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Dawning of a new ERA: Environmental Risk Assessment of antibiotics and their potential to select for antimicrobial resistance

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ABSTRACT

Antibiotics and antimicrobials are used, misused and overused in human and veterinary medicine, animal husbandry and aquaculture. These compounds can persist in both human and animal waste and then enter the environment through a variety of mechanisms. Though generally measured environmental concentrations (MECs) of antibiotics in aquatic systems are significantly lower than point of therapeutic use concentrations, there is increasing evidence that suggests these concentrations may still enrich antimicrobial resistant bacteria. In light of this evidence, a rigorous and standardised novel methodology needs to be developed which can perform environmental risk assessment (ERA) of antimicrobials in terms of their selective potential as well as their environmental impact, to ensure that diffuse and point source discharges are safe. This review summarises and critically appraises the current methodological approaches that study selection at below point of therapeutic use, or sub-inhibitory, concentrations of antibiotics. We collate and compare selective concentration data generated to date. We recommend how these data can be interpreted in line with current ERA guidelines; outlining and describing novel concepts unique to risk assessment of AMR (such as direct selection of AMR or increased persistence of AMR). We consolidate terminology used thus far into a single framework that could be adopted moving forward, by proposing predicted no effect concentrations for resistance (PNECRs) and predicted no effect concentrations for persistence (PNECPs) be determined in AMR risk assessment. Such a framework will contribute to antibiotic stewardship and by extension, protection of human health, food security and the global economy.

1. Introduction

Antimicrobial resistance (AMR) is one of the greatest threats to global health and the economy. It has been estimated that by 2050, antimicrobial resistant infections will be the leading cause of death worldwide and result in the loss of 100 trillion dollars of GDP (O'Neill 2014). The environmental dimension of AMR is being increasingly recognised as an area for potential mitigation, with several international efforts to raise the profile of this issue, particularly in a 'One Health' context (EU 2019; UNEP 2019; WHO 2015).

Antibiotics can enter the aquatic environment through a variety of interconnected pathways. These include: release from industrial and wastewater treatment plants, run off from agricultural fields fertilised with municipal human sewage sludge and/or animal manure and/or treated with antibiotic plant protection products; and in some countries, direct discharge of waste where there is no centralised wastewater treatment infrastructure. Antibiotic concentrations in the environment generally range from mg/L in the most impacted environments, such as industrial waste; to µg/L in wastewater and wastewater effluent; to ng/L in surface waters (aus der Beek et al. 2016; UmweltBundesamt 2019). This is of great concern regarding the development of AMR, given the growing body of research (outlined in this review) that demonstrates environmentally relevant concentrations of antibiotics can increase levels of AMR (i.e. select for resistance). In order to curb the rise in AMR, it is essential to recognise, assess and mitigate the risk antibiotics pose in the environment, as a direct result of anthropogenic activities. Development of an AMR specific environmental risk assessment (ERA) framework will be crucial, but has yet to be realised and is the focus of this review.

Currently, ERA is required for pharmaceuticals when the predicted environmental concentration (PEC) is greater than 10 ng/L,

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for members of the European Union (EMA 2006); or 100 ng/L, for the USA (FDA 1998a). The only ecotoxicological test required that specifically targets bacteria is the activated sludge respiration inhibition test (ASRIT) (EMA 2006). The ASRIT exposes an activated sludge bacterial community to a test compound and measures for significant decreases in respiration (OECD 2009). Concerns that the ASRIT and other eukaryotic ecotoxicological endpoints may not be protective of selection for AMR in the environment, and the subsequent need for novel methods to quantify selective endpoints, have been circulating for close to ten years (Ashbolt et al., 2013; Brandt et al., 2015; Gullberg et al., 2011; Le Page et al. 2017).

This review summarises, for the first time, the benefits and limitations of the different approaches to determining selective concentrations of antibiotics, alongside selective concentrations determined to date. Recommendations on how these data can be interpreted to determine selective endpoints for ERA, with minimal adjustment to current ERA approaches are outlined. Unique challenges posed by AMR through an ERA lens are also discussed.

2. Methods that determine selective concentrations of antibiotics

The potential selection for AMR in natural environments has been largely overlooked, in part, due to the unsupported hypothesis that very low concentrations of antimicrobials or antibiotics are unable to select for AMR. Gullberg et al. (2011) performed the first significant study that challenged this assumption, where they demonstrated that very low antibiotic concentrations could select for antibiotic resistant bacteria by conducting single species competition experiments. Isogenic bacterial species (genetically identical except for the presence of a resistance determinant on the chromosome of the resistant strain) were fluorescently tagged, allowing for their accurate enumeration. These isogenic strains were grown in defined ratios of resistant to susceptible cells, in the presence of multiple concentrations of antibiotics in a serial passage experiment where the bacteria were transferred into fresh media and antibiotic each day.

Enumeration after antibiotic exposure allowed calculation of selection coefficients, which represent the difference in fitness between resistant and susceptible bacteria (Otto and Day 2007). For example, selection coefficients greater than 0 indicate the bacteria harbouring the studied resistance gene are under positive selection, and their numbers will increase over time. Conversely, selection coefficients less than 0 indicate numbers of resistant bacteria will decrease over time (Day et al., 2015). Selection coefficients can be plotted against antibiotic concentration, and the x-axis intercept of the line of best fit through these data points indicates the minimal selective concentration (MSC). This is the lowest concentration at which resistance is predicted to be selected for (i.e. when numbers of resistant bacteria are expected to increase over time relative to non-resistant bacteria). Gullberg et al. (2011) found that the MSC can be over 200x lower than the Minimum Inhibitory Concentration (MIC) of the susceptible strain and confirmed antibiotics do not need to exceed the MIC of susceptible bacteria in order to have a selective effect. Gullberg et al. built upon their previous work by determining MSCs for antimicrobial resistance genes (ARGs) located on a mobile, clinically isolated plasmid. Again, MSCs were much lower than MICs of the susceptible strain (Gullberg et al., 2014).

These studies raised concerns regarding the potential selection of AMR occurring in the environment, as selection was observed at environmentally relevant concentrations, down to 2 ng/L (Gullberg et al., 2011). However, the relevance of these findings in relation to the environment is unclear, as single isogenic host species are unlikely to be representative of the complex competitive interactions occurring within, and other selective pressures encountered by, environmental communities of bacteria. This was confirmed by a study that found MSCs differ when isogenic strains are cultured in single species competition experiments, compared to within a bacterial community (Klümper et al., 2019). In this study, MSCs of gentamicin and kanamycin were determined using isogenic strains in single species competitions assays, as well as when the strains were embedded within a pig faecal bacterial community. The MSCs of gentamicin and kanamycin increased by 13 and 43x, respectively, when in the presence of the community; though the exact MSC values were not reported (Klümper et al., 2019).

Other work has attempted to determine selective concentrations within complex bacterial communities in an effort to increase environmental relevance. Strengths, weaknesses, opportunities and threats of these different approaches are detailed in Table 1, whilst experimental details are outlined below.

The first of the experimental approaches that used complex communities (Lundstrom et al., 2016) established a biofilm from untreated wastewater in a flow through system, which was continuously exposed to low concentrations of tetracycline, low nutrient media and untreated wastewater. A variety of methods were explored for determining the selective concentration, including phenotypic and culture-based methods; the pollution induced community tolerance (PICT) assay (Schmitt et al., 2005); and molecular methods including qPCR and metagenome analyses. The qPCR method was deemed the most sensitive, identifying the lowest tetracycline concentration where an effect was observed at 1 ug/L. Two different tetracycline resistance genes were quantified, alongside 16S rRNA copy number as a proxy for number of bacteria, enabling determination of molecular ARG 'prevalence' (ARG/16S rRNA copy number (Lundstrom et al., 2016)).

Further studies have also used untreated wastewater, but in closed experimental systems where bacterial communities are passed into fresh antibiotic media daily for 7 or 8 days. Metagenomic analyses were used to confirm suitable gene targets for qPCR, and molecular ARG prevalence was quantified with qPCR at the beginning and end of the experiment, which allowed determination of the MSC, as well as statistically defined selective concentrations (Murray et al., 2018; Stanton et al., 2020).

