Applied
Microbiology
International

CASE STUDY

NOVEMBER 2021

AMR case study: AMR in the environment

AMR case study: AMR in the environment

Applied Microbiology International is a global membership organization that seeks to bring the international microbiology community together to advance scientific impact. We are the oldest microbiology society in the UK and with more than half of our membership outside the UK, we are truly global, serving microbiologists based in universities, private industry and research institutes around the world.

The environment plays a critical role in the development, dissemination, and proliferation of AMR.

There is no structural, statutory surveillance dedicated to assessing the level of AMR in the environment in the UK.

Policymakers will need to support scientists' efforts in order to sufficiently reduce AMR in the environment. This support should include dedicating more resources to research, establishing sufficient reduction targets and measures, and developing policies that encourage multi-stakeholder collaboration.

Microbiologists play a crucial role in combatting AMR in the environment by investigating constant changes in the environment that promote the evolution of AMR and developing new technologies for monitoring, diagnosing, and mitigating AMR in all environmental sources.

INTRODUCTION

Most antimicrobial resistance (AMR) research and public policy, to date, has concentrated on human and animal health. However, the environment plays an equally critical role in the development, dissemination, and proliferation of AMR. The environment acts as a reservoir that provides ideal conditions for a myriad of resistance genes to evolve, then disseminate, and finally spread back to humans and animals. Given the extent of the environment, which encompasses the land, air, and sea, there are innumerable sources of, and ways AMR can spread. Some of those pathways include pharmaceutical or hospital wastewater, soils, manures, and agricultural runoff. Likewise, there are many AMR exposure risks for humans and animals such as through drinking water, irrigation water, food products, or even recreational sources like beaches and lakes.

Despite its importance, policy and research on AMR in the environment has been insufficient. Both the UK's 20-year vision (published in 2019) and the UK's 2019 One Health Report found that 'There is no structural, statutory surveillance dedicated to assessing the level of AMR in the environment in the UK'.[1,2] In June 2021, Lord Trees also raised concerns about AMR in the environment with the UK's Environment Bill noting, 'Currently there is no mandatory routine surveillance required for antimicrobials in the aquatic environment, nor is there routine surveillance for antibiotic resistance among bacteria in that environment. These seem to be essential data-collection functions which would help enable the national action plan to deliver its objectives. Moreover, it is not clear who will be responsible for setting environmental quality standards for antimicrobial environmental contamination'.[3]

The research questions that need to be addressed to better understand the role of AMR in the environment include:

- **What are the different drivers of AMR in the environment?**
- **What is the role of the environment in AMR's evolution and dissemination?**
- **What are the feasibility and success rates of various mitigation measures for containing AMR's spread in the environment?**
- **And how, when, and what should we be monitoring in order to assess AMR in the environment accurately?**

Without sufficient research, scientists cannot identify and implement successful solutions for containing AMR in the environment. Therefore, it is essential that research on AMR in the environment receives more support so that the UK can set suitable targets and establish ways to monitor adherence to those targets.

In addition, those solutions will require policy support for more coordinated, multi-stakeholder interactions to ensure that all sources and pathways are considered, and research outputs are as impactful as possible. Contributors, such as manufacturers, farmers, and policymakers, will all need to be included in this process.

The impact of climate change will also need to be considered as it is exacerbating the rising threat of AMR through increasing global temperature and humidity, conditions that favour bacterial growth and spread of infectious disease. It is also leading to more frequent extreme weather events such as flooding, which increases exposure of humans and animals to environmental sources of AMR and antimicrobial pollution. These effects are compounded by increased intensive farming systems and rising use of antimicrobials in humans globally. It is therefore vital that researchers investigate these questions now as the effects of climate change are set to amplify the conditions that enable AMR evolution and dispersal.

NIHAL BANDARA
University of Bristol

Owing to the multidirectional linkage of AMR to humans, animals, plants and environment, holistic and multidisciplinary approaches are quintessential in managing this pandemic. Such approaches should include greater communication, coordination, and active contribution by clinicians, manufacturers, end users, policymakers and all other relevant sectors and stakeholders.

JAMES HAMUEL DOUGHARI
Modibbo Adama University

Implications of AMR in the environment include higher medical costs, prolonged hospital stays, and increased mortality, which collectively or individually impact negatively on the economy of individuals and the global community.

