Translating antibiotic prescribing into antimicrobial resistance in the environment: a hazard characterisation case study

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Abstract

The use and misuse of antibiotics in humans, animals and agriculture since penicillin's mass production in 1942 has contributed to the current global crisis of drug-resistant infections. Often overlooked are the unintended consequences of such widespread antibiotic use, such as the chronic environmental release of unmetabolised, bioactive antibiotics and antibiotic-resistance genes found in human and animals waste, particularly during and immediately after antibiotic treatment. Here, we report on the potential impact on antibiotic resistance gene selection in rivers from the antibiotic prescribing practice of the National Health Service (NHS) of England in 2015/16. Macrolide and fluoroquinolone prescription data were used to model the concentrations of antibiotics in a highly populous, sewage-impacted English catchment. Predicted antibiotic concentrations were compared against predicted no-effect concentrations (PNEC) to assess the risk of antibiotic resistance selection. The study revealed that PNECs for macrolide and fluoroquinolone were exceeded in 63% and 73% of the length of the modelled River Thames catchment, respectively. Foreseeable reductions in macrolide and fluoroquinolone prescriptions (e.g., reduction of 4% and 20%) were not shown to translate into meaningful reductions in the risk of AMR selection. Reductions in macrolide and fluoroquinolone prescribing of 77% and 85%, respectively, were necessary to protect 90+% of the modelled river catchment from resistance gene selection. Improved antibiotic stewardship, alone, is unlikely to alleviate the identified challenge; action is needed to: stimulate innovation in sewage-treatment, reduce non-antibiotic drivers of antibiotic resistance (e.g., biocides, metals), and treat DNA as a pollutant.

The drive to reduce antibiotic use and misuse in human medicine had gained considerable traction since 2011 when Dame Sally Davis, the Chief Medical Officer (CMO) for England and Chief Medical Advisor to the UK government published her Annual Report [1]. In Volume 2 of this report, she highlighted the “challenges and opportunities facing us in the prevention, diagnosis and management of infectious diseases”, which included the move to reduce inappropriate prescribing of antimicrobials. To this end, the National Health Service of England (NHS) proposed a series of targeted reductions in antibiotic prescriptions as part of a Quality Premium Programme (QPP) in 2015/16 [2].

The QPP in 2015/16 aimed to reduce antibiotic over-use and inappropriate prescribing through a reduction in:

1) the number of antibiotics prescribed in primary care by ≥1% from each Clinical Commissioning Group (CCG’s) 2013/14 value;
2) the proportion of broad-spectrum antibiotics prescribed in primary care. Specifically, the QPP aims to reduce prescriptions of co-amoxiclav, cephalosporins and fluoroquinolones by 10% (from each CCG’s 2013/14 value) as a percentage of the total number of antibiotics prescribed in primary care, or to be below the 2013/14 median proportion for English CCGs (11.3%), whichever represents the smallest reduction.

As a result of the QPP, in 2015-16 the NHS of England, for the first time, reduced their prescriptions of antibiotics by 7.3% (37.03 million items to 34.34 million) as compared to 2014-15. The NHS also saw a reduction of 16% in broad-spectrum antibiotics (3.94 million items to 3.3 million, inclusive of cephalosporins, co-amoxiclav and quinolones). The goal for 2016-17 was set to reduce total antibiotic use by another 4% and 20% for broad-spectrum antibiotics.

The reduction in antibiotic prescribing in 2015/16 resulted in the use of nearly 3 million fewer antibiotic items, resulting in a proportional reduction in antibiotics released into wastewater. This is because a significant proportion of antibiotics are conserved as they pass through the body before being excreted as a mixture of the parent compound and metabolites in the urine and faeces [3]. The gut bacteria from hundreds of thousands of NHS patients would have been enriched in antibiotic resistance, a phenomenon that is unavoidable upon consumption of antibiotics [4]. It is this chronic release of antibiotic and antibiotic-resistant bacteria from sewage effluent into freshwater that is thought to increase the prevalence of resistance genes in the environment substantially. This is most evident downstream a sewage treatment plant (STP) discharge point as compared to upstream [5–10]. Recent evidence shows that even trace levels of sewage effluent can elevate the prevalence of markers of antibiotic resistance, i.e., class 1 integrons [11].

This study focused on two questions that explore the link between antibiotic use and environmental impact:

1) Would reductions in antibiotic use, such as the ones witnessed in England in 2015/16, result in a reduction in antibiotic resistance in English sewage-impacted rivers? and
2) How much of a reduction in antibiotic use might be required to alleviate the risk posed by sewage effluent on antimicrobial resistance selection in rivers? These are the two questions that this research aims to examine.

This study focused on two classes of antibiotics: macrolides and fluoroquinolones. Table 1 highlights the pharmacokinetics of these classes of antibiotics. These were specifically selected because: 1) they are two of the more persistent classes of antibiotics in the environment; 2) they are found in nearly all antibiotic surveillance studies [12–17]; 3) they

have significant clinical relevance [18]; and, 4) macrolides are on the EU Watch List of Decision 2015/495/EU [19,20].

The River Thames catchment (i.e., Thames Basin) in southern England was selected for this study as it is the most highly populous catchment in the UK (nearly 4 million people). It also might be seen as a realistic worst-case scenario, as on average, this part of England is in the 25th quartile of predicted values of annual dilution factors, i.e., between 2.24 and 6.26 [21]. As such, the river offers little opportunity for dilution, maximising the impact of antibiotics found in the discharged sewage. Furthermore, the catchment has previously been the subject of a number of studies investigating predicted environmental concentrations of pharmaceuticals [16,22–24].

Materials and Methods

Prescription data

Macrolide prescribing data for each month of 2015/16 were acquired from the NHS Business Service Authority (NHSBSA), i.e., azithromycin, clarithromycin and erythromycin (Figure 1a). Fluoroquinolone prescribing data for each month of 2015/16 were also acquired from the NHSBSA, i.e., ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin and ofloxacin (Figure 1b). Prescription data was acquired from four Clinical Commissioning Groups (CCGs): Oxfordshire, Gloucestershire, Swindon and Wiltshire, all of which are situated in the upper River Thames catchment (Figure 2). It was not possible to reliably assign CCG prescriptions to STPs in the lower Thames catchment owing to the small geographic size of the CCGs and high population density. As such, all other CCGs within the catchment were assigned the prescription rate of NHS Oxfordshire CCG as a pragmatic solution—reinforcing the importance of it being the largest CCG this is completely contained within the River Thames catchment.

NHS prescription data for each CCG per month in 2014/15 were converted to units of kg of macrolide and fluoroquinolone (Table 2) and then moles (Table 3) and subsequently normalised to the population within each CCG (Table 4). In the case of erythromycin, the three forms of erythromycin (erythromycin, erythromycin ethyl succinate and erythromycin stearate) were converted individually and summed.

Antibiotic Excretion Rate

The excretion of antibiotics in the faeces and urine depends on its metabolism *in vivo*, which is a function of health, age, gender, ethnicity, as well as dosage and mode of administration, e.g., capsule, injection. Given the large uncertainty associated with estimating excretion rates across diverse populations, an average rate acquired from the literature was used: macrolide excretion rate of 32.2% and 64.2% for fluoroquinolones (Table 1).

Antibiotic Loss in STPs

The antibiotic loss in STPs was modelled using STPWIN model within the Estimation Program Interface (EPI) Suite^{TM} 4.0, using the Biowin/EPA draft method for determining half-life data as previously described [23]. Estimates of loss in STPs was also acquired from the literature [25–30], with some relevant data acquired from STPs within the River Thames catchment found in Table 5. These data support the use of 50% loss during STP passage, which is a compromise between measured loss and the high variability reported within the literature.

Modelled Environmental Concentrations: Low Flows 2000 – Water Quality eXtension

Low Flows 2000 Water Quality eXtension (LF2000-WQX) model [24,31] is an extension to the LF2000 [32] . LF2000 is a decision support tool designed to estimate river flow at gauged and ungauged sites and to assist regional water resources and catchment management. The LF2000-WQX software is a geographical information-based system that combines

hydrological models with a range of water-quality models, including a catchment-scale water-quality model. This model generates spatially explicit statistical distributions of down-the-drain chemicals for both conservative and degradable compounds. It uses a Monte Carlo model approach to combine statistical estimates of chemical loads at specific emission points (e.g., STP) with estimated river flow distributions for the whole river network of interconnected model reaches (a reach is the river stretch between model features, e.g., major tributaries, STPs). The hydrometric area of the River Thames catchment and the STPs included in LF2000-WQX (black points) can be seen in Figure 2. Thus, working from the upstream reaches at the head of the river network to the outlet of the river basin, the model accounts for the accumulation of point antibiotic loads and the water in which these loads are diluted. Degradable chemicals might be removed from STPs and the river water. The latter is represented by a non-specific dissipation process assuming first-order kinetics. To provide a realistic worst-case scenario, antibiotics were assumed to persist in the river for at least one day.

