the present work (μg/mL)
Carbamazepine	Carbatrol, Tegretol	Antiepileptic drug	298-46-4	302 ng/L	Tremblay & Northcott [25]	0.05, 0.5, 5, 10
Clotrimazole	Canesten	Antifungal agent	23593-75-1	0.2–142 ng/L	Assress et al. [29]	0.5, 1, 2, 4
Diclofenac	Voltaren	Anti-inflammatory	15307-86-5	19.35 ng/L	Tremblay & Northcott [25]	0.005, 0.05, 0.5, 5
Fluoxetine	Prozac	Antidepressant	54910-89-3	14.7–58.7 ng/L	Tremblay et al. [30]	2.5, 5, 10, 15
Ibuprofen	Brufen, Nurofen, Advil	Anti-inflammatory	15687-27-1	6.05 ng/L	Tremblay & Northcott [25]	0.05, 0.5, 5, 50
Methyl paraben	E218	Preservative	99-76-3	0.17–0.46 ng/L	Tremblay & Northcott [13]	0.1, 1, 5, 10
Glyphosate (in Roundup formulation)	Roundup	Herbicide	1071-83-6	40-1000 ng/g	Tremblay et al. [30]	0.001, 0.002, 0.005, 0.009
Sodium benzoate	E211	Preservative	532-32-1	0.02–10e6 ng/L	Wibbertmann et al. [31]	0.1, 1, 5, 10
Triclocarban	NA	Antimicrobial	101-20-2	40.6–13,700 ng/L	Tremblay et al. [30], WERF Report [32]	10, 100, 1000
Triclosan	NA	Antimicrobial	3380-34-5	8.90–630 ng/L	WERF Report [32], Tremblay & Northcott [25]	0.005, 0.01, 0.025, 0.05

[a] Environments included wastewater, groundwater, drinking water and landfill leachate.
[b] References provide information on relevant concentrations in the environment.

transferred to LB agar medium supplemented with antibiotics to enumerate recipients and transconjugants (rifampicin, 100 μg mL^{-1}), donors (streptomycin, 100 μg mL^{-1}), and transconjugants (tetracycline, 8 μg mL^{-1} + rifampicin, 100 μg mL^{-1}). Transmission frequency was cfu mL^{-1} of transconjugants divided by the cfu mL^{-1} of the least numerous parent (the limiting parent). In parallel, the experiment was performed with the addition of DMSO, equal to the highest concentration of DMSO needed to achieve the equivalent concentration of EC, where applicable.

Inter-species crosses were between donor strain SL1344_RP4 and recipient CMB44_Rif^R [33] (Table 1). Both parental strains were cultured to OD_{600} of approximately 0.05 in 10 ml LB broth with aeration [34]. Donors were cultured at 43 °C and recipients at 37 °C. Cells were pelleted by centrifugation, washed in PBS, and resuspended in the original volume of PBS. A 10 μl volume of donor and recipient culture was mixed on solid LB medium containing the EC at appropriate concentrations and incubated at 37 °C for 3 h. Bacteria were washed with 1 mL PBS, diluted, and plated on selective media to enumerate donors (streptomycin, 100 μg mL^{-1} + tetracycline 15 μg mL^{-1}), recipients and transconjugants (rifampicin, 100 μg mL^{-1}), and transconjugants (rifampicin, 100 μg mL^{-1} + tetracycline, 15 μg mL^{-1}). DMSO controls containing DMSO at the highest concentration used were included in the experimental setup. Plates were incubated for 16 h at 37 °C, before enumeration and calculation of the transmission frequency.

2.4. Statistical analysis

We used R Studio for all statistical analysis [35]. We used *ggplot2* to produce graphical figures [36]. We used a linear model to measure the effect of ECs on EoP of *S. enterica* on gentamicin and ampicillin. Antibiotic concentration was treated as a factor to evaluate the significance of ECs on EoP at every concentration of the antibiotic separately. The response variable was EoP, predicted by the interaction between antibiotic and the presence or absence of EC. Because we had two controls with and without DMSO, the model was fitted twice for both data sets (EoP calculated against control with DMSO and EoP calculated against control without DMSO). Residual plots were used to check that the data fitted the assumptions of a linear model. All EoP values were $\log_{10}$-transformed prior to model fitting to fulfil the model assumptions.

To test for a significant effect of EC on plasmid transmission frequency (i.e., an increase in the number of transconjugants per limiting donor in the presence of a given EC), we used Student's T-tests Data were first checked for normalcy using the Shapiro-Wilk test [37], and for equal variances. A p-value of <0.05 was considered significant in all cases. The statistical analyses were performed using R Studio core package code [35]. We converted p-values into significance codes according to the R Studio standard as follows: '***', when the p-value was between 0 and 0.001; '**', when the p-value was between 0.001 and 0.01; '*', when the p-value was between 0.01 and 0.05; and '.', when the p-value was between 0.05 and 0.1 [35].