More recently, selection experiments were performed using the flow through system described by Lundstrom et al. (2016) and a batch microcosm system within the same study (Kraupner et al., 2018). Both used the same minimal medium, though batch microcosms were incubated at 25 °C and the flow through system was maintained at 20 °C. Percentages of *Escherichia coli* resistant to ciprofloxacin (the test antibiotic) were calculated, and the lowest concentration where an increase was observed relative to the control occurred at 5 µg/L and 10 µg/L for the flow through system and batch microcosm, respectively. Though this differs by a factor of two, the no observed effect concentration (NOEC; the concentration directly below the lowest observed effect concentration) for both systems was 1 µg/L. Therefore in this instance, the two systems were comparable, and may even have yielded the same result were the same concentrations tested in both systems (Kraupner et al., 2018).

A comparison was also made between the flow through system (Lundstrom et al., 2016) and the high nutrient, batch microcosm (Murray et al., 2018) in a study by Stanton et al. (2020). Both Lundstrom et al. (2016) and Stanton et al. (2020) quantified increases in tetracycline resistance genes using qPCR, and when data were analysed in the same way, the same NOEC was observed. However, as hypothesised previously (Murray et al., 2018), the observed effect was likely increased persistence of resistance genes as opposed to positive selection (Stanton et al., 2020). This minimal increased persistence concentration (MIPC) is still relevant for human health risk assessment, as it results in higher numbers of

Table 1
Strengths, weakness, opportunities and threats (SWOT) analysis of different methodological approaches to determining effect concentrations of antibiotics.

Approach	Strengths	Weaknesses	Opportunities	Threats
Isogenic single species competition assays e.g. Gullberg et al. (2011, 2014) and N. Kraupner et al. (2020)	High resolution determination of MSCs due to low experimental variance. Definitive observation of positive selection as initial prevalence of resistance quantified. High nutrient and temperature conditions enable rapid data generation. Use of well characterised model bacterial species (e.g. *E. coli*) enhances replicability. Single spikes of exposure antibiotic per 24 h may represent peak load effluents from wastewater treatment plants in the morning or evening	Single species used is unrepresentative of complex, mixed communities existing in the environment. Does not capture intra-species or inter-species competition/mutualism which may influence selection. Unable to account for horizontal gene transfer within/between species. Specialised equipment and personnel required (e.g. molecular microbiologists, fluorescence activated cell sorting machine). Chemical quantification of antibiotics not usually performed (though possible)	Nutrient and temperature conditions can be easily modified to increase environmental relevance, which should be paired with additional chemical data to understand degradation dynamics. Study enrichment of chromosomal mutations/genes or plasmid-borne resistance genes with minimal confounding variables possible. Could use more ecologically relevant species to represent particular environments or ecosystem services provided by prokaryotes. System can be applied to look at mixture or combined exposure effects	Possible under or overestimation of selective endpoints due to simplicity of system. High cost for routine performance due to requirement for continual development of novel isogenic strains may impact feasibility of roll out. Use of different isogenic strains may alter MSC, leading to potential under or overestimation of risk
Biofilm established from mixed bacterial community in a flow through system e.g. Lundstrom et al. (2016) and Kraupner et al. (2018 & 2020)	Use of mixed bacterial community captures some intra- and inter-species competition expected in natural environments. Low temperature and nutrient conditions also emulate environmentally realistic conditions. Biofilm with flow through represents formation of natural biofilms, e.g. in wastewater effluent pipes and trickling filter beds. Chemical quantification performed in liquid phase of system	Flow through systems are technically demanding and face issues such as wash out or antibiotic degradation if flow rate is set too high or too low. Chemical quantification is technically difficult in biofilms, this is complicated further as penetration of antibiotics is likely to be lower leading to overestimation of selective endpoints if only liquid phase is quantified. Horizontal gene transfer frequencies are not determined. Likely low replicability	Peak loads and dynamics of antibiotic concentrations released in wastewater following different treatment processes could be emulated in a flow through system to increase realism. Metagenomics facilitates quantification of changes in resistance genes and in community composition providing information on co-selection and potential loss of ecosystem services. System could be applied to look at mixture or combined exposure effects. Possibility to study community resilience	Continual addition of community (i.e. wastewater) means observed effects may be due to changes in community and not solely due to exposure to test antibiotic. Unable to establish a starting prevalence of resistance means observed effects may be due to increased persistence of resistance, rather than positive selection (could overestimate risk). Currently are limited in terms of detecting selection for mutations; however these pose relatively lower risk compared to mobile genes
Mixed bacterial community (sewage) batch microcosms e.g. Murray et al. (2018 & 2020) and Stanton et al. (2020)	Use of mixed bacterial community captures some intra- and inter-species competition expected in natural environments. High nutrient and temperature conditions enable rapid data generation. Definitive observation of positive selection as initial prevalence of resistance quantified. Medium to high replicability. Single spikes of exposure antibiotic per 24 h may represent release from wastewater treatment plants in the morning or evening. Straightforward chemical quantification allows accurate selective endpoint determination. Similar effect concentrations observed to flow through systems	Horizontal gene transfer frequencies are not determined. Currently, only high temperature, high nutrient microcosms have been used which may not represent natural environments. Higher variation than single species methods, due to variation inherent in the community and potential founder population biases and small sample volume. Metagenomics are recommended to determine a representative qPCR target – increased expense	Nutrient and temperature conditions can be easily modified to increase environmental relevance, which should be paired with additional chemical data to understand degradation dynamics. Different bacterial inocula can be tested to quantify risk for different aquatic environments. Metagenomics facilitates quantification of changes in resistance genes and in community composition providing information on co-selection and potential loss of ecosystem services. Both MSC and statistically significant selective endpoints can be determined. System could be applied to look at mixture or combined exposure effects. Possibility to study community resilience	Potential exclusion of ecologically relevant microorganisms and impacts on selection of ARGs due to high nutrient and high temperature. Possible under or overestimation of real-world risk due to high nutrient, high temperature conditions, though results tend to agree with more environmentally realistic flow through systems. Currently are limited in terms of detecting selection for mutations; however these pose relatively lower risk compared to mobile genes

(*continued on next page*)

Table 1 (*continued*)

E. coli multi-isolate batch microcosms e.g. N. Kraupner et al. (2020)	Strongly reflects intra-species competition, reducing biases that may result from media/temperature preferences of other species. *E. coli* already monitored with well-established isolation and screening protocols available. Relatively low cost method e.g. compared to qPCR. Confirms increases in phenotypic resistance at a clinical level. Straightforward chemical quantification allows accurate selective endpoint determination. Similar results to other approaches	Horizontal gene transfer frequencies are not determined. Plating is a less sensitive technique than others, e.g. qPCR. Single species used is unrepresentative of complex, mixed communities existing in the environment. Does not determine specific resistance mechanisms enriched, preventing assessment of relative risk posed by mobile or chromosomal resistance mechanisms	Possible to determine both persistence and selection concentrations. System could be applied to look at mixture or combined exposure effects. Possibility to study community resilience. Relatively accessible, e.g. to lower to middle income countries	Risk may be over or underestimated as inter-species competition eliminated from test system. Risk may be overestimated due to relative insensitivity of plating method. Not suitable for antibiotics that are ineffective against *E. coli* / gram negatives (i.e. for antibiotics where clinical breakpoint data are not available). Screening at clinical breakpoint concentrations may underestimate risk due to lack of genetic diversity. Similar results to other approaches, but only one antibiotic tested so far
SELECT method e.g. A.K. Murray et al. (2020)	Cheapest and least labour intensive of the experimental methods by far. Use of mixed bacterial community captures some intra- and inter- species competition expected in natural environments. High nutrient and temperature conditions enable rapid data generation. High replicability. Single spikes of exposure antibiotic per 24 h may represent release from wastewater treatment plants in the morning or evening. Similar effect concentrations observed to other methods, particularly mixed bacterial community batch microcosms. Captures selection acting on all resistance mechanisms within the population	Horizontal gene transfer frequencies are not determined. Does not determine specific resistance mechanisms enriched, preventing assessment of relative risk posed by mobile or chromosomal resistance mechanisms. Cannot determine concentrations that increase persistence of resistance	Straightforward chemical quantification would allow accurate selective endpoint determination. Initial validation suggests endpoints largely unaffected by sewage inoculum used. Initial validation suggests endpoints largely unaffected by changes to experimental conditions (temperature, media). Highly suitable for ring testing/further validation. Highly accessible, e.g. to lower to middle income countries. Could be adapted for environmental surveillance/whole effluent testing. System could be applied to look at mixture or combined exposure effects. Possibility to study community resilience	Possible under or overestimation of real-world risk due to high nutrient, high temperature conditions, though initial validation suggests limited effect further testing is still required. Possible over or underestimation of risk without further validation as a proxy
Estimation of selective endpoints using minimum inhibitory concentration (MIC) data e.g. Kummerer & Henninger (2003), Tello et al. (2012) and Bengtsson-Palme & Larsson (2016)	Rapid and cost-effective data generation. High clinical relevance applicable to human health risk assessment. MIC data routinely generated, updated and easily accessed	MIC data are based on single species responses – community dynamics are not represented. Conversion from MIC to MSC is arbitrary and has little scientific basis; the difference between. MIC and MSC may be species and/or antibiotic specific	Selective endpoints can be validated in experimental studies. Experimental concentrations can be informed by estimated endpoints to reduce cost	Many species within the database do not have ecological cut off (ECOFF) values determined, so risk may be overestimated. MIC data change over time, usually increasing - with this approach, $PNEC^R$s would be higher, this is counterintuitive as when resistance increases, mitigation efforts should also increase