In summary, LF2000-WQX was parameterised with the highest monthly fluoroquinolone and macrolide prescription rate for each of the four CCGs (see bolded rates in Table 4). Macrolide and fluoroquinolone loss was accounted for upon excretion (32.2% and 64.2%, respectively) and before discharge from STPs into the receiving river (50%). As such, the mass of macrolide and fluoroquinolone consumed was reduced by 83.9% and 67.9%, respectively, upon discharge into the adjacent river. The antibiotic load was subsequently diluted within the river, parameterised with the mean annual river flows within LF2000-WQX.

Environmental Thresholds for Antibiotics

Management targets for antibiotics in freshwater would ideally be set at concentrations that are below the lowest concentration that allows antibiotics to select for antibiotic resistance genes. Such effect concentrations have been determined experimentally in a limited number of lab-based studies, operationally termed minimum selection concentrations (MSC). MSCs typically range between 0.1 to 10 µg/L [33–45].

The literature lacks MSCs for all fluoroquinolones and macrolides, hence, modelled predicted no-effect concentrations (PNECs) were acquired from Bengtsson-Palme and Larsson (2016). The PNECs represent the threshold concentration of an antibiotic, above which, there is a heightened risk of antibiotic resistance selection. In this latter study, PNECs were derived from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database of minimum inhibitory concentrations to form species sensitivity distributions. The authors selected the concentration of each antibiotic representing the 1% potentially effective fraction (PAF). A safety factor of 10 was added to this 1% PAF to account for the observation that experimentally-derived resistance selection thresholds tend to be approximately an order of magnitude lower than the MIC, while also offering an added level of protection to the estimate. The 111 antibiotic thresholds reported in Bengtsson-Palme and Larsson 2016 ranged from 0.008 µg/L to 64 µg/L. The PNECs derived in Bengtsson-Palme and Larsson (2016) were recently chosen to inform antibiotic discharge limits from pharmaceutical manufacturing [46].

PNECs for fluoroquinolones ranged from 0.064 µg/L to 0.5 µg/L and for macrolides 0.25 µg/L to 1 µg/L (Table 6). When modelled concentrations of macrolides and fluoroquinolones exceeded the PNEC for the most ‘potent’ antibiotic within each class, e.g., ciprofloxacin for fluoroquinolones and azithromycin/clarithromycin for macrolides, the stretch of river was denoted as being ‘at risk’ for resistance selection. When the modelled concentrations exceeded the PNEC for the least ‘potent’ antibiotic within each class, e.g., norfloxacin/ofloxacin for fluoroquinolones and erythromycin for macrolides, the stretch of river was denoted as being at a ‘critical risk’ of resistance selection.

Results

This study aimed to understand the degree of resistance selection that might be occurring in the River Thames catchment under current macrolide and fluoroquinolone prescribing practice by the NHS as well as after foreseeable and aspirational reductions in prescribing. The objective was to estimate the level of reduction needed in NHS antibiotic prescribing that might be necessary to reduce the macrolide and fluoroquinolone-resistance selection burden created in the river environment.

Predicted environmental concentration (PEC) for macrolides and fluoroquinolones prescribed in 2015/16 revealed a maximum concentration of 4.3 µg/L and 2.0 µg/L, respectively. Hazard characterisation revealed 279 out of 457 reaches in the catchment are 'at risk' or 'critical' for macrolide resistance selection (Figure 3a)—equating to 1155 of 1398 km of the modelled River Thames Catchment (63.2% of the total catchment length modelled). Similarly, 311 reaches out of 457 were 'at risk' or 'critical' for fluoroquinolone resistance selection (Figure 3b)—equating to 1026 of 1398 km (73.4% of the modelled river length in the catchment).

Reflecting the 2015/16 goals, a 4% reduction in macrolide prescribing saw a 3.8% reduction in the length of the modelled Thames Catchment 'at risk' for macrolide resistance selection (273 reaches and 830 km, equal to 59.4% of the catchment), and a 0.1% reduction in the length of the modelled Thames Catchment 'at risk' for fluoroquinolone resistance selection (310 reaches and 1025.1 km, equal to 73.3% of the catchment).

Reflecting the 2015/16 goals, a 20% reduction of the 2015/16 prescription rate of macrolides resulted in a 8.9% reduction in the length of the modelled Thames catchment 'at risk' for macrolide resistance selection (254 reaches and 759 km, equal to 54.3% of the catchment). A 20% reduction in fluoroquinolones resulted in a 5.4% reduction in the length of the modelled Thames catchment 'at risk' for fluoroquinolone resistance selection (296 reaches and 949 km, equal to 68.0% of the catchment).

A sensitivity analysis was conducted to identify the NHS prescribing rate that protects ≥90% of the length of the modelled Thames catchment from macrolide and fluoroquinolone resistance selection. A target of 90% was set instead of 100% as many rivers in England are highly impacted by, or solely composed of, sewage effluent—particularly in headwaters. Hence, a target of 100% is unrealistic and would necessitate even more dramatic reductions in antibiotic use or zero effluent discharge.

The sensitivity analysis revealed that macrolide prescriptions must decline by 77% to protect 90.4% of the length of the Thames catchment from macrolide resistance selection (Table 6; Figure 4a). Even at this much-reduced prescription rate, there were still 13 reaches with a total length of 8.5 km (0.6%) remaining 'critical' for macrolide resistance selection (Figure 4a). Fluoroquinolone prescriptions would need to be reduced by 85% to alleviate the selection risk in 90% of the catchment (Table 6; Figure 4b).

Discussion

The goal of this study was to evaluate how antibiotic prescribing, alone, contributes to antibiotic resistance selection in the aquatic environment. Although such a perspective is fundamentally naïve given the knowledge of antibiotic use in animals and other pollutants that can co-select for antibiotic resistance, it provides a conservative estimate of our current state of the environment and a starting point for discussing mitigation, which can then begin to include additional antibiotic sources and chemical drivers of resistance [47–49].

Selection for antibiotic resistance in the environment is ancient but is exacerbated by the chronic emissions of antibiotics and co-selecting chemicals from anthropogenic sources [50,51]. However, a non-trivial proportion of the resistance genes found downstream of STPs originate from human gut bacteria, many of which have acquired resistance *in vivo*, perhaps during antibiotic treatment of human infections. Antibiotic-resistance genes disseminated from anthropogenic sources into the environment are pollutants [52,53] maintained and spread through: 1) survival of enteric bacteria in the environment and wildlife [54–56]; 2) horizontal gene transfer of resistance genes from enterics into indigenous environmental bacteria [57]; and 3) selection and co-selection taking place *in situ* [58,59].

Evidence of these processes occurring in the River Thames catchment has been reported in the literature. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* ($bla_{CTX\text{-}M\text{-}14}$) belonging to the clinically important O25b:H4-ST131 lineage was isolated from water from the River Thames in west London [60]. Sediment samples from 13 sites across the River Thames basin were analysed for class 1 integron prevalence and cefotaxime- (third-generation cephalosporin)-resistant *E. coli* [5]. The authors reported both metrics of antibiotic resistance were strongly correlated with proximity to and size of upstream STPs. Manipulation of the level of sewage present within a clean sub-catchment within the Thames basin showed a minor change in the bacterial community composition, but a significant and rapid increase in class 1 integrons—a strong metric of horizontal gene transfer and antibiotic resistance [11]. The evidence in the literature supports this study's contention that the river environment contains, maintains and selects for antibiotic resistance, a phenomenon that is strongly correlated with chemical and DNA pollution of sewage origin.

Strengths and Limitations

To our knowledge, this study is the first attempt to model the impact of current and aspirational antibiotic prescribing in England on antibiotic resistance selection in freshwater rivers. The study's approach reflects the state of our knowledge at this time, and as such, is limited in many ways.

Heterogeneity of population. The results do not reflect the granular differences in prescribing that are seen between small CCGs as there would be no way to attribute an antibiotic user to a particular STP confidently. It is expected that if such an effort were possible, it would highlight elevated antibiotics in STPs that have: older demographics (e.g., care homes [61]), hospitals [62], and areas of inhabitants with elevated international travel [63,64]. Should these STPs be located at or near headwaters, it would likely result in elevated risks for antibiotic resistance selection. Conversely, when these high-antibiotic users are located in stretches where there is sufficient dilution, the risks would be alleviated somewhat.