3. Results

3.1. Emerging contaminants alter responses of S. enterica to antibiotics

All three previously reported antibiotic x EC x species effects were observed for the combinations we tested. Most ECs decreased tolerance to ampicillin (Fig. 1). Most ECs increased tolerance to gentamicin (Fig. 2). Exceptions were an EoP decrease for co-exposures to gentamicin and carbamazepine, ibuprofen, Roundup, triclocarban or triclosan. Finally, there was no or only a weak observable effect for the combinations of ampicillin and diclofenac and methyl paraben (Fig. 1).

Aside from Roundup, each of the ECs effected a different response to the two different antibiotics. Clotrimazole accentuated ampicillin toxicity (Fig. 1) but decreased gentamicin toxicity (Fig. 2). Roundup significantly decreased EoP when combined with either ampicillin or gentamicin, except at the lowest concentration of the herbicide.

Unexpectedly, the EC x antibiotic effect changed direction at different ampicillin concentrations for two ECs (Fig. 1). The EoP trend switched from indicating lesser toxicity of ampicillin to enhanced toxicity between 1.5 μg mL^{-1} and 2 μg mL^{-1} ampicillin (Fig. 1). This was also observed for triclocarban between the same two concentrations of ampicillin. The same outcome was observed for triclocarban x gentamicin. The lower concentration of gentamicin (2 μg mL^{-1}) appeared to be made less toxic by the addition of triclocarban, but the toxicity of the highest concentration of gentamicin (4 μg mL^{-1}) was enhanced by triclocarban (Fig. 2).

EoP increased for co-exposures with clotrimazole, fluoxetine, methyl paraben, sodium benzoate triclocarban and triclosan at 4 μg mL^{-1} gentamicin (Fig. 2). EoP decreased for co-exposures to either carbamazepine or Roundup at 4 μg mL^{-1} gentamicin. Gentamicin concentrations of 2 μg mL^{-1} also increased EoP on clotrimazole, diclofenac, methyl paraben sodium benzoate, triclosan and triclocarban. Conversely, EoP decreased on carbamazepine, ibuprofen, Roundup, triclocarban and triclosan.

3.2. DMSO alters responses to antibiotics

Our methodology included a control for the solvent DMSO, used to dissolve most of the ECs (Figs. S1 and S2). Solvent controls for DMSO were not required for Roundup or sodium benzoate, which are water-soluble.

In many cases, the measure of the effect using the linear model was different depending on whether or not the series of exposures to the antibiotics was also supplemented with 0.25% DMSO. When the control series was solid LB medium supplemented with both ampicillin (1.5 μg mL^{-1}) and clotrimazole without DMSO (Fig. 1), EoP significantly increased. In contrast, no EoP change was seen in the equivalent parallel series with DMSO. A similar observation was made for the combination of clotrimazole and gentamicin.

DMSO also had a significant effect on EoP as measured in the linear model (Fig. S3). We compared the EoP of *S. enterica* on plates prepared with both 0.25% DMSO and an antibiotic, and plates prepared with only the antibiotics. Supplementation of the medium with DMSO significantly decreased EoP at 1, 1.5 and 2 μg mL^{-1} ampicillin. DMSO switched effects on EoP on gentamicin from a decrease at at 4 μg mL^{-1}, to an increase at 2 μg mL^{-1}.