resistant bacteria being present in a given microbiome in the presence of antibiotic than in the absence, which increases the potential of human or animal exposure to these bacteria (Stanton et al., 2020). In addition, there could be increased likelihood of horizontal gene transfer, as there would be a greater number of ARG donors. A selective endpoint for ciprofloxacin was also determined by Stanton et al. (2020) as 7.6 µg/L, very similar to the 5 - 10 µg/L observed in Kraupner et al. (2018). Together, these points indicate highly replicable, high throughput batch microcosms can produce very similar if not identical selective concentration data as more expensive, technically demanding flow through systems.

A further study by N. Kraupner et al. (2020) compared selective endpoints of trimethoprim in different systems. Changes in taxonomic composition were determined in an effluent sewage community biofilm microcosm, but the study primarily focused on resistant *E. coli* in three different experimental systems (biofilms derived from treated effluent, a community of 149 *E. coli* strains isolated from sewage and isogenic strains of *E. coli* in competition ex-

periments). At 100 µg/L of trimethoprim, significant increases in percentage of resistant *E. coli* in the biofilm and the *E. coli* community were observed, whereas only a trend for significance was observed in taxonomic composition. For the engineered *E. coli*, positive selection coefficients were determined at 100 µg/L, but not 10 µg/L. However, the authors indicate that the cost of the resistance gene was significantly reduced at 10 µg/L (N. Kraupner et al. 2020) (i.e. there would be increased persistence at this concentration (Stanton et al., 2020)) when statistically comparing percentages of engineered resistant E. coli carrying four of the five genes tested. An exposure limit of 1 µg/L was suggested based on this observation.

The most recent experimental method to determine selective endpoints is the SELECT (SELection Endpoints in Communities of bacTeria) method (A.K. Murray et al. 2020). This method exposes a community of sewage bacteria to two-fold dilutions of antibiotics in a 96 well plate and measures optical density in a plate reader. During exponential growth phase of the community, a significant dose-response relationship can be observed. The selective endpoint is the antibiotic concentration that significantly reduces the growth of the community, at the time point where that dose-response relationship is strongest. This has been demonstrated to be a reliable proxy for selection for resistance genes, determined using qPCR in previous studies (Murray et al., 2018; Stanton et al., 2020). Due to the rapidity of the method, selective endpoints for several antibiotics were determined, including trimethoprim at 1.56 µg/L, extremely similar to the 1 µg/L determined previously (N. Kraupner et al. 2020). In the SELECT study, lower temperature and nutrient conditions (use of artificial sewage as the growth media, as in the ASRIT (OECD 2009)) were also tested and had minimal effect on the endpoints derived. This study was also the first to directly compare different sewage inocula for the same antibiotic, and found this too, had minimal effect on the endpoints derived, indicating a single endpoint may be suitable for different geographical locations or environments (A.K. Murray et al. 2020).

Finally, there have been attempts to consider selection by estimating selective endpoints, as opposed to using experimental approaches. The first of these used MIC_{50} values for susceptible pathogens as surrogates for selective endpoints to perform risk assessment of antibiotics in hospital effluent (Kümmerer and Henninger 2003). An assessment factor (AF) of 100 was applied; this relatively large AF was used for two reasons. The first was to estimate the MIC_{10} value, given that NOECs can be considered as the concentration where only a 10% effect is observed (EU 1996). The second was because the PECs were multiplied by 10 to estimate maximum peak loads (Kümmerer and Henninger 2003). The second paper to estimate selective endpoint predicted species sensitivity distributions using MIC data, and extrapolated these selective concentrations across bacterial genera through phylogenetic analyses. By comparing to MECs, it was estimated $\leq$7% of environmental genera could be under selection (Tello et al., 2012). Finally, selective concentrations have been estimated using the EUCAST MIC database to estimate predicted no effect concentrations for resistance ($PNEC^R$s). All MICs for all the species were extracted and MICs above the wildtype were removed. These were adjusted against the numbers of species per antibiotic. Finally, the lowest 1% of all MICs were taken and an AF of 10 applied. This resulted in $PNEC^R$s ranging from 8 ng/L to 64 ug/L (Bengtsson-Palme and Larsson 2016).

All of these studies have not yet determined how community resilience may affect selection for AMR. Community resilience is the ability of a bacterial community to return to its original state, or a new stable state, following the removal of a disturbance (Brandt et al., 2015). The functionality of bacterial communities may be compromised for example, if diversity is reduced and this could be further compounded by exposure to other pollution sources, or changes in temperature, making it particularly pertinent in light of climate change. AMR adds an additional facet to community resilience, as resistance normally results in an evolutionary cost to the host bacterium. However, there are numerous evolutionary mechanisms to mitigate this cost such as presence of compensatory mutations, cost-free resistance traits, or location of resistance genes on plasmids which confer a fitness benefit (Andersson and Hughes 2011). Arguably, following exposure to antibiotics, bacterial communities may never return to a pre-exposure state due to these compensatory effects. Furthermore, it is unclear whether communities that have evolved at selective concentrations of antibiotics are then 'primed' to face future episodes of exposure due to these evolutionary compensatory mechanisms. However, whether communities do become 'primed' will likely dependant on many factors. These include whether the resistance mechanism is 'selfish' or produces 'public goods' (Bottery et al., 2016; Murray et al., 2018). Public good resistance mechanisms, such as antibiotic degradation by enzymes produced by resistant bacteria, could be negatively selected because the degradation of antibiotics is beneficial to all bacteria in the population, whether they produce the enzyme or not. Whether a resistance mechanism is readily mobilisable (i.e. plasmid-borne) will impact the extent of transmission within the community, and the fitness of the bacterial host in the absence of exposure is also key. If future studies point toward a lack of community resilience, then different safe release limits may be required for different environments (e.g. pharmaceutical waste vs municipal waste) or limits may need to change over time depending on the history of exposure.

3. AMR specific era – adjusting and consolidating with current guidelines

All of the above studies have determined selective concentrations, but there are differences in how these are derived, what they are termed and what they mean. Each has different implications for both environmental and human health/exposure risk assessment.

The standardised ERA approach determines the Lowest Observed Effect Concentration (LOEC) on the basis this test concentration has a significantly different response compared to the control (ECHA, 2008, EMA 2006, 2018, FDA 1998b). The concentration below this, the NOEC, is used to derive the Predicted No Effect Concentration (PNEC) by dividing it by an AF (which differs based on chronic or acute exposure and number of studies (ECHA, 2008), see AF section). Environmental risk is then determined by dividing the Predicted Environmental Concentration (PEC) or Measured Environmental Concentration (MEC) by the PNEC, to generate a Risk Quotient (RQ) (EMA 2006). A recent meta-analysis (Le Page et al. 2017) compared the relative sensitivity of environmental species used to derive PNECs in regulatory approaches to ERA.