Varied antibiotic stewardship within antibiotic class. In this model, we applied any reductions in antibiotic prescribing, i.e., 4 or 20%, uniformly across all antibiotics within the class. However, if the antibiotics within a class were unevenly reduced, this could delay or hasten the time to achieve 'low risk'. For example, ciprofloxacin is the most 'potent' fluoroquinolone in its class, with a PNEC of 64 ng/L, whereas ofloxacin and norfloxacin are less 'potent', with a PNEC of 500 ng/L. In other words, ciprofloxacin is 7.8125 times more potent than ofloxacin and norfloxacin. Hence, the benefit gained from reducing 1 unit of ciprofloxacin necessitates a reduction in 7.8125 units of ofloxacin or norfloxacin. As ciprofloxacin represents the majority of fluoroquinolone prescribed (96.9%; Table 2), any reduction in ciprofloxacin is likely to have the maximum impact on fluoroquinolone resistance selection. Macrolides, however, do not differ as much in their capacity to select for resistance. Azithromycin and clarithromycin have PNECs of 250 ng/L, four times more potent than erythromycin (1000 ng/L). Prescription of macrolides varies considerably between the four study CCGs (Tables 2 and 4), with clarithromycin (Gloucestershire and Wiltshire CCG) or erythromycin (Oxfordshire and Swindon) being the most frequently prescribed macrolide. Hence, the CCGs that are overprescribing clarithromycin will find reduced macrolide prescriptions

potentially more impactful owing to the higher potency of clarithromycin relative to erythromycin.

Reliability of PNEC. The PNECs employed in this study have recently emerged as modelled estimates for assessing the risk of resistance selection [33]. To date, modelled PNECs are consistent with the growing literature base [33–45], and as such, have been adopted by the AMR Industry Alliance for establishing regulatory thresholds in antibiotic manufacturing waste effluent [46]. There is a risk that the modelled PNECs are too protective, which would elevate the perception of risk and encourage potentially costly mitigation where it might not be necessary. Conversely, modelled estimates of single antibiotics might also underestimate selection risk, as antibiotic (pollutant) mixtures have been shown to be synergistic, discussed below.

Mixture effects. Recent experimental evidence suggests antibiotics can act synergistically and antagonistically when in mixtures [65,66]. Tekin *et al.* report synergistic and antagonistic interactions increase in frequency with the number of drugs in the bacteria's environment [66]. Hence, a non-trivial number of the modelled PNECs used in this study could be over- and under-estimating the threshold for resistance selection when applied to sewage-impacted rivers.

Brochado *et al.* demonstrated that many synergies were found within the same class of antibiotic, in particular, where the same cellular process was targeted. The authors postulated that by targeting the same functional process at different steps, drug combinations of the same class could bypass the apparent redundancy of having multiple antibiotics within the same class and exhibit a synergistic effect [65]. The authors also demonstrated that many of the drug antagonisms involve antibiotic resistance mechanisms that modulate intracellular drug concentrations and not direct interactions of the primary drug targets. Hence, some antibiotic combinations were less inhibitory than expected (i.e., non-additive) because the cell's response to one drug helped to buffer the effects from the second drug. For example, a decrease in the intracellular concentration of one drug by an efflux pump, can potentially also decrease the intracellular concentration of the interacting drug [65]. Notably, the authors found that the drug-drug interactions were often conserved within species (70%), but with 13–32% of the interactions strain-specific, and poor conservation across species (5% of drug-drug interactions). It is premature to apply any specific drug-drug interactions to this model, but future efforts should attempt to account for mixture effects as part of a holistic risk assessment.

Mixture Effects with Non-Antibiotics. Another limitation of this study is that it does not take into account the presence of many potentially relevant chemicals that are known to be present in sewage and sewage-impacted rivers, such as:

1) antibiotics from agriculture/animal use [67];
2) biocides, which have been shown to co-select for antibiotic resistance genes [68–70];
3) metals, which have been shown to co-select for antibiotic resistance genes [71–74];
4) other classes of pharmaceuticals that might have synergistic or antagonistic impacts on resistance selection [65,75,76], and
5) herbicides and pesticides that have been shown to co-select for resistance [77,78].

It is impractical to experimentally test all the chemicals found in sewage in isolation and mixtures for the threshold concentration that allows for resistance selection, as there are simply too many. However, our environment can be assayed for such insight, by measuring the diversity and quantity of antibiotic resistance genes in the river environment and their relationship to STPs, farms, hospitals, aquaculture, urban runoff, etc. Where 'pristine' environments still exist, they might serve as benchmarks for qualifying and quantifying 'natural' resistance gene prevalence and biogeography. However, this might prove difficult

as wildlife have been implicated in the dissemination of human clinical resistance genes, making ‘pristine’ locations potentially unattainable [55]. Nevertheless, such an understanding would be critical for risk assessment and establishing mitigation targets.

LF2000-WQX Limitations. LF2000-WQX generates PECs from STPs characterised by dry weather flows of >5000 m^3/d. A majority of the small STPs not included in the model are located in the upper-most reaches of the catchment where flows are typically very low. Headwaters impacted by small STPs are likely to have a disproportionate impact on seeding the catchment with pharmaceuticals as well as antibiotic-resistant bacteria, the former of which would further add to the total load of macrolides and fluoroquinolones in the catchment. The omission of these STPs is unlikely to increase the antibiotic load in the catchment substantially, however, it might impact the seeding and maintenance of antibiotic resistance.

Aspirational Reductions in Prescribing. In this study, we projected a decline in prescribing without considering the likely/inevitable change in other important factors, such as, increasing and ageing UK population that will take more pharmaceuticals per capita. The resulting increase in antibiotic load from an ageing population might not have the same level of dilution in the rivers that we ‘enjoy’ today owing potentially dryer summer, resulting from climate change [79,80]. More intense storms predicted in a changing climate would lead to more frequent and longer combined sewage overflows (CSOs), which deposit raw sewage into the adjacent river, thereby elevating the release of resistant bacteria, genes and chemical pollutants into the environment. CSOs, septic tanks, and farm runoff represent potentially significant sources of antibiotics and resistance genes which would benefit from being included in future models. However, such data is not currently available, representing a large knowledge gap for risk assessment.

Validation of PECs. Previous research using LF2000-WQX to generate PECs, e.g., steroid estrogens [24,81], glucocorticoids [82], cytotoxic chemotherapy drugs [83] and antivirals [23], provides reassurance that the river network representation within LF2000-WQX is accurate (i.e., network, flows, dilution and STPs). The modelled antibiotic concentrations from LF2000-WQX, denoted by the PNEC thresholds in Table 6, are within the same range as measured environmental concentrations detailed in Table 7, lending credibility to the model outputs.

Antibiotic Stewardship. Reduction of antibiotics in the environment can be achieved through a range of mitigating actions, the first of which can be through improved antibiotic stewardship. The results of this study indicate that current goals for antibiotic stewardship do not go far enough [84,85]. In Europe, the Netherlands has among the most restricted antibiotic stewardship, with nearly 50% fewer antibiotics prescribed in the community/primary care sector (10.06 DDD/1000 inhabitants/d) than in the UK (19.09), in 2017 (https://ecdc.europa.eu/en/antimicrobial-consumption/database/country-overview). Macrolide prescribing in the Netherlands (1.38 DDD/1000/d) was only 47% that of the UK (2.90 DDD/1000/d). The extent to which the Netherlands still overprescribes and misprescribes macrolides would be highly instructive in defining safe limits for further reductions in macrolide prescribing. Notably, the UK has a lower fluoroquinolone prescribing rate in the community (0.44 DDD/1000/d) as compared to the Netherlands (0.75). Hence, there are important lessons to be mutually shared across Europe and more widely.

The UK has reported between 8.8% and 23.1% of all systemic antibiotic prescriptions in English primary care as inappropriate, with some high prescribing practices in England capable of reducing antibiotic prescriptions by as much as 52.9% [86]. In a linked study where the authors and an expert panel explored the ‘appropriateness’ of antibiotic prescription, the authors found that substantially higher proportions of patients received antibiotics than was deemed ‘appropriate’, with the respiratory ailments showing the largest

contrast between actual and ideal: acute cough (41% vs 10%, respectively), bronchitis (82%:13%); sore throat (59%:13%); rhinosinusitis (88%:11%); and acute otitis media in 2- to 18-year-olds (92%:17%) [87]. Such a reduction in prescribing will be beneficial to reducing resistance selection in humans, but, as shown in this paper, might not go far enough in reducing the environmental pressure for selection (Table 8).

Mitigation through Innovation and Investment in STPs. A step-change in the way we currently handle our wastewater in needed to tackle the challenge of antibiotic and DNA pollution. One option is to set emission limits on the concentration of antibiotics, in much the same way the pharmaceutical industry has voluntarily adopted for their manufacturing supply chain [46]. The setting of strict antibiotic emissions limits will have the desired effect of greatly reducing the environmental impact of antibiotics, with, arguably, several serendipitous implications for other chemical hazards. For example, any technological solution for the treatment of wastewater that is effective in removing a wide range of antibiotics will also likely reduce the load of estrogens and estrogen-mimicking chemicals which has been a growing environmental concern for several decades [88–90]. As such, it is entirely likely that the substantial cost associated with tackling estrogens and estrogen-mimicking chemicals [91] can be shared with the challenge of reducing AMR in the environment.