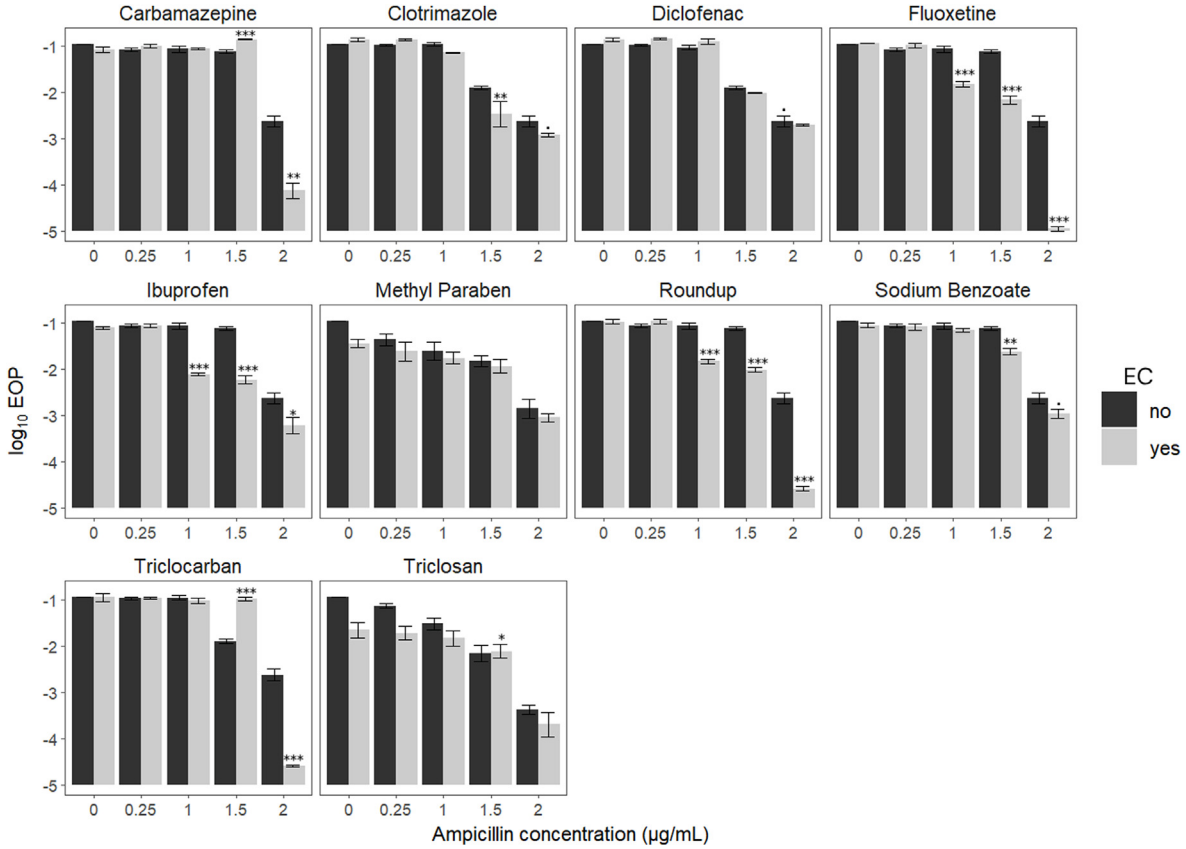

Fig. 1. Efficiency of plating (EoP) of *S. enterica* on increasing concentrations of ampicillin, with and without an emerging contaminant (EC). Error bars represent mean standard deviation.