There are currently four antibiotics (amoxicillin, ciprofloxacin, trimethoprim and sulfamethoxazole) included on the third version of the Water Framework Directive's (WFD) Watch List (Gomez Cortes et al. 2020). These are surface water pollutants where the PEC or MEC regularly exceeds the PNEC, which are being monitored with the European Union to determine if Environmental Quality Standards (EQS) are required (Carvalho et al., 2015; Loos et al., 2018). Until this most recent iteration, these PNECs were based on ecotoxicological endpoints, though AMR data are now being considered (Gomez Cortes et al. 2020).

To enable rapid ERA and human health risk assessment of the selective potential of compounds released into our environments, it would be best to align with current methodologies. However, it has been established that ecotoxicological PNECs are not always protective against selection for AMR (Bengtsson-Palme and Larsson 2016; Le Page et al. 2017; Tell et al., 2019). Therefore, it is rec-

ommended that PNEC^{R}s are used when referring specifically to selective concentrations of antibiotics (which may not always have ecotoxicological effects at ecologically relevant PECs or MECs). Furthermore, as there are several experimental methodologies, several different definitions of selective concentrations also exist.

The first of these methods used was the MSC (Gullberg et al., 2011), as described above. However, it is debatable whether a PNEC should be derived from MSCs as this endpoint is a modelled estimate that can be greatly impacted by the number of test concentrations included in the model and the intervals between these test concentrations. In addition, the MSC is defined as the concentration "where the fitness cost of the resistance is balanced by the antibiotic-conferred selection for the resistant mutant" (Gullberg et al., 2011); or in other words, where resistant strains are under neither positive nor negative selection and there is no longer an evolutionary cost to resistance. Only when stochastic events occur or further increases in selective pressure are introduced, will resistant bacteria be positively selected. Therefore, in terms of protection against selection, the MSC is likely conservative and may marginally overestimate risk if used to calculate a PNEC^R. MSCs are also likely to change when different isogenic strains are used, which could lead to over or underestimation of risk.

The MSC method also presents difficulties as the no antibiotic control may also show positive selection for resistance (N. Kraupner et al. 2020; Stanton et al., 2020) – as all selection coefficients are >0, the intercept (MSC) cannot be calculated. Although this could be overcome by subtracting the no antibiotic control selection coefficients from the exposure selection coefficients, this may introduce bias. The MSC method does, however, require quantification of resistance at the beginning of the exposure experiment, as this is crucial for understanding risk. This allows clarification between increases in total number of resistance genes/resistant bacteria over time (i.e. positive selection), vs reduced loss of resistance genes compared to the control (i.e. increased persistence), which is needed for interpretation of risk.

Increased persistence of resistance has been referred to as the MIPC, which is below the MSC (Stanton et al., 2020). However, it is unclear how an MIPC could be determined using an MSC approach, as the MIPC lies beneath the intercept of the x-axis (i.e. below the MSC). This phenomenon has also been referred to as a significant reduction in fitness cost (N. Kraupner et al. 2020). Persistence could be defined statistically as the concentration where a significant increase in antibiotic resistance gene prevalence is observed, compared to the control, at the end of the experiment and irrespective of starting prevalence (Fig. 1). It would be unnecessary to first calculate selection coefficients and then perform statistical tests, when the same tests could be used directly on the antibiotic resistance endpoint data (e.g. antibiotic resistance gene prevalence).

To closer align to current ERA methods, a statistical method should be used to determine the selective concentration as opposed to an estimate, like the MSC. Given the differences highlighted in this review and elsewhere between selection and persistence (N. Kraupner et al. 2020; Murray et al., 2018; Stanton et al., 2020), we propose new terms to clarify these endpoints. The Lowest Observed Selection Concentration (LOSC) represents the concentration where a significant increase in resistance gene prevalence/other endpoint is observed, over time, compared to the control. A No Observed Selection Concentration (NOSC) would then be the concentration below this, and a PNEC^R derived through applying an AF as before. In terms of human exposure, it may be more protective to consider overall numbers of resistant bacteria (Stanton et al., 2020). The concentration at which persistence is observed could be considered as the LOEC, which could be used to derive a predicted no effect concentration for persistence (PNEC^P) using the NOEC. Compared to the MSC (and by extension, MIPC) that may overestimate risk for the reasons described above, the LOSC and LOEC may slightly underestimate risk but are statistically robust. Adjustment with AFs would further reduce the possibility of risk underestimation.

Whether safe release limits should be based on the PNEC^R or PNEC^P is a matter for debate, but broadly, the former may be more suited to ERA and the latter to human health risk assessment. The relationship between these values are shown in Fig. 1. It is important to define and distinguish between the two in order to 1) facilitate compliance, by not imposing overly stringent targets in areas with minimal human exposure whilst still preventing increases in resistance and 2) recognise the difference in risk posed by overall resistant numbers of bacteria/genes in an environment to which humans or animals may be readily exposed. However, use of the PNEC^P for both environmental and human health risk assessment would be the most protective.

There are also further considerations when considering risk of individual genes/bacteria, such as mobilisable potential of different genes, which have been reviewed elsewhere (Bengtsson-Palme and Larsson 2015; Martinez et al., 2014). However generally, we recommend a mixed community approach for the experimental determination of PNEC^{R}s or PNEC^{P}s, to be more environmentally representative and to provide a diverse collection of bacteria and ARGs that selection could act upon.

The options of LOSCs, LOECs and MSCs have been discussed but the question remains over what an appropriate assessment factor might be, in order to convert these into PNEC^{R}s or PNEC^{P}s. Recently, the EMA revised their guidance on ERA of medicines approved for human use. An AF of 10 was recommended for surface water and sewage treatment plant PNECs (EMA 2018). In addition, the WFD used an AF of 10 when determining which substances to include on the Watch List (Carvalho et al., 2015). Given these current approaches and the lack of understanding regarding effects of acute or chronic exposure to antibiotics of bacterial communities and community resilience, this AF seems appropriate for the time being. However, it has been suggested previously that microbial community-level studies may require greater assessment factors to account for the greater degree of uncertainty within these systems (Brandt et al., 2015). Therefore, the community based studies described above could utilise larger AFs. The inclusion of more prokaryotic taxa within the revised EMA ERA guideline (EMA 2018) attempts to address some aspects of this uncertainty by recognising the limited value of fish studies and testing more relevant taxa.

We applied this framework to interpret all the concentration data generated thus far (Table 2), in the experimental studies already described. There are 33 concentration data generated to date, for 11 antibiotics, spanning 6 antibiotic classes. We determined the PNEC^R or PNEC^P as follows. Selective concentration data determined using the MSC approach we left unadjusted as this is already a modelled estimate, as described above. Where statistics were used to assign the selective or persistence concentration, the test concentration below this (the NOSC or NOEC) was adjusted with an AF of 10. Where the methodology was insufficient to determine the concentration as being the selective or persistence concentration, we assigned this as 'unknown', applied an AF of 10 and assigned as either a potential PNEC^R or PNEC^P (see Table 2).

Using this approach and the data currently available, the antibiotic likely to pose the greatest risk to the environment is ciprofloxacin (PNEC^{R}s 0.05 and 0.78 µg/L, MSC 0.004 - 10.77 µg/L, n studies =3, unknown potential PNEC^R or PNEC^P using metagenomics of 0.01 µg/L). Trimethoprim is also of potential concern (PNEC^{R}s 1 – 5 µg/L, n studies =3; MSCs 33 – 49.6 µg/L, n studies =2). These two compounds are already on the current version of

Table. 2
Revised.