Moreover, there is a rapidly growing list of chemicals that are found within sewage effluent that have been shown to select or co-select for ARGs, i.e., antiepileptics [75], biocides/disinfectants [92], metals [93]. Hence, engineering solutions to antibiotic removal from wastewater can spread the cost across a very wide range of pollutants, thereby alleviating a substantial range of ecotoxicological hazards, in addition to resistance selection. Spreading the mitigation costs across a range of challenges might also be more politically and socially tractable, owing to the significant cost associated with improving our treatment of wastewater.

Although not immediately within the scope of this paper, it is relevant to highlight that any engineering solutions employed for the removal of antibiotics from sewage effluent might be ineffective in reducing the DNA pollution that ARGs represent. Co-development of engineering solutions to tackle the chemical and biological drivers of ARGs in the environment is required to reduce the environmental pressure caused by our wastewater. In addition, the ‘waste’ by-products of STP, sludge, which contains antibiotic resistance, antibiotics and non-antibiotic chemical drivers of resistance are currently amended to land, representing additional risks to humans and the environment [47], but are out of the study’s scope.

Conclusion. This study aims to catalyze a more holistic discussion about antibiotic stewardship and its impact on the environment. The extent to which further reductions in antibiotic prescribing needs to intensify is estimated, and provides an aspirational target for the NHS. A One Health approach to tackling AMR must consider, more broadly, the factors that can limit the environmental selection and dissemination of antibiotic resistance genes, including co-selecting chemicals, e.g., metals, biocides, and their varied sources. Tackling antimicrobial resistance necessitates the inclusion of interdisciplinary perspectives to ensure a holistic assessment and management of risk.

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References

1. Davies SC. Annual Report of the Chief Medical Officer, Volume Two, 2011, Infections and the rise of antimicrobial resistance [Internet]. Department of Health; 2013 Mar. Available: https://www.gov.uk/government/publications/chief-medical-officer-annual-report-volume-2
2. NHS England Contracts and Incentives Team. Quality Premium: 2015/16 guidance for CCGs [Internet]. 3rd ed. 2015 Sep. Available: https://www.england.nhs.uk/resources/resources-for-ccgs/ccg-out-tool/ccg-ois/qual-prem/
3. Lienert J, Güdel K, Escher BI. Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environ Sci Technol. 2007;41: 4471–4478. doi:10.1021/es0627693
4. Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One. 2010;5: e9836. doi:10.1371/journal.pone.0009836
5. Amos GCA, Gozzard E, Carter CE, Mead A, Bowes MJ, Hawkey PM, et al. Validated predictive modelling of the environmental resistome. ISME J. 2015;9: 1467–1476. doi:10.1038/ismej.2014.237
6. Amos GCA, Ploumakis S, Zhang L, Hawkey PM, Gaze WH, Wellington EMH. The widespread dissemination of integrons throughout bacterial communities in a riverine system. ISME J. 2018;12: 681–691. doi:10.1038/s41396-017-0030-8
7. Marti E, Jofre J, Balcazar JL. Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. PLoS One. 2013;8: e78906. doi:10.1371/journal.pone.0078906
8. Berglund B, Fick J, Lindgren P-E. Urban wastewater effluent increases antibiotic resistance gene concentrations in a receiving northern European river. Environ Toxicol Chem. 2015;34: 192–196. doi:10.1002/etc.2784
9. Karkman A, Pärnänen K, Larsson DGJ. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nat Commun. 2019;10: 80. doi:10.1038/s41467-018-07992-3
10. Hu A, Ju F, Hou L, Li J, Yang X, Wang H, et al. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. Environ Microbiol. 2017; doi:10.1111/1462-2920.13942
11. Lehmann K, Bell T, Bowes MJ, Amos GCA, Gaze WH, Wellington EMH, et al. Trace levels of sewage effluent are sufficient to increase class 1 integron prevalence in freshwater biofilms without changing the core community. Water Res. 2016;106: 163–170. doi:10.1016/j.watres.2016.09.035
12. Bai Y, Meng W, Xu J, Zhang Y, Guo C. Occurrence, distribution and bioaccumulation of antibiotics in the Liao River Basin in China. Environ Sci Process Impacts. 2014;16: 586–593. doi:10.1039/c3em00567d
13. Sabri NA, Schmitt H, Van der Zaan B, Gerritsen HW, Zuidema T, Rijnaarts HHM, et al. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. Journal of Environmental Chemical Engineering. 2018; doi:10.1016/j.jece.2018.03.004
14. Gao L, Shi Y, Li W, Liu J, Cai Y. Occurrence, distribution and bioaccumulation of antibiotics in the Haihe River in China. J Environ Monit. 2012;14: 1248–1255. doi:10.1039/c2em10916f
15. Watkinson AJ, Murby EJ, Kolpin DW, Costanzo SD. The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. Sci Total Environ. 2009;407: 2711–2723. doi:10.1016/j.scitotenv.2008.11.059
16. Singer AC, Järhult JD, Grabic R, Khan GA, Lindberg RH, Fedorova G, et al. Intra- and inter-pandemic variations of antiviral, antibiotics and decongestants in wastewater

treatment plants and receiving rivers. PLoS One. 2014;9: e108621. doi:10.1371/journal.pone.0108621

17. Johnson AC, Keller V, Dumont E, Sumpter JP. Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers. Sci Total Environ. 2015;511: 747–755. doi:10.1016/j.scitotenv.2014.12.055
18. Murray GL, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerging Infect Dis. 2017;23: 809–812. doi:10.3201/eid2305.161745
19. Sousa JCG, Ribeiro AR, Barbosa MO, Pereira MFR, Silva AMT. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J Hazard Mater. 2017;344: 146–162. doi:10.1016/j.jhazmat.2017.09.058
20. Barbosa MO, Moreira NFF, Ribeiro AR, Pereira MFR, Silva AMT. Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495. Water Res. 2016;94: 257–279. doi:10.1016/j.watres.2016.02.047
21. Keller VDJ, Williams RJ, Lofthouse C, Johnson AC. Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors. Environ Toxicol Chem. 2014;33: 447–452. doi:10.1002/etc.2441
22. Singer AC, Nunn MA, Gould EA, Johnson AC. Potential risks associated with the proposed widespread use of Tamiflu. Environ Health Perspect. 2007;115: 102–106. doi:10.1289/ehp.9574
23. Singer AC, Colizza V, Schmitt H, Andrews J, Balcan D, Huang WE, et al. Assessing the ecotoxicologic hazards of a pandemic influenza medical response. Environ Health Perspect. 2011;119: 1084–1090. doi:10.1289/ehp.1002757
24. Williams RJ, Keller VDJ, Johnson AC, Young AR, Holmes MGR, Wells C, et al. A national risk assessment for intersex in fish arising from steroid estrogens. Environ Toxicol Chem. 2009;28: 220–230. doi:10.1897/08-047.1
25. Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson BAV. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environ Sci Technol. 2005;39: 3421–3429. doi:10.1021/es048143z
26. Jia A, Wan Y, Xiao Y, Hu J. Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. Water Res. 2012;46: 387–394. doi:10.1016/j.watres.2011.10.055
27. Göbel A, McArdell CS, Joss A, Siegrist H, Giger W. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ. 2007;372: 361–371. doi:10.1016/j.scitotenv.2006.07.039
28. Radjenović J, Petrović M, Barceló D. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 2009;43: 831–841. doi:10.1016/j.watres.2008.11.043
29. Watkinson AJ, Murby EJ, Costanzo SD. Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. Water Res. 2007;41: 4164–4176. doi:10.1016/j.watres.2007.04.005
30. Michael I, Rizzo L, McArdell CS, Manaia CM, Merlin C, Schwartz T, et al. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 2013;47: 957–995. doi:10.1016/j.watres.2012.11.027
31. Keller V, Young AR. Development of an Integrated Water Resources and Water Quality Modelling System. 2004;
32. Young AR, Grew R, Holmes MG. Low Flows 2000: a national water resources assessment and decision support tool. Water Sci Technol. 2003;48: 119–126.

33. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environ Int. 2016;86: 140–149. doi:10.1016/j.envint.2015.10.015
34. Sandegren L. Selection of antibiotic resistance at very low antibiotic concentrations. Ups J Med Sci. 2014;119: 103–107. doi:10.3109/03009734.2014.904457
35. Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio. 2014;5: e01918–14. doi:10.1128/mBio.01918-14
36. Strukova EN, Portnoy YA, Zinner SH, Firsov AA. Predictors of bacterial resistance using in vitro dynamic models: area under the concentration-time curve related to either the minimum inhibitory or mutant prevention antibiotic concentration. J Antimicrob Chemother. 2016;71: 678–684. doi:10.1093/jac/dkv387
37. Khan S, Beattie TK, Knapp CW. The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria. Ecotoxicology. 2017;26: 283–292. doi:10.1007/s10646-017-1762-y
38. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, et al. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 2011;7: e1002158. doi:10.1371/journal.ppat.1002158
39. Liu A, Fong A, Becket E, Yuan J, Tamae C, Medrano L, et al. Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. Antimicrob Agents Chemother. 2011;55: 1204–1210. doi:10.1128/AAC.01182-10
40. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol. 2014;12: 465–478. doi:10.1038/nrmicro3270
41. Andersson DI, Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist Updat. 2012;15: 162–172. doi:10.1016/j.drup.2012.03.005
42. Mezger A, Gullberg E, Göransson J, Zorzet A, Herthnek D, Tano E, et al. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. J Clin Microbiol. 2015;53: 425–432. doi:10.1128/JCM.02434-14
43. Hughes D, Andersson DI. Selection of resistance at lethal and non-lethal antibiotic concentrations. Curr Opin Microbiol. 2012;15: 555–560. doi:10.1016/j.mib.2012.07.005
44. Kraupner N, Ebmeyer S, Bengtsson-Palme J, Fick J, Kristiansson E, Flach C-F, et al. Selective concentration for ciprofloxacin resistance in' ' Escherichia coli grown in complex aquatic bacterial biofilms. Environ Int. 2018;116: 255–268. doi:10.1016/j.envint.2018.04.029
45. Murray AK, Zhang L, Yin X, Zhang T, Buckling A, Snape J, et al. Novel Insights into Selection for Antibiotic Resistance in Complex Microbial Communities. MBio. 2018;9. doi:10.1128/mBio.00969-18
46. AMR Industry Alliance. AMR Industry Alliance Antibiotic Discharge Targets. In: AMR Alliance Recommended PNECs for Risk Assessments [Internet]. 2018 [cited 14 Jan 2019]. Available: https://www.amrindustryalliance.org/wp-content/uploads/2018/09/AMR_Industry_Alliance_List-of-Predicted-No-Effect-Concentrations-PNECs.pdf
47. Singer A, Shaw H, Rhodes V, Hart A. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. Front Microbiol. 2016;7: 1728. doi:10.3389/fmicb.2016.01728
48. Bürgmann H, Frigon D, H Gaze W, M Manaia C, Pruden A, Singer AC, et al. Water and sanitation: an essential battlefront in the war on antimicrobial resistance. FEMS Microbiol Ecol. 2018;94. doi:10.1093/femsec/fiy101
49. International Environmental AMR Forum. Initiatives for Addressing' ' Antimicrobial Resistance in the Environment Current: Situation and Challenges. Wellcome Trust, U.S. CDC, UK Science & Innovation Network; 2018.
50. Graham DW, Knapp CW, Christensen BT, McCluskey S, Dolfing J. Appearance of β-lactam Resistance Genes in Agricultural Soils and Clinical Isolates over the 20th Century. Sci Rep. 2016;6: 21550. doi:10.1038/srep21550

51. Perry J, Waglechner N, Wright G. The prehistory of antibiotic resistance. Cold Spring Harb Perspect Med. 2016;6. doi:10.1101/cshperspect.a025197
52. Pruden A, Pei R, Storteboom H, Carlson KH. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ Sci Technol. 2006;40: 7445–7450. doi:10.1021/es060413l
53. Gillings MR. DNA as a pollutant: the clinical class 1 integron. Curr Pollution Rep. 2018;4: 49–55. doi:10.1007/s40726-018-0076-x
54. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental Escherichia coli: ecology and public health implications-a review. J Appl Microbiol. 2017;123: 570–581. doi:10.1111/jam.13468
55. McCann CM, Christgen B, Roberts JA, Su J-Q, Arnold KE, Gray ND, et al. Understanding drivers of antibiotic resistance genes in High Arctic soil ecosystems. Environ Int. 2019; doi:10.1016/j.envint.2019.01.034
56. Furness LE, Campbell A, Zhang L, Gaze WH, McDonald RA. Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. Environ Res. 2017;154: 28–34. doi:10.1016/j.envres.2016.12.014
57. Amos GCA, Hawkey PM, Gaze WH, Wellington EM. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J Antimicrob Chemother. 2014;69: 1785–1791. doi:10.1093/jac/dku079
58. Chait R, Palmer AC, Yelin I, Kishony R. Pervasive selection for and against antibiotic resistance in inhomogeneous multistress environments. Nat Commun. 2016;7: 10333. doi:10.1038/ncomms10333
59. Zhang Y, Gu AZ, Cen T, Li X, He M, Li D, et al. Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. Environ Pollut. 2018;237: 74–82. doi:10.1016/j.envpol.2018.01.032
60. Dhanji H, Murphy NM, Akhigbe C, Doumith M, Hope R, Livermore DM, et al. Isolation of fluoroquinolone-resistant O25b:H4-ST131 Escherichia coli with CTX-M-14 extended-spectrum β-lactamase from UK river water. J Antimicrob Chemother. 2011;66: 512–516. doi:10.1093/jac/dkq472
61. Dhanji H, Doumith M, Rooney PJ, O'Leary MC, Loughrey AC, Hope R, et al. Molecular epidemiology of fluoroquinolone-resistant ST131 Escherichia coli producing CTX-M extended-spectrum beta-lactamases in nursing homes in Belfast, UK. J Antimicrob Chemother. 2011;66: 297–303. doi:10.1093/jac/dkq463
62. Weingarten RA, Johnson RC, Conlan S, Ramsburg AM, Dekker JP, Lau AF, et al. Genomic analysis of hospital plumbing reveals diverse reservoir of bacterial plasmids conferring carbapenem resistance. MBio. 2018;9. doi:10.1128/mBio.02011-17
63. McNulty CAM, Lecky DM, Xu-McCrae L, Nakiboneka-Ssenabulya D, Chung K-T, Nichols T, et al. CTX-M ESBL-producing Enterobacteriaceae: estimated prevalence in adults in England in 2014. J Antimicrob Chemother. 2018;73: 1368–1388. doi:10.1093/jac/dky007
64. Dhanji H, Patel R, Wall R, Doumith M, Patel B, Hope R, et al. Variation in the genetic environments of bla(CTX-M-15) in Escherichia coli from the faeces of travellers returning to the United Kingdom. J Antimicrob Chemother. 2011;66: 1005–1012. doi:10.1093/jac/dkr041
65. Brochado AR, Telzerow A, Bobonis J, Banzhaf M, Mateus A, Selkrig J, et al. Species-specific activity of antibacterial drug combinations. Nature. 2018;559: 259–263. doi:10.1038/s41586-018-0278-9
66. Tekin E, White C, Kang TM, Singh N, Cruz-Loya M, Damoiseaux R, et al. Prevalence and patterns of higher-order drug interactions in Escherichia coli. npj Syst Biol Appl. 2018;4: 31. doi:10.1038/s41540-018-0069-9
67. European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), European Medicines Agency (EMA). ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and

occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. EFSA Journal. 2017;15. doi:10.2903/j.efsa.2017.4872

68. European Commission. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides,. 2010;

69. Carey DE, McNamara PJ. The impact of triclosan on the spread of antibiotic resistance in the environment. Front Microbiol. 2014;5: 780. doi:10.3389/fmicb.2014.00780

70. Veterinary Medicines Directorate. UK Veterinary Antibiotic' ' Resistance and Sales Surveillance Report UK - VARSS 2017. Veterinary Medicines Directorate; 2018 Oct.

71. Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. Trends Microbiol. 2006;14: 176–182. doi:10.1016/j.tim.2006.02.006

72. Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol. 2012;3: 399. doi:10.3389/fmicb.2012.00399

73. Beane SJ, Comber SDW, Rieuwerts J, Long P. Abandoned metal mines and their impact on receiving waters: A case study from Southwest England. Chemosphere. 2016;153: 294–306. doi:10.1016/j.chemosphere.2016.03.022

74. Comber SDW, Gardner MJ, Churchley J. Aluminium speciation: implications of wastewater effluent dosing on river water quality. Chem Spec & Bioavail. 2005;17: 117–128. doi:10.3184/095422905782774874

75. Wang Y, Lu J, Mao L, Li J, Yuan Z, Bond PL, et al. Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera. ISME J. 2018; doi:10.1038/s41396-018-0275-x

76. Comber S, Gardner M, Sörme P, Leverett D, Ellor B. Active pharmaceutical ingredients entering the aquatic environment from wastewater treatment works: A cause for concern? Sci Total Environ. 2017;613-614: 538–547. doi:10.1016/j.scitotenv.2017.09.101

77. Kurenbach B, Marjoshi D, Amábile-Cuevas CF, Ferguson GC, Godsoe W, Gibson P, et al. 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. 2015;6. doi:10.1128/mBio.00009-15

78. Kurenbach B, Gibson PS, Hill AM, Bitzer AS, Silby MW, Godsoe W, et al. Herbicide ingredients change Salmonella enterica sv. Typhimurium and Escherichia coli antibiotic responses. Microbiology (Reading, Engl). 2017; doi:10.1099/mic.0.000573