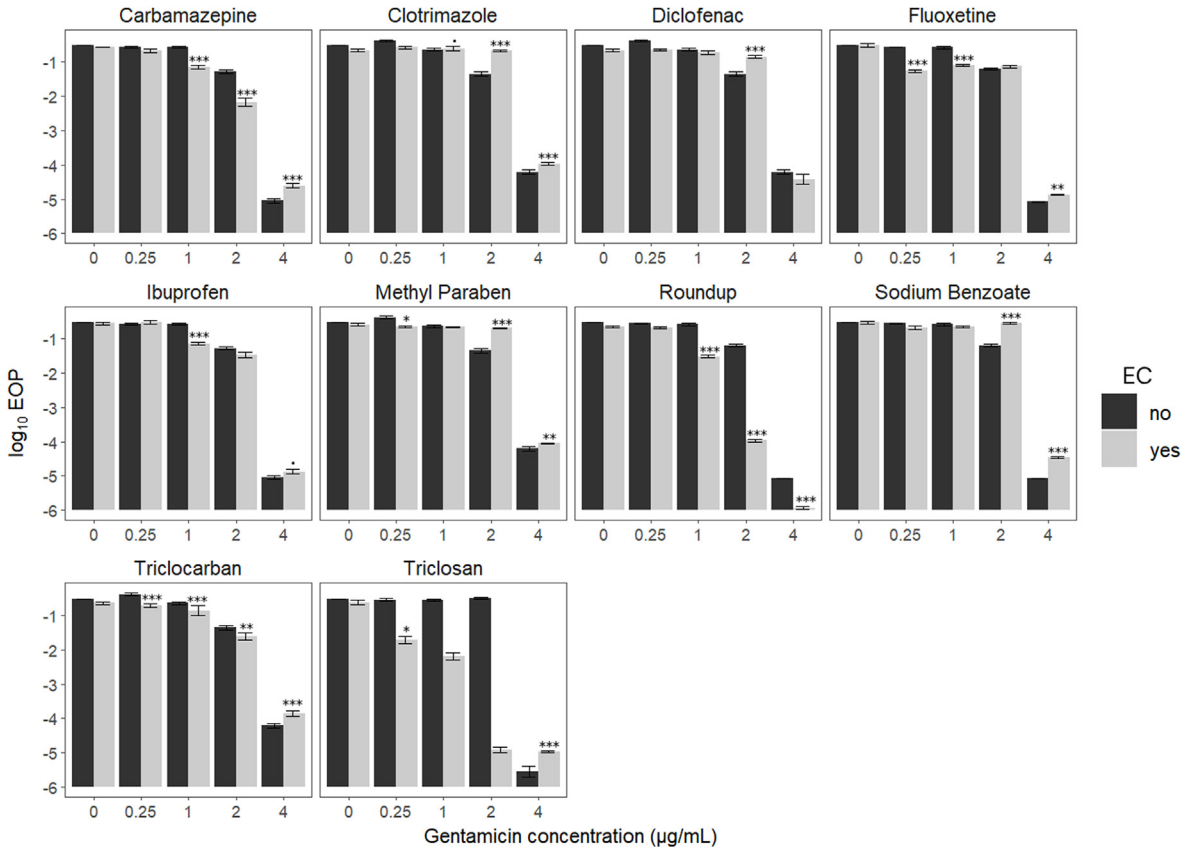

Fig. 2. Efficiency of plating (EoP) of *S. enterica* on increasing concentration of gentamicin, with and without an emerging contaminant (EC). Error bars represent mean standard deviation.

3.3. Emerging contaminants can alter plasmid transmission frequencies

All ECs tested, except triclocarban, increased plasmid transmission frequency in intra-species conjugation (Fig. 3). Triclocarban did not have an effect on RP4 transmission frequency, but there was a consistent dose-response trend (Fig. 3). DMSO alone did not have a significant effect on transmission frequency (data not shown).

There were some subtle differences in the kinetics. In general, transmission frequencies were consistent with doses in exposures to carbamazepine, clotrimazole, fluoxetine, methyl paraben, Roundup or triclosan. For Roundup, at the highest concentration tested, plasmid transmission frequencies fell to levels observed without Roundup.

Following 3-h exposures to carbamazepine, clotrimazole or fluoxetine, the donor population was reduced relative to the control (data not shown). The donor numbers were unaffected by 3-h exposures to Roundup. Likewise, DMSO did not affect the survival of donor or recipient exconjugants.

We also measured the transmission frequency of RP4 between bacteria of different species during exposure to five selected ECs (carbamazepine, clotrimazole, diclofenac, Roundup or triclocarban) on a solid substrate. In contrast to intra-species transmission frequencies, three out of five of these ECs did not significantly increase plasmid transmission frequency in matings between a donor *S. enterica* and recipient *E. coli* (Fig. S4). Increased transmission was observed at some concentrations of carbamazepine or diclofenac.

4. Discussion

4.1. The effect of emerging contaminants on antibiotic resistance was variable and unpredictable

We observed different responses to antibiotics when *S. enterica* was simultaneously exposed pairwise to ten ECs. Combined with the different ECs, sometimes the bacteria were phenotypically more or less responsive to the antibiotic. Ampicillin was often lethal at lower concentrations when combined with one of the ECs. More varied effects were observed in combinations including gentamicin.

In addition, we observed both of these effects with the same EC, but at different concentrations of the same antibiotic. For example, the EoP decreased in combination with the anti-epileptic drug carbamazepine at 2 μg mL^{-1}, but increased at 4 μg mL^{-1}. To our knowledge, this concentration-dependent switch in phenotypic responses is a novel observation. This may mean that at low concentrations of gentamicin and carbamazepine, such as those possible in the wastewater environment, *Salmonella* is more susceptible to gentamicin.

From previous work we hypothesise that with combinations of EC and antibiotic that increase the toxicity of the antibiotic, resistance is more likely to evolve in environments with lower concentrations of the antibiotic [16]. A bi-modal EoP as observed here suggesting an even more complex outcome is possible. Fitness of competing strains may vary both because of their individual MIC phenotypes and antibiotic/EC concentration pockets. Currently, it is difficult to explain this observation as the mechanisms of increased susceptibility to antibiotics in combination with ECs are not fully characterised and likely to involve multiple biochemical processes.