Antibiotic	Experimental system	Inoculum	Method	Method Endpoint	Risk	Concentration (µg/L)	Reference	$PNEC^R$ (µg/L)	$PNEC^P$ (µg/L)
Azithromycin	Sewage microcosm	Influent	qPCR statistics	*ermF*	Selection	750	Stanton 2020	50	
	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	1000	Murray 2020	50	
Cefotaxime	Sewage microcosm	Influent	qPCR MSC	*CTX-M*	Selection	4	Murray 2018	4*	
	Sewage microcosm	Influent	SELECT statistics	Growth	Selection**	15.63	Murray 2020	0.78	
	Sewage microcosm	Influent	qPCR statistics	*CTX-M & intI1*	Selection	125	Murray 2020	6.25	
Ciprofloxacin	Isogenic competition	*E. coli*	Ratio MSC	*gyrA1*	Selection	0.1	Gullberg 2011	0.1*	
	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	0.98	Murray 2020	0.05	
	Biofilm microcosm	Effluent	Metagenome statistics	*qnrD*	Unknown	1	Kraupner 2018	0.01	0.01
	Sewage microcosm	Influent	qPCR MSC	*intI1*	Selection	10.77	Stanton 2020	10.77*	
	Isogenic competition	*E. coli*	Ratio MSC	Unknown mutant	Selection	0.004	Vos 2020	0.004*	
	Sewage microcosm	Influent	qPCR statistics	*intI1*	Selection	15.625	Stanton 2020	0.78	
Chloramphenicol	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	250	Murray 2020	12.5	
	Sewage microcosm	Influent	qPCR statistics	*intI1*	Selection	500	Murray 2020	25	
Clarithromycin	Sewage microcosm	Influent	qPCR statistics	*ermF*	Selection	750	Stanton 2020	50	
	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	5000	Murray 2020	250	
Erythromycin	Sewage microcosm	Influent	qPCR MSC	*ermF*	Selection	514.1	Stanton 2020	514.1*	
	Sewage microcosm	Influent	qPCR statistics	*ermF*	Selection	750	Stanton 2020	50	
	Isogenic competition	*E. coli*	Ratio MSC	*mph*	Selection	3000	Gullberg 2014	3000*	
	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	25,000	Murray 2020	12500	
Gentamicin	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	250	Murray 2020	12.5	
	Sewage microcosm	Influent	qPCR statistics	*intI1*	Selection	250	Murray 2020	12.5	
Kanamycin	Isogenic competition	*E. coli*	Ratio MSC	Not stated	Selection	470	Gullberg 2014	470*	
Streptomycin	Isogenic competition	*E. coli*	Ratio MSC	*rspL105*	Selection	1	Gullberg 2011	1*	
Tetracycline	Biofilm microcosm	Effluent	qPCR statistics	*tetA/tetG*	Unknown	1	Lundstrom 2016	0.09†	0.09†
	Sewage microcosm	Influent	qPCR statistics	*tetG*	Persistence	1	Stanton 2020		0.01
	Isogenic competition	*E. coli*	Ratio MSC	*cobA367::Tn10dTet*	Selection	15	Gullberg 2011	15*	
	Isogenic competition	*E. coli*	Ratio MSC	*tetRA*	Selection	45	Gullberg 2014	45*	
Trimethoprim	SELECT	Influent	SELECT statistics	Growth (OD)	Selection**	31.25	Murray 2020	1.56	
	Isogenic competition	*E. coli*	Ratio MSC	*dhfr*	Selection	33	Gullberg 2014	33*	
	Sewage microcosm	Influent	qPCR statistics	*intI1*	Selection	62.5	Murray 2020	3.13	
	Isogenic competition	*E. coli*	Ratio MSC	*dfrA12*	Selection	42.7	Kraupner 2020	42.7*	
	E. coli microcosm	*E. coli*, mixed population	CFU statistics	% resistant *E. coli*	Selection	100	Kraupner 2020	1	
	Biofilm microcosm	Effluent	CFU statistics	% resistant *E. coli*	Unknown	100	Kraupner 2020	1	1

Experimental concentrations determined to date. The 'Risk' column denotes whether the measured outcome is definitive positive selection, persistence, or cannot be known from the experimental design and analyses reported ('Unknown'). 'Concentration' column reports either the minimal selective concentration or the concentration where a significant difference was observed (i.e. the LOEC, not the NOEC and before any application of AFs). Concentrations reported are the lowest within each study, and within each experimental system within each study, where comparisons were made. $PNEC^R$s are calculated by taking the NOSC and applying an assessment factor of 10. $PNEC^P$s are calculated by taking the NOEC and applying an assessment factor of 10. † indicates the LOSC/LOEC was the lowest concentration tested, so an arbitrary 0.1 ug/L below this is used as the NOSC/NOEC. Selection** denotes that this is a proxy for direct selection only and not confirmed within the experimental design and analyses. * against a $PNEC^R$ indicates this is a MSC and therefore unadjusted by an AF.

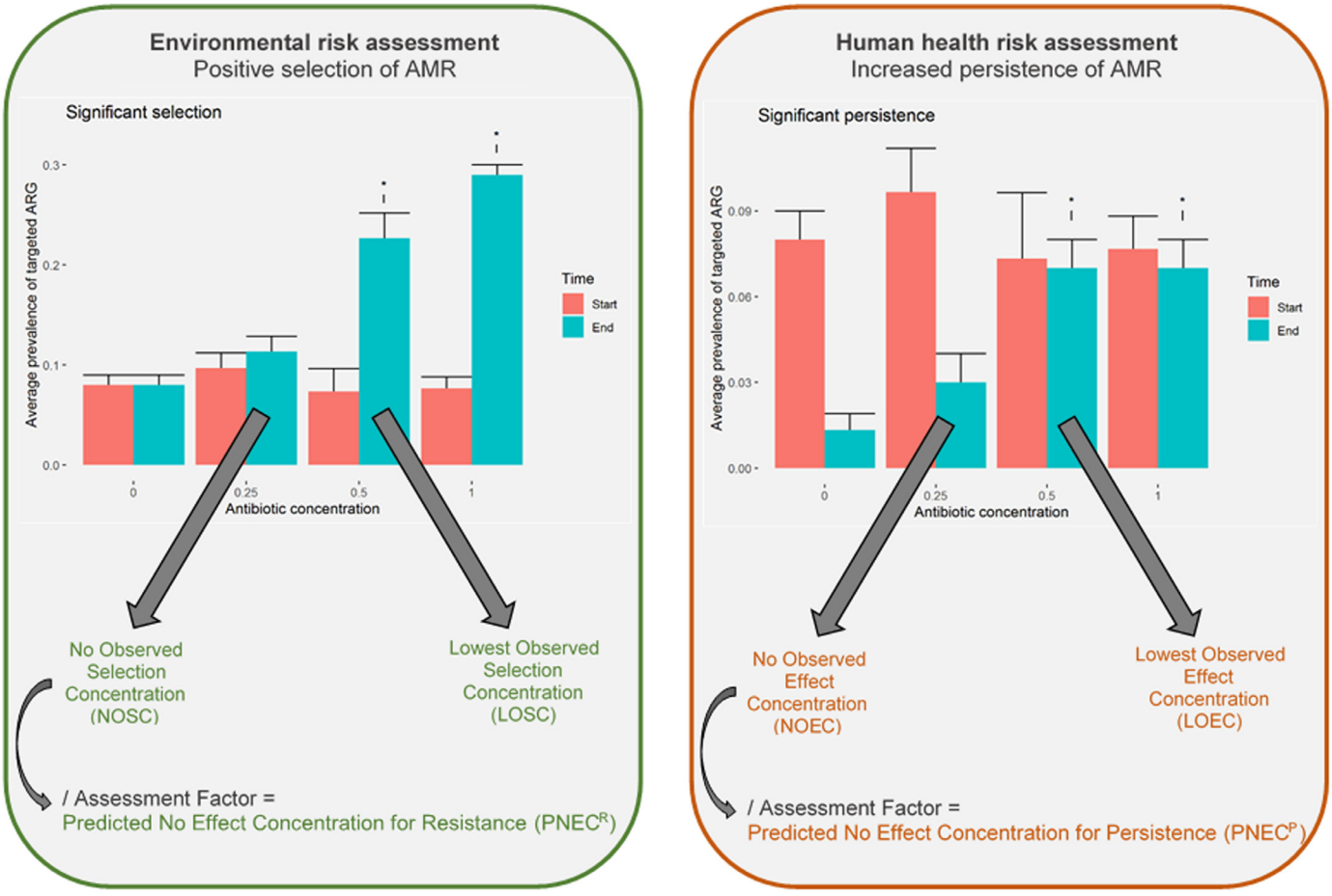

Fig. 1. Different approaches to determining endpoints to use for risk assessment. Of relevance to environmental risk assessment (left hand box) - selection for AMR. Example data set shows significant, positive selection at 0.5 and 1 concentrations of the exposure antibiotic (indicated by '*'), i.e. significantly different to the control at the end of the experiment and with higher average prevalence than at the beginning. This enables derivation of a Predicted No Effect Concentration for Resistance. Of relevance to human health risk assessment (right hand box) - persistence of AMR. Example data set shows significant, increased persistence at 0.5 and 1 concentrations of the exposure antibiotic (indicated by '*'), i.e. significantly different to the control at the end of the experiment, but still with a lower average prevalence than at the beginning. This enables derivation of a Predicted No Effect Concentration for Persistence.

the WFD Watch List (Gomez Cortes et al. 2020), and are the most well studied in terms of selective concentrations (Table 2). Tetracycline may also pose a risk, but further work is needed as currently only $PNEC^P$ or 'unknown' data (0.01 and 0.09 µg/L, respectively) are available alongside the MSC data (15 and 45 µg/L). By comparison, $PNEC^R$s determined to date for the three macrolides (azithromycin, clarithromycin and erythromycin) indicate these pose relatively low risk of selection for resistance, with all lowest $PNEC^R$s determined as 50 µg/L, but ranging from 50 to 12,500 µg/L for erythromycin (n studies =4). These macrolides were on previous versions of the WFD Watch List (Carvalho et al., 2015; Loos et al., 2018) but have since been removed.