79. Johnson AC, Acreman MC, Dunbar MJ, Feist SW, Giacomello AM, Gozlan RE, et al. The British river of the future: how climate change and human activity might affect two contrasting river ecosystems in England. Sci Total Environ. 2009;407: 4787–4798. doi:10.1016/j.scitotenv.2009.05.018

80. Keller VDJ, Lloyd P, Terry JA, Williams RJ. Impact of climate change and population growth on a risk assessment for endocrine disruption in fish due to steroid estrogens in England and Wales. Environ Pollut. 2015;197: 262–268. doi:10.1016/j.envpol.2014.11.017

81. Johnson AC, Williams RJ. A Model To Estimate Influent and Effluent Concentrations of Estradiol, Estrone, and Ethinylestradiol at Sewage Treatment Works. Environ Sci Technol. 2004;38: 3649–3658. doi:10.1021/es035342u

82. Kugathas S, Williams RJ, Sumpter JP. Prediction of environmental concentrations of glucocorticoids: the River Thames, UK, as an example. Environ Int. 2012;40: 15–23. doi:10.1016/j.envint.2011.11.007

83. Johnson AC, Jürgens MD, Williams RJ, Kümmerer K, Kortenkamp A, Sumpter JP. Do cytotoxic chemotherapy drugs discharged into rivers pose a risk to the environment and human health? An overview and UK case study. J Hydrol (Amst). 2008;348: 167–175. doi:10.1016/j.jhydrol.2007.09.054

84. Davies SC. Reducing inappropriate prescribing of antibiotics in English primary care: evidence and outlook. J Antimicrob Chemother. 2018;73: 833–834. doi:10.1093/jac/dkx535
85. HM Government. Tackling antimicrobial resistance 2019–2024: The UK's five-year national action plan. HM Government; 2019 Jan.
86. Smieszek T, Pouwels KB, Dolk FCK, Smith DRM, Hopkins S, Sharland M, et al. Potential for reducing inappropriate antibiotic prescribing in English primary care. J Antimicrob Chemother. 2018;73: ii36–ii43. doi:10.1093/jac/dkx500
87. Pouwels KB, Dolk FCK, Smith DRM, Robotham JV, Smieszek T. Actual versus "ideal" antibiotic prescribing for common conditions in English primary care. J Antimicrob Chemother. 2018;73: 19–26. doi:10.1093/jac/dkx502
88. Harris CA, Hamilton PB, Runnalls TJ, Vinciotti V, Henshaw A, Hodgson D, et al. The consequences of feminization in breeding groups of wild fish. Environ Health Perspect. 2011;119: 306–311. doi:10.1289/ehp.1002555
89. Johnson AC, Dumont E, Williams RJ, Oldenkamp R, Cisowska I, Sumpter JP. Do concentrations of ethinylestradiol, estradiol, and diclofenac in European rivers exceed proposed EU environmental quality standards? Environ Sci Technol. 2013;47: 12297–12304. doi:10.1021/es4030035
90. Jobling S, Williams R, Johnson A, Taylor A, Gross-Sorokin M, Nolan M, et al. Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. Environ Health Perspect. 2006;114 Suppl 1: 32–39. doi:10.1289/ehp.8050
91. Owen R, Jobling S. Environmental science: The hidden costs of flexible fertility. Nature. 2012;485: 441. doi:10.1038/485441a
92. Yazdankhah SP, Scheie AA, Høiby EA, Lunestad B-T, Heir E, Fotland TØ, et al. Triclosan and antimicrobial resistance in bacteria: an overview. Microb Drug Resist. 2006;12: 83–90. doi:10.1089/mdr.2006.12.83
93. Di Cesare A, Eckert E, Corno G. Co-selection of antibiotic and heavy metal resistance in freshwater bacteria. J Limnol. 2016;75. doi:10.4081/jlimnol.2016.1198
94. Zhang Q-Q, Ying G-G, Pan C-G, Liu Y-S, Zhao J-L. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ Sci Technol. 2015;49: 6772–6782. doi:10.1021/acs.est.5b00729
95. Kümmerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin Microbiol Infect. 2003;9: 1203–1214. doi:10.1111/j.1469-0691.2003.00739.x
96. Besse J-P, Kausch-Barreto C, Garric J. Exposure assessment of pharmaceuticals and their metabolites in the aquatic environment: application to the french situation and preliminary prioritization. Human and Ecological Risk Assessment: An International Journal. 2008;14: 665–695. doi:10.1080/10807030802235078
97. Monteiro SC, Boxall ABA. Occurrence and fate of human pharmaceuticals in the environment. Rev Environ Contam Toxicol. 2010;202: 53–154. doi:10.1007/978-1-4419-1157-5_2
98. Nightingale CH. Moxifloxacin, a new antibiotic designed to treat community-acquired respiratory tract infections: a review of microbiologic and pharmacokinetic-pharmacodynamic characteristics. Pharmacotherapy. 2000;20: 245–256. doi:10.1592/phco.20.4.245.34880
99. Johnson AC, Jürgens MD, Nakada N, Hanamoto S, Singer AC, Tanaka H. Linking changes in antibiotic effluent concentrations to flow, removal and consumption in four different UK sewage treatment plants over four years. Environ Pollut. 2017;220: 919–926. doi:10.1016/j.envpol.2016.10.077

Table 1. Human excretion of fluoroquinolones and macrolides as a percentage of the parent compound.

Class of Antibiotic	Antibiotics	Excretion (% of parent compound)
Fluoroquinolones	Ciprofloxacin	53.8 [94], 40 [95], 50 [96], 45-60 [97]
	Levofloxacin	71 (60-80) [94], 70 [95]
	Moxifloxacin	60 [98]
	Norfloxacin	61.5 [94], 30 [95]
	Ofloxacin	75.8 (60-80) [94], 100 [96]
Macrolides	Azithromycin	50 [96], 50 [97], 8 [95]
	Clarithromycin	33.7 (14.4-60) [94], 20 [95], 0.18 [96]
	Erythromycin	35 (3.5-98) [94], 8 [95], 12-15 [97]

Table 2. Mass of macrolides and fluoroquinolones prescribed in the study CCGs in 2015/16.

		Oxfordshire (kg)	Gloucestershire (kg)	Swindon (kg)	Wiltshire (kg)
Macrolides	Azithromycin	21.8	18.9	4.5	12.6
	Clarithromycin	179.5	322.7	66.7	198.0
	Erythromycin	97.3	80.7	50.6	82.3
	Erythromycin ethyl succinate	120.8	79.0	32.3	34.4
	Erythromycin stearate	12.4	5.4	4.3	5.0
Total of macrolides (kg)		431.8	506.7	158.4	332.3
Fluoroquinolones	Ciprofloxacin	93.8	94.6	29.5	72.2
	Levofloxacin	0.3	4.6	0	0.3
	Moxifloxacin	0.5	0.1	0.1	0.2
	Norfloxacin	0	0	0.1	0
	Ofloxacin	2.2	4.1	0.7	1.0
Total of fluoroquinolones (kg)		96.8	103.4	30.4	73.7

Table 3. Molecular weights of macrolides and fluoroquinolones.

Macrolides	Molecular weight (g/mol)	Quinolones	Molecular weight (g/mol)
Azithromycin	748.98	Ciprofloxacin	331.34
Clarithromycin	747.95	Levofloxacin	361.36
Erythromycin	733.90	Moxifloxacin	401.43
Erythromycin ethyl succinate	862.10	Norfloxacin	319.33
Erythromycin stearate	1018.40	Ofloxacin	361.36

Table 4. Moles of macrolides (M) and fluoroquinolones (F) prescribed capita^{-1} day^{-1} month^{-1} within each CCG

CCGs	Oxfordshire[1]		Swindon[2]		Gloucestershire[3]		Wiltshire[4]	
Population	666100		231277		624000		500000	
Antibiotics	M	F	M	F	M	F	M	F
	$\times 10^{-7}$ mol							
January	**30.4**	**13.3**	24.8	**12.5**	27.9	12.7	23.4	11.5
February	28.7	12.8	28.4	11.2	36.7	**16.4**	**30.2**	**13.9**
March	28.1	11.7	**30.5**	11.8	**36.8**	15.5	28.0	11.8
April	23.6	11.9	26.0	11.1	30.4	12.5	25.8	11.8
May	21.3	11.1	22.8	9.50	26.9	13.6	23.1	10.9
June	22.5	12.0	25.6	12.9	28.8	13.1	24.2	11.8
July	20.5	11.5	24.8	11.3	26.7	13.6	22.5	12.3
August	10.2	11.6	18.2	9.40	22.0	12.0	19.9	12.4
September	20.9	11.2	22.3	9.30	25.2	13.7	22.3	12.4
October	22.0	12.3	22.1	10.9	27.8	13.6	22.3	12.8
November	22.2	12.1	22.1	9.80	29.1	12.5	21.6	11.4
December	23.4	12.6	25.4	10.0	31.8	14.4	25.6	12.9

Population statistics are provided by the respective Annual Report for the CCG. Bolded values in each column represent the month with the maximum prescription rate in 2015. These were selected for use in the model to ensure a realistic worst-case scenario.