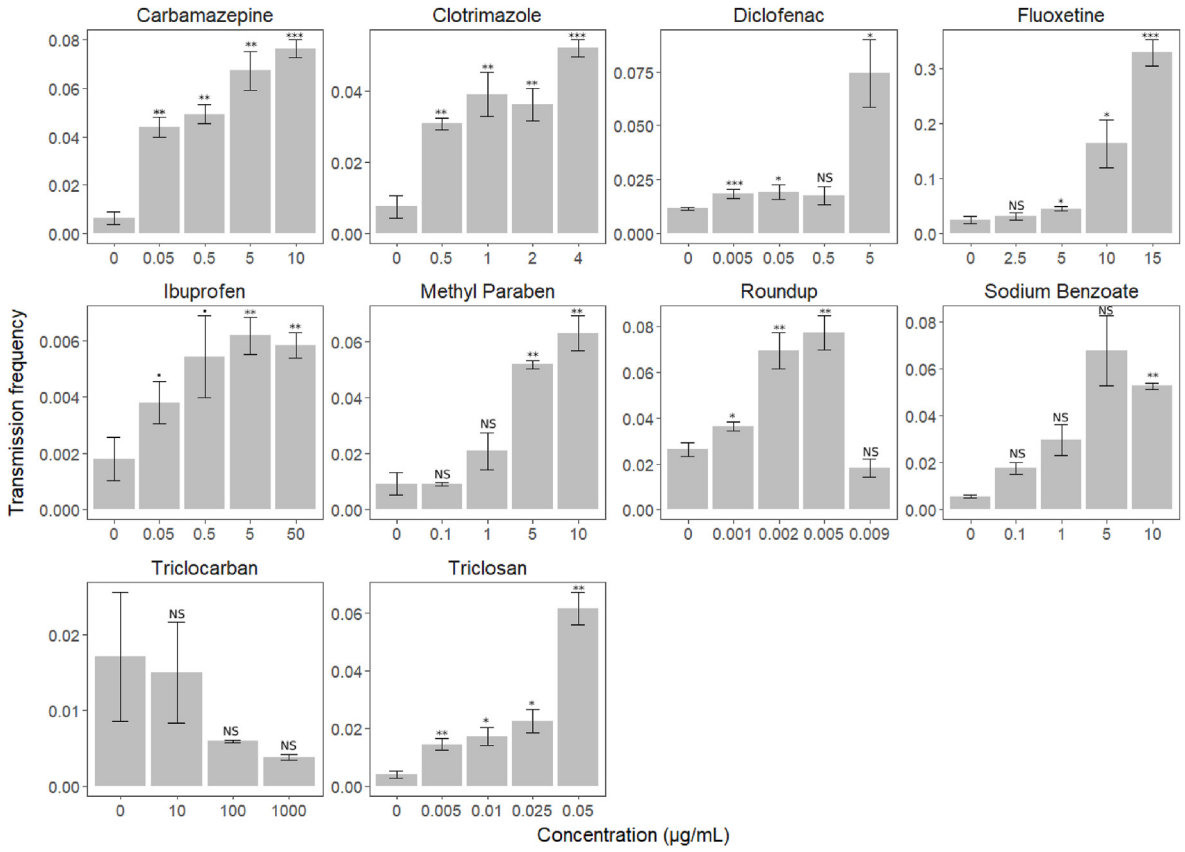

Fig. 3. Transmission frequency of RP4 between an isogenic *E. coli* pair during conjugant exposure to different concentrations of ECs. Transmission frequency is reported in transconjugants per limiting parent. NS: not significant, p-value was greater than 0.1. Error bars represent mean standard deviation.

What remains an important lesson from the totality of these observations, those made by us and others previously [18,19,38], is that empirical testing of combinations will not be displaced by simple predictions based on observation of different ECs and antibiotics in single exposures. For example, the antimicrobial triclocarban and triclosan, which are analogues, elicited different responses when combined with ampicillin, but not gentamicin. As another example, sodium benzoate increased susceptibility to ampicillin, but increased resistance to gentamicin.

The relevance of the effect cannot be assumed to be the same in all environments and all contexts. For example, there is a tendency to see synergy or additive effects as opportunities to increase therapeutic options [39]. Such ideas need to be considered with caution. Because the EoP decreased when ampicillin was combined with the ECs also means that the selection of resistant genotypes begins at lower concentrations of the antibiotic [16]. At the clinic, this may not be relevant, but it may be in a waste stream. The concentrations of the ECs that made a statistically significant effect on the response to an antibiotic may be above normal background contamination levels. However, they provide an insight into the potential to cause a biological effect, even one that would not meet the magnitude necessary for laboratory experiments to confirm, and which could be relevant in places where an EC was concentrated.

4.2. Solvents used in studies of emerging contaminants might affect interpretation of results

We observed different EoPs depending on the comparator used. Without controlling for DMSO, we could have in some combination exposures mistaken a response to the solvent for a response to the EC. Conversely, the lack of a DMSO control would have in other cases masked some of the significant effects. Prior studies, although using DMSO and other solvents sparingly, rarely reported results of DMSO-only exposures when measuring responses to antibiotics. However, our results and prior studies indicate that DMSO can alter the response of bacteria to various antibiotics [40,41]. This may be an important aspect to consider for comparisons between studies.

It is interesting to note that DMSO is available as an at home treatment in the form of a gel [42]. DMSO is also found in wastewater and could be considered an EC [43].

4.3. Emerging contaminants increased plasmid transmission frequency

Like prior studies, we showed that exposure to a range of ECs resulted in increased plasmid transmission [6,7,22,44]. Transmission frequencies were generally dose responsive.

In some cases, our results differed from previous studies. Plasmid transmission frequency decreased at higher concentrations of triclocarban in our study, but others demonstrated an increase in transmission with triclocarban exposure [6]. Because we only used an *inc*P plasmid, the question of how generalisable our results are to conjugative plasmids from different incompatibility groups requires further investigation.