These data indicate $PNEC^R$ determination and ERA of other quinolones and fluoroquinolones should be of high priority, followed potentially by cephalosporins (cefotaxime MSC 4 µg/L; $PNEC^R$ 0.78 and 6.25 µg/L) and further consolidation work on tetracyclines. There are still two classes of non-combination antibiotic classes without any data: penicillin beta-lactams and sulfonamides (though trimethoprim is usually grouped with these as they are commonly used in combination). These should also be prioritised for determination of further AMR endpoints. However, more data are still required for the other antibiotics (e.g. macrolides, aminoglycosides) in order to draw definitive conclusions about the risk they pose. Currently available data suggest AMR endpoints will not always be more protective than ecotoxicological endpoints and vice versa, so both are needed to move toward maximum environmental and human health protection (A.K. Murray et al. 2020).

4. Further considerations

For determination of any PNEC, chemical quantification of the test compound is required. This is essential for antibiotics, as many antibiotic resistance mechanisms are degradative in nature. For example, almost complete degradation of cefotaxime at clinical concentrations (2 mg/L) was observed in the presence of a wastewater community; whereas in sterile culture degradation was around 60% (Murray et al., 2018). It is possible that the relatively low MECs of antibiotics such as cefotaxime (to which there are numerous degradative resistance mechanisms) have resulted from degradation by resistant bacterial populations present in the environment, as opposed to high lability alone. This raises questions about the robustness of chemical based EQS used under the WFD to protect human health; they will be adequate for ecological protection, but high levels of resistance that could pose a human health threat may occur where there are low levels of antibiotic, if the resistance mechanism is degradative. This is most relevant for the beta-lactam class of antibiotics, including the World Health Organisation critically important humans medicines such as third generation cephalosporins and last-line carbapenems (WHO 2017), to

which clinical resistance is often conferred by degradative enzymes such as the extended spectrum beta-lactamases (Canton and Coque 2006) or carbapenemases (Nordmann et al., 2011). This is problematic as low MECs could actually indicate high levels of resistance. Monitoring of resistance genes in soil was recently proposed by the European Commission (EU 2019) and so this could also be recommended for monitoring surface waters, for example in future amendments to the WFD Watch List. However, quantitatively linking the presence of resistance genes in the environment to colonisation, subsequent infection and adverse clinical outcomes remains a significant challenge.

When determining $PNEC^R$s for highly labile antibiotics, it may be necessary to introduce antibiotic continuously in a flow through system; though this may still not be recommended if the required flow rate exceeds the maximum growth rate of bacteria (as this would result in wash out and reduced ecological relevance (Ziv et al., 2013)). In batch systems, degradation experiments are recommended to enable extrapolation of the average exposure concentration over the test period to determine a more accurate NOEC. For either system, understanding of compound lability is essential to prevent $PNEC^R$ overestimation.

Chemical quantification is complicated further when considering biofilms and heterogeneous penetration by antibiotics. Very little is understood regarding the antibiotic concentration within bacterial cells or at the bacterial cell surface and how this may affect selection. Furthermore, there is some evidence to suggest intra and extracellular pH concentrations can lead to different antimicrobial activity of the same antibiotic in different bacteria (Tappe et al., 2008). Studying these phenomena is now possible due to the development of single cell microfluidic systems, such as the mother machine microfluidic device (Bamford et al., 2017).

In addition to quantification of antibiotics within experimental systems, greater effort is required to generate PECs of antibiotics with high spatial resolution; and to determine MECs of antibiotics in a more systematic manner, looking at catchments and inputs over time. There are many physiochemical considerations that may affect fate and transport of antibiotics, which are reviewed elsewhere (Jafari Ozumchelouei et al. 2020). The largest open access database of MECs was collated by the Umweltbundesamt in 2016 (UmweltBundesamt 2016) and was recently updated (aus der Beek et al. 2016, UmweltBundesamt 2019). These data are essential for determining RQs that are protective of selection.

Better understanding of the pathways of human exposure to these MECs is also needed. For example, the relative significance of these pathways for colonisation/infection by resistant bacteria (which may be pathogenic, opportunistically pathogenic or even non-pathogenic); or their impacts on the existing commensal bacteria that form the human microbiome, such as potential horizontal gene exchange between the pre-existing microbiome and ingested bacteria. This should be included within an integrated human and environmental health risk framework (Ashbolt et al., 2013; Larsson et al., 2018).

A significant factor affecting all aspects of ERA, not just ERA of AMR, is the inescapable fact that pharmaceuticals exist in the environment as a complex mixture with other chemicals and stressors. It is likely that in the majority of cases, selective concentrations of individual antibiotics will be reduced in antibiotic mixtures, especially as some antibiotics are used in combination clinically for their synergistic effects, such as trimethoprim and sulfamethoxazole. Within class antibiotics are likely to have additive effects. Given the lack of methods to experimentally quantify ecotoxicological effects of mixtures in general, and the missing requirement for mixtures assessment in current ERA guidelines, a tiered approach has been suggested (Backhaus 2016). Firstly, RQs derived for individual compounds could be summed, using readily available individual compound PNEC data and secondly, mixture-specific AFs could also be used (Backhaus 2016). However, as previously highlighted, still comparatively few $PNEC^R$ data are available for AMR ERA. Furthermore, how antibiotics may interact with other co-selective compounds, such as metals (Baker-Austin et al., 2006) or biocides (Pal et al., 2015) may be less predictable and more research is needed in this area. Co-selection is the process whereby resistance to a compound can be indirectly selected for, in the absence of that compound or when it is only present at a sub-selective concentration. This can be through genetic 'hitch hiking' of co-localised resistance genes, for example on mobile genetic elements (co-resistance) or if one gene confers resistance to both compounds (cross-resistance), such as multidrug efflux pumps. Only one compound needs to be present in order to select for the entire mobile element or gene conferring cross-resistance to be selected for (Baker-Austin et al., 2006). Generally, it is assumed both metals and biocides could co-select for antibiotic resistance and therefore may have an additive or synergistic effect. However, there are some data to suggest antagonistic effects may also occur. For example, one recent study demonstrated the selective concentration of ciprofloxacin increased in the presence of zinc, possibly due to chelation (Vos et al., 2020). The 'baseline' MSC of ciprofloxacin of 0.004 μg/L increased to 0.011 μg/L and 0.022 μg/L in the presence of 0.5 mM and 1 mM concentrations of Zinc ($^{2+}$), respectively (Vos et al., 2020). However generally, it is assumed that risks posed by mixtures are greater than the individual constituent compounds (Backhaus 2016). Revision of current ERA guidelines, such as those by the EMA that progress to Phase II toxicity testing based on PEC data for individual compounds may also be required (Backhaus 2016). If and when mixture effects are incorporated into current guidelines, any AMR-specific ERA guidelines should be updated.

One possible approach to quantify mixture effects could be whole effluent toxicity (WET). This is currently used in the USA to determine if all the compounds present in effluents are 'safe' or have a toxic effect (EPA 2002). This could be adapted to examine the selective potential of effluents through exposure experiments as outlined above, where a range of diluted effluents are used in place of a single test antibiotic. The most selective effluents can then be prioritised for further analyses (e.g. chemical quantification, prevalence of antibiotic resistant bacteria and/or genes) or mitigation.