[1] NHS Oxfordshire CCG Annual Report

[2] NHS Swindon CCG Annual Report

[3] NHS Gloucestershire CCG Annual Report

[4] NHS Wiltshire CCG Annual Report

Table 5. Measured antibiotics concentration in STPs and rivers within the Thames Catchment (ng/L).

	Macrolide			Fluoroquinolone		
	CLAR	ERY	AZO	CIP	NOR	OFL
Eff – Oxford*	98 (338 – 1504)		156 (69 – 264)			
Eff – Didcot*	104 (160 – 305)		110 (74 – 216)			
Eff – Cholsey*	181 (128 – 321)		91 (48 – 135)			
Eff – Benson*	152 (87 – 254)		76 (35 – 133)			
Eff – Benson**	50	244	34	14	25	195
Eff – Oxford**	91	236	30	52	21	23
Thames var.***	292, 30	448, 58	51, 21	46, 20	45, 9	17, 11

Eff sewage effluent, CLAR clarithromycin, ERY erythromycin, AZO azithromycin, CIP ciprofloxacin, NOR norfloxacin, OFL ofloxacin

* Mean (highest – lowest) measured [99]

**24-h mean concentration [16]

***Max and mean concentration for 21 locations at 7 time points [16]

Table 6. Modelled PNECs for macrolides and fluoroquinolones [33] **.**

Antibiotic class	Antibiotic	PNEC (µg/L)	PNEC (10^{-10} mol/L)
Fluoroquinolones	Ciprofloxacin	0.064	1.9
	Levofloxacin	0.25	6.9
	Moxifloxacin	0.125	3.1
	Norfloxacin	0.5	15.7
	Ofloxacin	0.5	13.8
Macrolides	Azithromycin	0.25	3.3
	Clarithromycin	0.25	3.3
	Erythromycin	1	9.8

Table 7. Sensitivity analysis for level of reduction in prescriptions needed to protect 90+% of the length of the modelled River Thames catchment from resistance gene selection.

	% Reduction in Prescriptions	Number of reaches 'at risk' or 'critical'	Length 'at risk' or 'critical' (km)	% Length 'at risk' or 'critical'
Macrolides	80	69	110	7.9
	77	**82**	**134**	**9.6**
	76	86	157	11.2
	75	93	169	12.1
Fluoroquinolones	**85**	**82**	**135**	**9.6**
	84	88	157	11.2
	83	96	175	12.5
	80	118	215	15.4

Table 8. Summary of modelled impact of antibiotic prescribing on antibiotic resistance gene selection.

Scenario	% of Modelled Catchment 'at risk' and/or 'critical' (rounded to nearest %)	
	Macrolide	Fluoroquinolone
Antibiotics from 2015-16	63	73
4% Reduction both antibiotic classes	59	73
20% Reduction both antibiotic classes	54	68
90% of catchment at 'low risk'	77	85

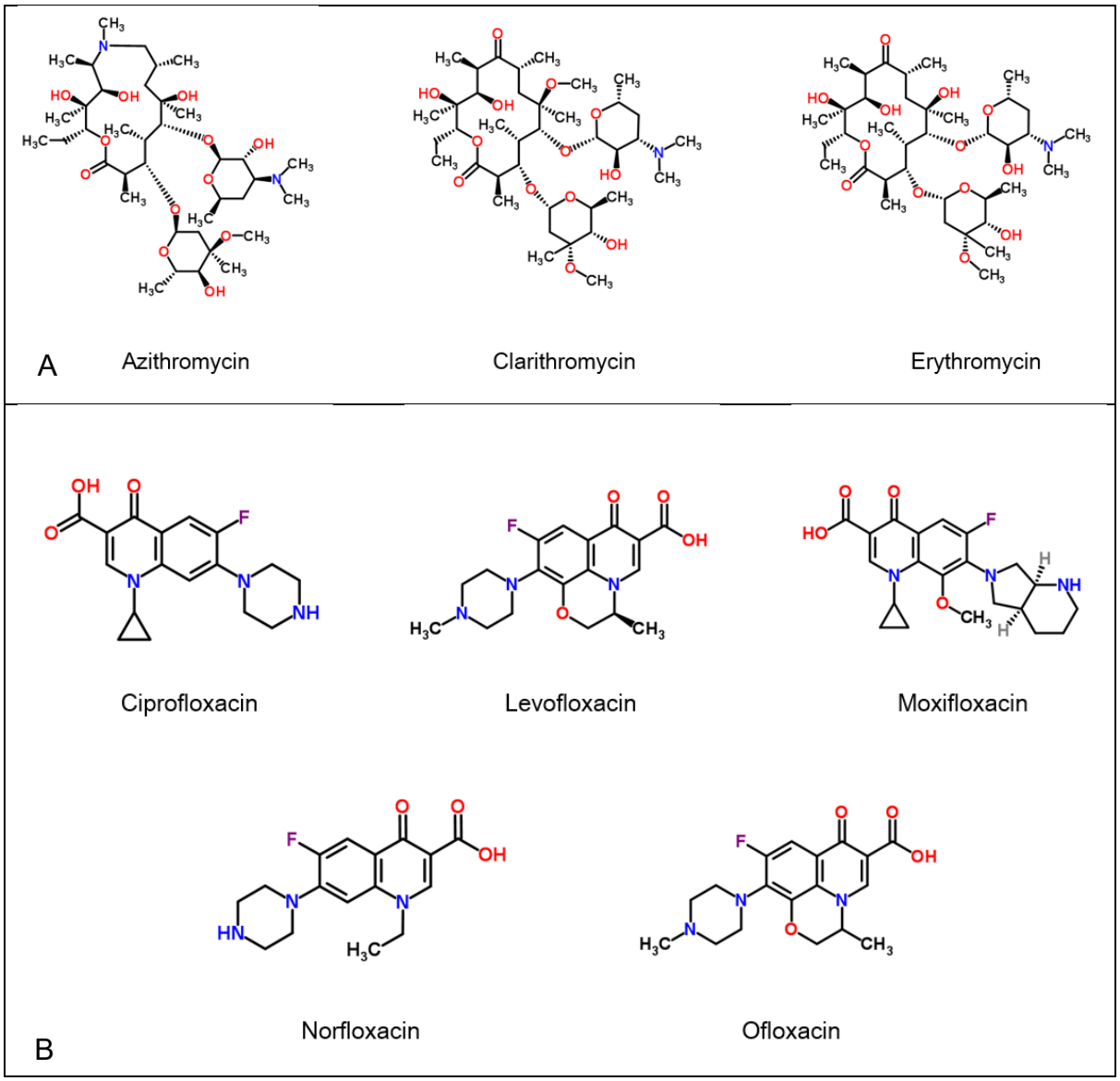

Figure 1. Antibiotics: A) macrolides and B) fluoroquinolones included in the study

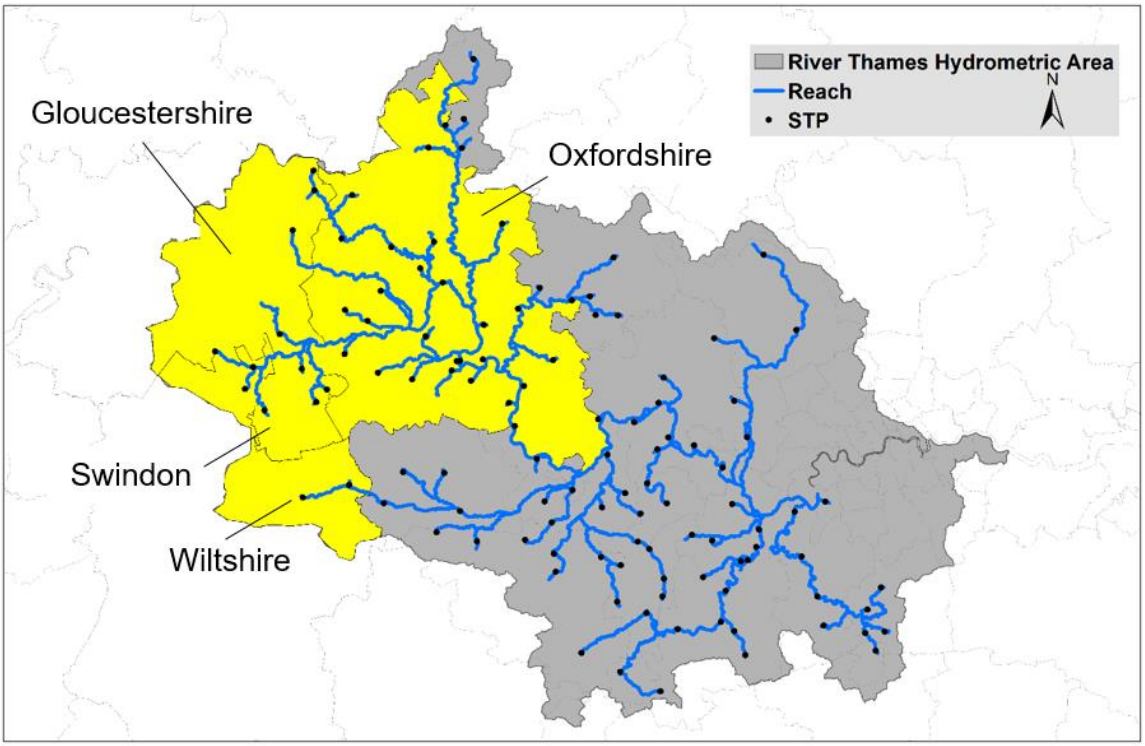

Figure 2. Hydrometric area of Thames Region with the four CCGs in the upper Thames Catchment denoted in yellow.