4.4. Conclusions

Exposure to the ECs used in our study was variously associated with increase in resistance, or increase in susceptibility to

ampicillin, gentamicin, or both. These observations were expected, but the magnitude and direction of the effect could not be predicted *a priori* using information about single exposures. Further, ECs generally increased transmission of plasmids with AMR genes. The ECs we studied here are either present, or likely to be present, in the environment (Table 2). We have shown that they may contribute to an increased prevalence of AMR. We also demonstrated that the effect of EC on AMR is dependent on antibiotic concentration. This is important because it highlights the significance of variable environmental conditions in the development of AMR. In situations where antibiotics are in low concentrations, such as contaminated sites and wastewater, selection for resistant individuals can still occur. Ongoing improvements to legislation to regulate and reduce the use of problematic ECs are crucial.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare no competing interests. The funding sources played no role in the experimental design or conclusions of this study.

Acknowledgements

This research was funded through the New Zealand Ministry for Business Innovation and Employment (MBIE) Endeavour grant: Managing the Risk of Emerging Contaminants (CAWX1708).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2023.100257.

References

[1] A. Cassini, L.D. Högberg, D. Plachouras, A. Quattrocchi, A. Hoxha, G.S. Simonsen, M. Colomb-Cotinat, M.E. Kretzschmar, B. Devleesschauwer, M. Cecchini, Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis, Lancet Infect. Dis. 19 (1) (2019) 56–66, https://doi.org/10.1016/S1473-3099(18)30605-4.

[2] E. Tacconelli, M.D. Pezzani, Public health burden of antimicrobial resistance in Europe, Lancet Infect. Dis. 19 (1) (2019) 4–6, https://doi.org/10.1016/s1473-3099(18)30648-0.

[3] I. Alderton, B.R. Palmer, J.A. Heinemann, I. Pattis, L. Weaver, M.J. Gutiérrez-Ginés, J. Horswell, L.A. Tremblay, The role of emerging organic contaminants in the development of antimicrobial resistance, Emerging Contam. 7 (2021) 160–171, https://doi.org/10.1016/j.emcon.2021.07.001.

[4] D.E. Carey, P.J. McNamara, The impact of triclosan on the spread of antibiotic resistance in the environment, Front. Microbiol. 5 (2015) 780, https://doi.org/10.3389/fmicb.2014.00780.

[5] J. Lu, Y. Wang, J. Li, L. Mao, S.H. Nguyen, T. Duarte, L. Coin, P. Bond, Z. Yuan, J. Guo, Triclosan at environmentally relevant concentrations promotes horizontal transfer of multidrug resistance genes within and across bacterial genera, Environ. Int. 121 (2018) 1217–1226, https://doi.org/10.1016/j.envint.2018.10.040.

[6] T. Cen, X. Zhang, S. Xie, D. Li, Preservatives accelerate the horizontal transfer of plasmid-mediated antimicrobial resistance genes via differential mechanisms, Environ. Int. 138 (2020) 105544, https://doi.org/10.1016/j.envint.2020.105544.

[7] Y. Wang, J. Lu, S. Zhang, J. Li, L. Mao, Z. Yuan, P.L. Bond, J. Guo, Non-antibiotic pharmaceuticals promote the transmission of multidrug resistance plasmids through intra- and intergenera conjugation, ISME J. 15 (9) (2021) 2493–2508, https://doi.org/10.1038/s41396-021-00945-7.

[8] M. Jin, J. Lu, Z. Chen, S.H. Nguyen, L. Mao, J. Li, Z. Yuan, J. Guo, Antidepressant fluoxetine induces multiple antibiotics resistance in Escherichia coli via ROS-mediated mutagenesis, Environ. Int. 120 (2018) 421–430, https://doi.org/10.1016/j.envint.2018.07.046.

[9] J.E. Cavanagh, K. Trought, C. Mitchell, G. Northcott, L.A. Tremblay, Assessment of endocrine disruption and oxidative potential of bisphenol-A, triclosan, nonylphenol, diethylhexyl phthalate, galaxolide, and carbamazepine, common contaminants of municipal biosolids, Toxicol. Vitro 48 (2018) 342–349, https://doi.org/10.1016/j.tiv.2018.02.003.

[10] P. Emnet, A.S. Mahaliyana, G. Northcott, S. Gaw, Organic micropollutants in wastewater effluents and the receiving coastal waters, sediments, and biota of Lyttelton Harbour (Te Whakaraupō), New Zealand, Arch. Environ. Contam. Toxicol. 79 (4) (2020) 461–477, https://doi.org/10.1007/s00244-020-00760-9.

[11] G.L. Northcott, J.-A. Cavanagh, O. Champeau, L.A. Tremblay, in: Australasian Bulletin of Ecotoxicology and Environmental Chemistry (Ed.), The Effects of the Mixtures of Three Micro-contaminants Commonly Found in Biosolids on Earthworm Reproduction, 2019, pp. 1–12. https://www.researchgate.net/publication/336868278.