5. Conclusions and future directions

A novel framework for ERA of antibiotics is required for antibiotic stewardship and by extension, protection of human health, food security and the global economy. We have outlined such a potential framework, and recommended determination of $PNEC^R$s through experimentally defined LOSC/NOSCs and $PNEC^P$s through experimentally defined LOECs/NOECs. These should be determined for both new and old antibiotic compounds and we have highlighted quinolone, cephalosporins, beta-lactams and sulphonamide antibiotics should be priority candidates moving forward. Whilst environmental monitoring of MECs is crucial, derivation of reliable PEC data is particularly important for novel compounds, as MEC data will be unavailable.

By drawing on current ERA guidelines, we hope to accelerate standardisation and adoption of this new ERA, which addresses the selective effect of antibiotics. Inclusion of antibiotics on the WFD Watch List presents opportunities to compare ecological and selective endpoints for ERA. However, monitoring of ARGs may also be required at this scale to complement chemical monitoring data. New antibiotics approved for use in humans may provide novel opportunities to track resistance development. By monitoring chemical and resistance data in human sewage, wastewater treatment plants and surface waters in populations that experience either

high or low use, the environment could become an early indicator for clinical resistance. There is already a body of evidence demonstrating that current, clinically important resistance genes originated in the environment (Humeniuk et al., 2002; Poirel et al., 2008; Poirel et al., 2004; Potron et al., 2011). Such environmental surveillance data could inform and complement routine clinical surveillance efforts.

Selection for resistance in the environment and human or animal exposure to resistant bacteria are interlinked phenomena. Better understanding of the human and animal health effects of exposure to resistant environmental bacteria/genes is required (Larsson et al., 2018) as this will help drive development and adoption of AMR relevant EQS. However, appropriate EQS will be required to mitigate the health effects of such exposure. The precautionary principle recommends that given the alarming impacts on human health and the global economy estimated as a result of AMR (O'Neill 2014), ERA which considers selection for AMR cannot, and should not, wait.

There are also opportunities to reduce the environmental risk of selection for AMR in the short term, whilst longer term solutions such as implementation of a novel ERA are underway. Some recommended immediate actions are summarised:

1) Appropriate use of antibiotics – i.e. improved diagnosis, appropriate prescribing and reduction and elimination of over the counter use of antibiotics and their use in growth promotion.
2) Controlling release of selective compounds into the environment. It is now more than 10 years since the Larsson et al. measured alarmingly high concentrations of ciprofloxacin in pharmaceutical manufacturing effluent (Larsson et al., 2007). Safe release limits for antibiotics were reported recently (Tell et al., 2019) and meeting these targets should be a top priority for antibiotic manufacturers and industry.
3) Sanitation. Basic levels of sanitation are still lacking in many parts of the world, particularly in lower to middle income countries. Though wastewater treatment in areas like Europe and the USA are still not completely effective, removal of antibiotics and resistant bacteria is still significant. Provision of this in all countries would reduce opportunities for environmental selection and human exposure; but also reduce prevalence of communicable disease and thereby antibiotic use.

Declaration of Competing Interest

Jason Snape is an employee and shareholder of AstraZeneca PLC. All remaining authors declare no competing interests.

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References

Andersson, D.I., Hughes, D, 2011. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol. Rev. 35 (5), 901–911.
Ashbolt, N.J., Amezquita, A., Backhaus, T., Borriello, P., Brandt, K.K., Collignon, P., Coors, A., Finley, R., Gaze, W.H., Heberer, T., Lawrence, J.R., Larsson, D.G., McEwen, S.A., Ryan, J.J., Schonfeld, J., Silley, P., Snape, J.R., Van den Eede, C., Topp, E., 2013. Human Health Risk Assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ. Health Perspect. 121 (9), 993–1001.
and aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A., 2016. Pharmaceuticals in the environment–global occurrences and perspectives. Environ. Toxicol. Chem. 35 (4), 823–835.
Backhaus, T., 2016. Environmental risk assessment of pharmaceutical mixtures: demands, gaps, and possible bridges. AAPS J. 18 (4), 804–813.
Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V., 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol. 14 (4), 176–182.
Bamford, R.A., Smith, A., Metz, J., Glover, G., Titball, R.W., Pagliara, S., 2017. Investigating the physiology of viable but non-culturable bacteria by microfluidics and time-lapse microscopy. BMC Biol. 15 (1), 121.
Bengtsson-Palme, J., Larsson, D.G., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ. Int. 86, 140–149.
Bengtsson-Palme, J., Larsson, D.G.J, 2015. Antibiotic resistance genes in the environment: prioritizing risks. Nat. Rev. Microbiol. 13 (6) 396-396.
Bottery, M.J., Wood, A.J., Brockhurst, M.A., 2016. Selective Conditions for a Multidrug Resistance Plasmid Depend on the Sociality of Antibiotic Resistance. Antimicrob. Agents Chemother. 60 (4), 2524–2527.
Brandt, K.K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J.R., Lazorchak, J., Schönfeld, J., Snape, J.R., Zhu, Y.-.G., Topp, E., 2015. Ecotoxicological assessment of antibiotics: a call for improved consideration of microorganisms. Environ. Int. 85, 189–205.
Canton, R., Coque, T.M., 2006. The CTX-M beta-lactamase pandemic. Curr. Opin. Microbiol. 9 (5), 466–475.
Carvalho, R.N., Ceriani, L., Ippolito, A., Lettieri, T., 2015. Development of the First Watch List Under the Environmental Quality Standards Directive. European Commission.
Day, T., Huijben, S., Read, A.F., 2015. Is selection relevant in the evolutionary emergence of drug resistance? Trends Microbiol. 23 (3), 126–133.
ECHA, 2008. Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of Dose [concentration]-response for Environment. European Chemicals Agency (ECHA) Helsinki, Finland.
EMA, E., 2006. Guideline On the Environmental Risk Assessment of Medicinal Products For Human use. First Version.. Committee for Medicinal Products for Human Use (CHMP), London, UK.
EMA, E. (2018) Guideline on the environmental risk assessment of medicinal products for human use.
EPA (2002) Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms U.S. Environmental Protection Agency, Office of Water, Washington.
EU, 1996. Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances. Commission Regulation (EC) no. 1488/94 On Risk Assessment For Existing substances, Part II.. Brussels: EU.
EU, 2019. Communication from the Commission to the European Parliament, the Council and the European Econominc and Social Committee: European Union Strategic Approach to Pharmaceuticals in the Environment. European Commission, Brussels.
FDA (1998a) Guidance for industry: environmental assessment of human drug and biologics applications, pp. 1–39.
FDA (1998b) Guidance for industry: environmental assessment of human drug and biologics applications. Center for Biologics Evaluation and Research, CDER/CBER, CMC 6, 1–39.
Gomez Cortes, L., Marinov, D., Sanseverino, I., Navarro Cuenca, A., Niegowska, M., Porcel Roderiguez, E., Lettieri, T., 2020. Selection of Substances For the 3rd Watch List Under the Water Framework Directive. Publications Office of the European Union, Luxembourg 2020.
Gullberg, E., Albrecht, L.M., Karlsson, C., Sandegren, L., Andersson, D.I., 2014. Selection of a Multidrug Resistance Plasmid By Sublethal Levels of Antibiotics and Heavy Metals. mBio 5.
Gullberg, E., Cao, S., Berg, O.G., Ilback, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLoS Pathog. 7 (7).
Humeniuk, C., Arlet, G., Gautier, V., Grimont, P., Labia, R., Philippon, A., 2002. Beta-lactamases of Kluyvera ascorbata, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob. Agents Chemother. 46 (9), 3045–3049.
Jafari Ozumchelouei, E., Hamidian, A.H., Zhang, Y., Yang, M, 2020. Physicochemical properties of antibiotics: a review with an emphasis on detection in the aquatic environment. Water Environ. Res. 92 (2), 177–188.
Klümper, U., Recker, M., Zhang, L., Yin, X., Zhang, T., Buckling, A., Gaze, W.H., 2019. Selection for antimicrobial resistance is reduced when embedded in a natural microbial community. Isme J. 13, 2927–2937.
Kraupner, N., Ebmeyer, S., Bengtsson-Palme, J., Fick, J., Kristiansson, E., Flach, C.-.F., Larsson, D.G.J, 2018. Selective concentration for ciprofloxacin resistance in Escherichia coli grown in complex aquatic bacterial biofilms. Environ. Int. 116, 255–268.
Kraupner, N., Ebmeyer, S., Hutinel, M., Fick, J., Flach, C.-.F., Larsson, D.G.J, 2020. Selective concentrations for trimethoprim resistance in aquatic environments. Environ. Int. 144, 106083.
Kümmerer, K., Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin. Microbiol. Infect. 9 (12), 1203–1214.
Larsson, D.G.J., Andremont, A., Bengtsson-Palme, J., Brandt, K.K., de Roda Husman, A.M., Fagerstedt, P., Fick, J., Flach, C.F., Gaze, W.H., Kuroda, M., Kvint, K., Laxminarayan, R., Manaia, C.M., Nielsen, K.M., Plant, L., Ploy, M.C., Segovia, C., Simonet, P., Smalla, K., Snape, J., Topp, E., van Hengel, A.J., Verner-Jeffreys, D.W., Virta, M.P.J., Wellington, E.M., Wernersson, A.S, 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environ. Int. 117, 132–138.
Larsson, D.G.J., de Pedro, C., Paxeus, N, 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J. Hazard. Mater. 148 (3), 751–755.
Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R, 2017. Integrating human and en-

vironmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environ. Int. 109, 155–169.
Loos, R., Marinov, D., Sanseverino, I., Napierska, D., Lettieri, T., 2018. Review of the 1st Watch List Under the Water Framework Directive and Recommendations For the 2nd Watch List. Publications Office of the European Union, Luxembourg.
Lundstrom, S.V., Ostman, M., Bengtsson-Palme, J., Rutgersson, C., Thoudal, M., Sircar, T., Blanck, H., Eriksson, K.M., Tysklind, M., Flach, C.F., Larsson, D.G., 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Sci. Total Environ. 553, 587–595.
Martinez, J.L., Coque, T.M., Baquero, F., 2014. What is a resistance gene? Ranking risk in resistomes. Nat. Rev. Microbiol. 13, 116–123.
Murray, A.K., Stanton, I.C., Wright, J., Zhang, L., Snape, J., Gaze, W.H., 2020. The 'SELection End points in Communities of bacTeria' (SELECT) Method: a Novel Experimental Assay to Facilitate Risk Assessment of Selection for Antimicrobial Resistance in the Environment. Environ. Health Perspect. 128 (10), 107007.
Murray, A.K., Zhang, L., Yin, X., Zhang, T., Buckling, A., Snape, J., Gaze, W.H., 2018. Novel Insights into Selection for Antibiotic Resistance in Complex Microbial Communities 9 (4).
Nordmann, P., Poirel, L., Walsh, T.R., Livermore, D.M., 2011. The emerging NDM carbapenemases. Trends Microbiol. 19 (12), 588–595.
O'Neill (2014) O'Neill Report Wellcome Trust. Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations.
OECD (2009) Proposal for a revised Guideline 209 - Inhibition of respiration of activated sludge (carbon and/or ammonium oxidation).
Otto, S.P., Day, T., 2007. A Biologist's Guide to Mathematical Modelling in Ecology and Evolution. Princeton University Press, New Jersey.
Pal, C., Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G., 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16, 964.
Poirel, L., Figueiredo, S., Cattoir, V., Carattoli, A., Nordmann, P., 2008. Acinetobacter radioresistens as a Silent Source of Carbapenem Resistance for Acinetobacter spp. Antimicrob. Agents Chemother. 52 (4), 1252–1256.
Poirel, L., Heritier, C., Nordmann, P., 2004. Chromosome-encoded ambler class D beta-lactamase of Shewanella oneidensis as a progenitor of carbapenem-hydrolyzing oxacillinase. Antimicrob. Agents Chemother. 48 (1), 348–351.
Potron, A., Poirel, L., Nordmann, P., 2011. Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in Shewanella xiamenensis. Antimicrob. Agents Chemother. 55 (9), 4405–4407.
Schmitt, H., Haapakangas, H., van Beelen, P., 2005. Effects of antibiotics on soil microorganisms: time and nutrients influence pollution-induced community tolerance. Soil Biol. Biochem. 37 (10), 1882–1892.
Stanton, I.C., Murray, A.K., Zhang, L., Snape, J., Gaze, W.H., 2020. Evolution of antibiotic resistance at low antibiotic concentrations: selection below the minimal selective concentration. Commun. Biol. 3.
Tappe, W., Zarfl, C., Kummer, S., Burauel, P., Vereecken, H., Groeneweg, J., 2008. Growth-inhibitory effects of sulfonamides at different pH: dissimilar susceptibility patterns of a soil bacterium and a test bacterium used for antibiotic assays. Chemosphere 72 (5), 836–843.
Tell, J., Caldwell, D.J., Häner, A., Hellstern, J., Hoeger, B., Journel, R., Mastrocco, F., Ryan, J.J., Snape, J., Straub, J.O., Vestel, J., 2019. Science-based Targets For Antibiotics in Receiving Waters from Pharmaceutical Manufacturing Operations. Integrated Environmental Assessment and Management.
Tello, A., Austin, B., Telfer, T.C., 2012. Selective pressure of antibiotic pollution on bacteria of importance to public health. Environ. Health Perspect. 120 (8), 1100–1106.
UmweltBundesamt (2016) Pharmaceuticals in the environment. UBA (ed), https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-figures-0.
UmweltBundesamt (2019) Pharmaceuticals in the environment. UBA (ed), https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-figures-0.
UNEP, 2019. Frontiers 2018/19 Emerging Issues of Environmental Concern..
Vos, M., Sibleyras, L., Lo, L.K., Hesse, E., Gaze, W., Klümper, U., 2020. Zinc can counteract selection for ciprofloxacin resistance. FEMS Microbiol. Lett. 367 (3).
WHO (2015) Global Action Plan On Antimicrobial Resistance. WHO (ed), I. World Health Organisation., Geneva, Switzerland.
WHO (2017) WHO Model List of essential Medicines, 20th List, https://www.who.int/publications/i/item/eml-20.
Ziv, N., Brandt, N.J., Gresham, D, 2013. The use of chemostats in microbial systems biology. J. Visualized Exper. (80) 50168 JoVE.

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Corrigendum

Corrigendum to < Dawning of a new ERA: Environmental Risk Assessment of antibiotics and their potential to select for antimicrobial resistance'> <[Water Research 200 (2021) 117–233]>

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The authors regret <that it has been drawn to their attention that four of the PNECRs reported in Table 2 are reported incorrectly. Some of these errors are also repeated in the main body of text. The errors in Table 2 are as follows:

1. Ciprofloxacin, isogenic competition with *E. coli,* the ratio MSC taken from Vos et al. (2020) was reported as 0.004 in the 'Concentration μg/L' column, but the actual value should be 4. Therefore, the value in the 'PNECR μg/L' column should also be '4*'.
2. Erythromycin, isogenic competition with *E. coli*, the ratio MSC taken from Gullberg et al. (2014) was reported as 3000 in the 'Concentration μg/L' column, but the actual value should be 200. Therefore, the value in the 'PNECR μg/L' column should also be '200*'.
3. Erythromycin, SELECT assay with influent, the SELECT statistics value in the 'PNECR μg/L' column was reported as 12,500, but the actual value should be 1,250.
4. Streptomycin, isogenic competition with *E. coli*, the ratio MSC taken from Gullberg et al. (2011) was reported as 1 in the 'Concentration μg/L' column, but the actual value should be 1000. Therefore, the value in the 'PNECR μg/L' column should also be '1000*'.

The sentences where these errors were reproduced in the main body of text are as follows:

1. "Using this approach and the data currently available, the antibiotic likely to pose the greatest risk to the environment is ciprofloxacin (PNECRs 0.05 and 0.78 μg/L, MSC 0.004 - 10.77 μg/L, n studies =3". Here, the MSC should range from 4 – 10.77 μg/L.
2. "Relatively low risk of selection for resistance, with all lowest PNECRs determined as 50 μg/L, but ranging from 50 to 12,500 μg/L for erythromycin". Here, the range should be from 50 – 1,250 μg/L.
3. "The 'baseline' MSC of ciprofloxacin of 0.004 μg/L increased to 0.011 μg/L and 0.022 μg/L". Here, the units were incorrectly converted from the original reference, and should be 4, 11 and 22 μg/L. >.

All authors have double checked the values and would like to apologise for any inconvenience caused.

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