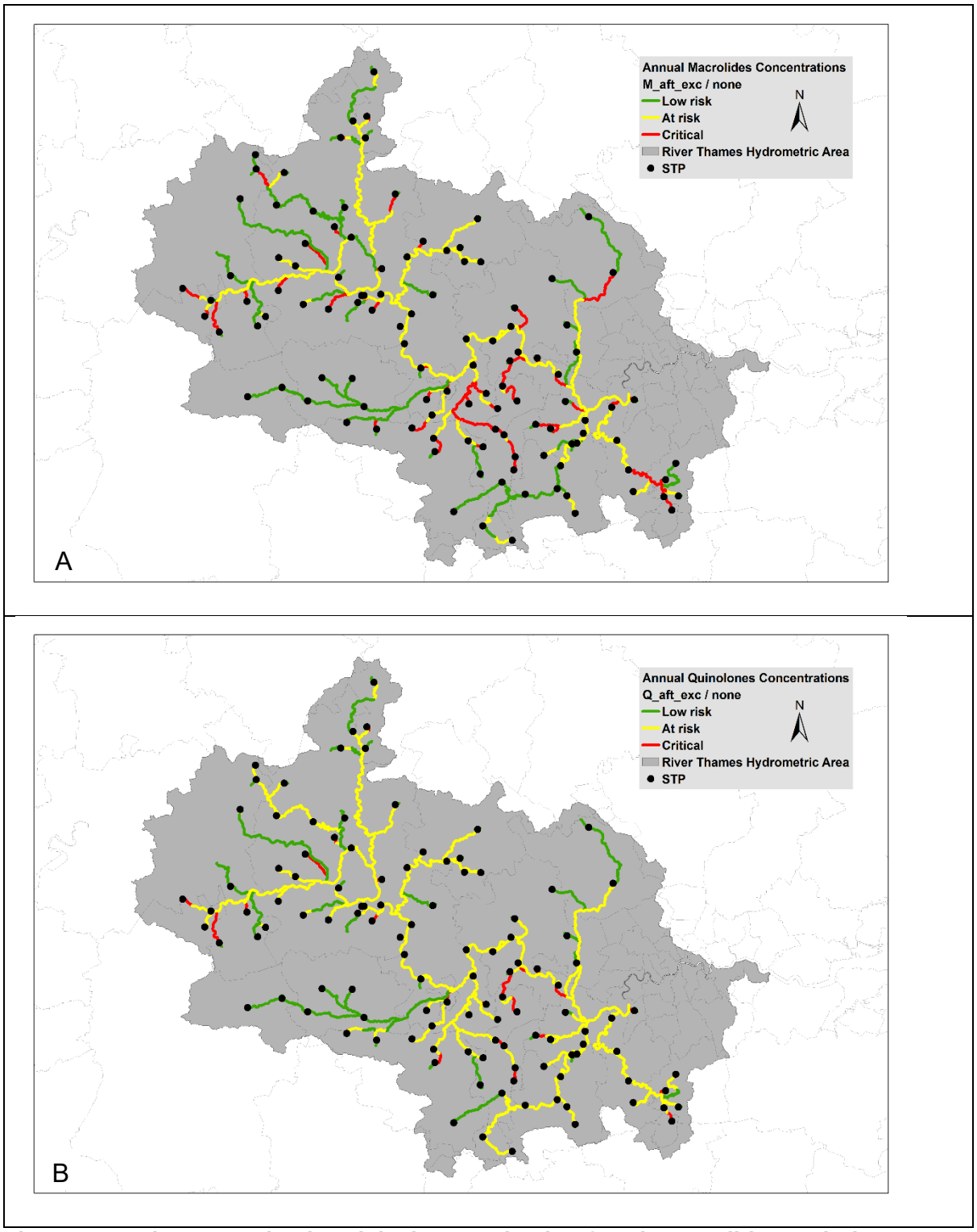

Figure 3. Resistance selection risk characterisation for A) macrolides and B) fluoroquinolones, using 2015/16 prescription statistics.

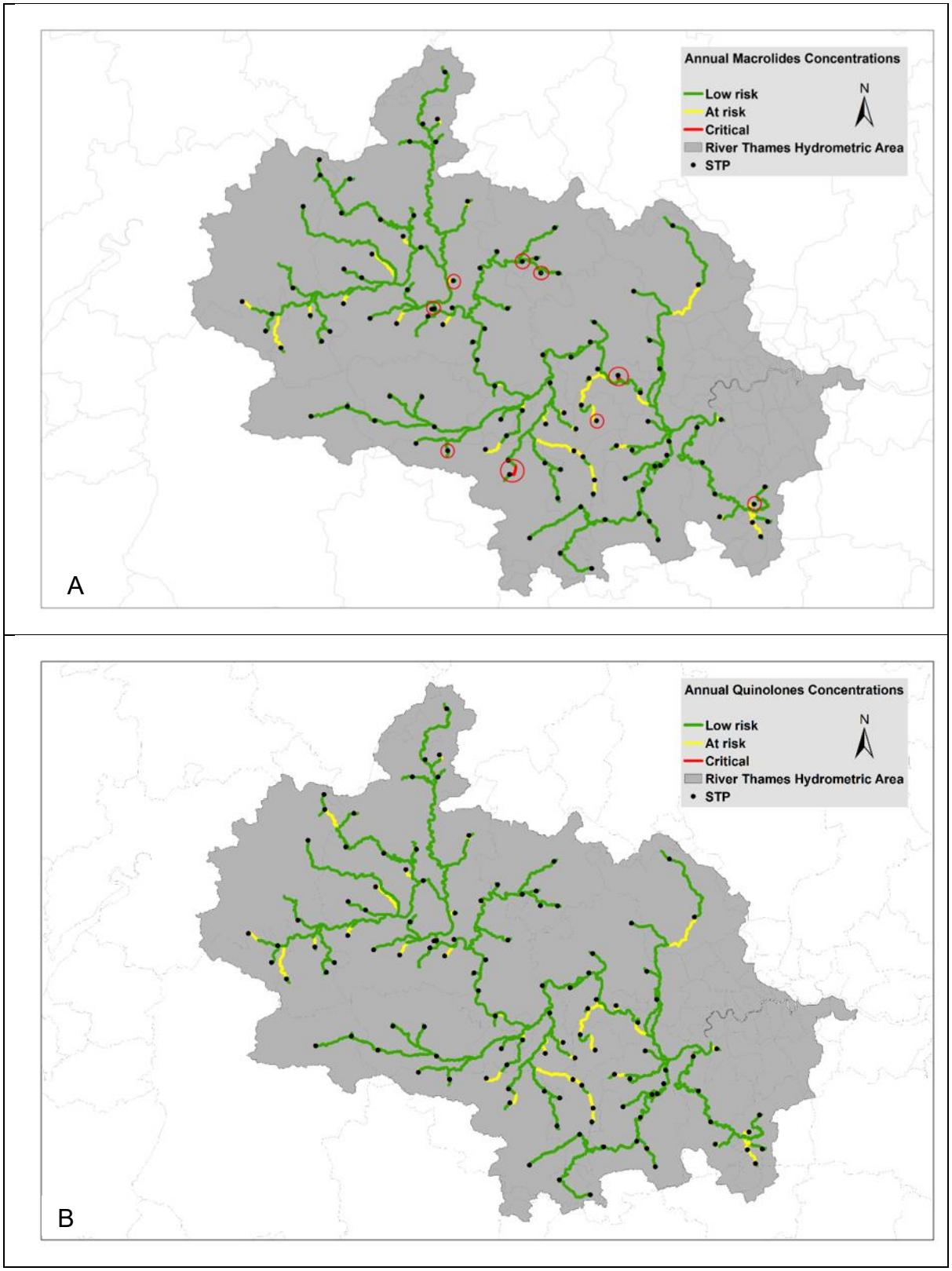

Figure 4. Hazard characterisation for (A) macrolide and (B) fluoroquinolone resistance selection after a reduction of 77% and 85% in prescriptions, respectively, on 2015/16 rates. Red circles in (A) indicate nine locations where the concentrations remained 'at critical' levels.