[12] A. Barrick, O. Champeau, J. Butler, T. Wiles, M. Boundy, L.A. Tremblay, Investigating the ecotoxicity of select emerging organic contaminants toward the marine copepod Gladioferens pectinatus, Environ. Toxicol. Chem. 41 (3) (2022) 792–799, https://doi.org/10.1002/etc.5275.

[13] L.A. Tremblay, G.L. Northcott, Analysis of Waikato River Water Samples for Selected Endocrine Disrupting Chemicals and Hormonal Activity, Waikato Regional Council, New Zealand, 2013. TR201355.pdf (waikatoregion.govt.nz).

[14] G.L. Northcott, J. Strong, L.A. Tremblay, A.L. Wilkins, WWTPs contribute significant loadings of emerging organic contaminants to New Zealand's aquatic environment, in: SETAC Australasia 'What's in Our Water Symposium', 2013 (Melbourne, Australia).

[15] M.L. Ledoux, N. Hettiarachchy, X. Yu, L. Howard, S.-O. Lee, Penetration of glyphosate into the food supply and the incidental impact on the honey supply and bees, Food Control 109 (2020) 106859, https://doi.org/10.1016/j.foodcont.2019.106859.

[16] B. Kurenbach, A.M. Hill, W. Godsoe, S. van Hamelsveld, J.A. Heinemann, Agrichemicals and antibiotics in combination increase antibiotic resistance evolution, PeerJ 6 (2018) e5801, https://doi.org/10.7717/peerj.5801.

[17] Y. Xing, S. Wu, Y. Men, Exposure to environmental levels of pesticides stimulates and diversifies evolution in Escherichia coli toward higher antibiotic resistance, Environ. Sci. Technol. 54 (14) (2020) 8770–8778, https://doi.org/10.1021/acs.est.0c01155.

[18] B. Kurenbach, P.S. Gibson, A.M. Hill, A.S. Bitzer, M.W. Silby, W. Godsoe, J.A. Heinemann, Herbicide ingredients change Salmonella enterica sv. Typhimurium and Escherichia coli antibiotic responses, Microbiology (Reading, Engl.) 163 (12) (2017) 1791–1801, https://doi.org/10.1099/mic.0.000573.

[19] B. Kurenbach, D. Marjoshi, C.F. Amábile-Cuevas, G.C. Ferguson, W. Godsoe, P. Gibson, J.A. Heinemann, Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium, mBio 6 (2015) 2, https://doi.org/10.1128/mBio.00009-15.

[20] Y. Wang, Z. Yu, P. Ding, J. Lu, L. Mao, L. Ngiam, Z. Yuan, J. Engelstädter, M.A. Schembri, J. Guo, Antidepressants can induce mutation and enhance persistence toward multiple antibiotics, Proc. Natl. Acad. Sci. U.S.A. 120 (5) (2023) e2208344120, https://doi.org/10.1073/pnas.2208344120.

[21] Y. Wang, J. Lu, L. Mao, J. Li, Z. Yuan, P.L. Bond, J. Guo, Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera, ISME J. 13 (2) (2019) 509–522, https://doi.org/10.1038/s41396-018-0275-x.

[22] H. Zhang, J. Liu, L. Wang, Z. Zhai, Glyphosate escalates horizontal transfer of conjugative plasmid harboring antibiotic resistance genes, Bioengineered 12 (1) (2021) 63–69, https://doi.org/10.1080/21655979.2020.1862995.

[23] L. Fernández, E.B. Breidenstein, R.E. Hancock, Creeping baselines and adaptive resistance to antibiotics, Drug Resist. Updates 14 (1) (2011) 1–21, https://doi.org/10.1016/j.drup.2011.01.001.

[24] E. Christaki, M. Marcou, A. Tofarides, Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence, J. Mol. Evol. 88 (1) (2020) 26–40, https://doi.org/10.1007/s00239-019-09914-3.

[25] L.A. Tremblay, G.L. Northcott, Analysis of Emerging Organic Contaminants in Effluent of the Bell Island Wastewater Treatment Plant (Cawthron Report; 3069) Prepared for the Nelson Regional Sewerage Business Unit, Prepared for the Nelson Regional Sewerage Business Unit, 2017, p. 14 (plus appendix).

[26] A.K. Sarmah, G.L. Northcott, F.D. Leusch, L.A. Tremblay, A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand, Sci. Total Environ. 355 (1–3) (2006) 135–144, https://doi.org/10.1016/j.scitotenv.2005.02.027.

[27] S. van Hamelsveld, B. Kurenbach, D.J. Paull, W.A. Godsoe, G.C. Ferguson, J.A. Heinemann, Indigenous food sources as vectors of Escherichia coli and antibiotic resistance, Environ. Pollut. 334 (2023) 122155, https://doi.org/10.1016/j.envpol.2023.122155.

[28] G.C. Ferguson, J.A. Heinemann, M.A. Kennedy, Gene transfer between Salmonella enterica serovar Typhimurium inside epithelial cells, J. Bacteriol. 184 (8) (2002) 2235–2242, https://doi.org/10.1128/JB.184.8.2235-2242.2002.

[29] H.A. Assress, H. Nyoni, B.B. Mamba, T.A.M. Msagati, Occurrence and risk assessment of azole antifungal drugs in water and wastewater, Ecotoxicol. Environ. Saf. 187 (2020) 109868, https://doi.org/10.1016/j.ecoenv.2019.109868.

[30] L.A. Tremblay, M. Stewart, B.M. Peake, J.B. Gadd, G.L. Northcott, Review of the Risks of Emerging Organic Contaminants and Potential Impacts to Hawke's

Bay, Hawke's Bay Regional Council New Zealand, 2011, p. 39. https://www.researchgate.net/publication/256498053.
[31] World Health Organisation and International Programme on Chemical Safety, Benzoic Acid and Sodium Benzoate, World Health Organization, Geneva, 2000.
[32] J.E. Drewes, E. Dickenson, S. Snyder, Contributions of Household Chemicals to Sewage and Their Relevance to Municipal Wastewater Systems and the Environment, vol. 8, IWA Publishing, 2009.
[33] J.A. Heinemann, R.G. Ankenbauer, Retrotransfer in Escherichia coli conjugation: bidirectional exchange or de novo mating? J. Bacteriol. 175 (3) (1993) 583–588, https://doi.org/10.1128/jb.175.3.583-588.1993.
[34] G. Reid, The Cell Envelope as a Barrier to Genetic Exchange, The Cell Envelope as a Barrier to Genetic Exchange A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Cellular and Molecular Biology at the University of Canterbury, University of Canterbury, Christchurch, New Zealand, 1996, https://doi.org/10.26021/11864.
[35] RStudio Core Software Development Team, RStudio: Integrated Development for R, RStudio (2020). https://www.r-project.org/.
[36] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag, New York, 2016. https://ggplot2.tidyverse.org. ISBN 978-3-319-24277-4.
[37] S.S. Shapiro, M.B. Wilk, An analysis of variance test for normality (complete samples), Biometrika 52 (1965) 591–611, https://doi.org/10.1093/biomet/52.3-4.591.
[38] D.K. Kleiner, S.E. Katz, P.M. Ward, Development of in vitro antimicrobial resistance in bacteria exposed to residue level exposures of antimicrobial drugs, pesticides and veterinary drugs, Chemotherapy 53 (2) (2007) 132–136, https://doi.org/10.1159/000100012.
[39] A. Karine de Sousa, J.E. Rocha, T. Gonçalves de Souza, T. Sampaio de Freitas, J. Ribeiro-Filho, H.D. Melo Coutinho, New roles of fluoxetine in pharmacology: antibacterial effect and modulation of antibiotic activity, Microb. Pathog. 123 (2018) 368–371, https://doi.org/10.1016/j.micpath.2018.07.040.
[40] H.C. Ansel, W.P. Norred, I.L. Roth, Antimicrobial activity of dimethyl sulfoxide against Escherichia coli, Pseudomonas aeruginosa, and Bacillus megaterium, J. Pharmaceut. Sci. 58 (7) (1969) 836–839, https://doi.org/10.1002/jps.2600580708.
[41] H. Mi, D. Wang, Y. Xue, Z. Zhang, J. Niu, Y. Hong, K. Drlica, X. Zhao, Dimethyl sulfoxide protects Escherichia coli from rapid antimicrobial-mediated killing, Antimicrob. Agents Chemother. 60 (8) (2016) 5054–5058, https://doi.org/10.1128/AAC.03003-15.
[42] I. Elisia, H. Nakamura, V. Lam, E. Hofs, R. Cederberg, J. Cait, M.R. Hughes, L. Lee, W. Jia, H.H. Adomat, E.S. Guns, K.M. McNagny, I. Samudio, G. Krystal, DMSO represses inflammatory cytokine Production from human blood Cells and reduces Autoimmune Arthritis, PLoS One 11 (3) (2016) e0152538, https://doi.org/10.1371/journal.pone.0152538.
[43] X. Cheng, M. Wodarczyk, R. Lendzinski, E. Peterkin, G.A. Burlingame, Control of DMSO in wastewater to prevent DMS nuisance odors, Water Res. 43 (12) (2009) 2989–2998, https://doi.org/10.1016/j.watres.2009.04.013.
[44] P. Ding, J. Lu, Y. Wang, M.A. Schembri, J. Guo, Antidepressants promote the spread of antibiotic resistance via horizontally conjugative gene transfer, Environ. Microbiol. 24 (11) (2022) 5261–5276, https://doi.org/10.1111/1462-2920.16165.