1 MONITORING ANTIBIOTIC RESISTANCE IN THE ENVIRONMENT

Antibiotic resistance is increasingly seen as an ecological problem, requiring significant measures to reduce the risks posed by the presence of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) within environmental compartments such as waste water, surface water and soils.[4, 5)] These measures must include the development of reliable surveillance systems, identification of critical control points during processing and solutions to prevent environmental contamination with both ARGs and ARB.[6]

To effectively monitor and mitigate the spread of AMR within the environment, significant research and knowledge gaps must be addressed. These include inconsistencies in ARG reporting units, a lack of standardised protocols, monitoring targets and agreed threshold values.[7]

HAZEL HATTON
Solent University
and
SUZY CLARE MOODY
Kingston University London

Our research concerns the impact of human activity on the presence of AMR genes in the Itchen River in Southampton. The Itchen River is a designated Site of Special Scientific Interest because it passes through both rural and urban areas as well as hosting fish farms,

watercress farms, and water treatment plants. It is also a popular site for water sport activities.

Our small pilot study sampled discarded items and natural solid surfaces along the urban length of the river. We found resistance genes to four commonly used antibiotics. Further studies are warranted to sample the river water for detection of antibiotic compounds, and wider sampling of the full river length and the shoreline to understand the broader context of this problem. Studies have shown that bacterial growth as a biofilm on solid surfaces in aquatic environments can harbour high levels of AMR genes, and our findings support the need to prevent solid waste items such as plastics reaching our river environment. This may reduce the prevalence of AMR genes present in the water, but ultimately the solution needs to be a reduction in entry into the watercourse of both antibiotic compounds and contaminated waste containing resistant bacteria.

SOPHIA BAKON

University College London

Regular monitoring is a key aspect for identifying hotspots and combatting AMR. We suggest not only monitoring faecal indicator pathogens but also ESKAPE* pathogens included in the WHO guidelines for Drinking Water Quality Standards.[8] The guidelines for monitoring major antibiotic residue levels in waterbodies include ß-lactams, tetracyclines, sulphonamides, aminoglycosides, fluoroquinolones, macrolides, trimethoprim and glycopeptides.[9] This extensive monitoring not only identifies the presence of commonly used antibiotics but also indicates where human activities are contributing to AMR in the surrounding environment.

DIANE PURCHASE

Middlesex University London

More than half of the world's population live in high-density urban areas,[10] which results in close contact of most inhabitants and increased risk of AMR transfer. Current AMR research in the urban environment is related mainly to wastewater treatment and hospital settings, other built environments (such as urban green space) are less well investigated. The majority of the epidemiological studies of urban green exposure focus on their availability and their health benefits;[11] there are few studies that address their potential relevance for the emergence and spread of AMR. Urban park soil microbiomes are rich in diversity and also distinct from non-urban samples in their gene composition.[12] They may acquire antibiotic resistomes via faecal shedding by animals (e.g. companion animals,[13] feral animals [14] or wild birds [15] and urban park irrigation with reclaimed water.[16] Horizontal gene transfer, mediated by mobile genetic elements, could facilitate the dissemination of ARGs across different bacterial phyla that become widespread within the microbial communities in non-domesticated and companion animal populations. In the urban environment, these companion animals also share close proximity with humans, resulting in the transference of antibiotic-resistant microorganisms between animals and cohabiting humans.

The UK's 5-year national action plan on tackling AMR highlights the need to 'support research to reduce evidence gaps and improve understanding of the hazards and risks from AMR in the environment.' Therefore, awareness needs to be raised to address the complexity in the urban environment, increase our understanding of the spread of AMR in this multifaceted ecosystem and broaden the reach of the current surveillance programme.

** ESKAPE is an acronym comprising the scientific names of six highly virulent and antibiotic resistant bacterial pathogens including: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*

2 SELECTION FOR ANTIBIOTIC RESISTANCE IN THE ENVIRONMENT

The variables that influence the evolution of bacterial resistance are yet to be fully elucidated.[17] Antibiotic resistance develops through complex interactions, typically through mutations or by the acquisition of resistance genes present within the environment.[18] Traditionally, research on the evolution of resistance focused on the selection of resistant organisms using an antimicrobial concentration range termed the mutant selection window, where resistant bacteria are selected at concentrations exceeding the minimum inhibitory concentration (MIC) of susceptible bacteria and below that of the resistant bacteria.[19] Standardised procedures for quantifying the MIC involve monoculture growth assays to determine the ability of a bacterial strain to survive a given concentration of antibiotic.[20]

Despite this, recent studies have revealed that the selection for antibiotic resistance occurs at sublethal antibiotic concentrations below that of the MIC.[21, 22] Conclusively, to understand the relevance of such findings and to fully understand the evolution of resistance, we must study the evolution of resistance within the context of complex microbial communities as found in the natural environment.[23, 24]

JOY WATTS

University of Portsmouth

Pollution (current and historical) may be discharged into environmental systems, for example in marine sediments, resulting in complex mixtures of human and/or animal waste, mixed with industrial contaminants, including heavy metals. Many of these heavy metals are known co-selective factors for antibiotic resistance in bacteria in soils, water and sediment [25] and also promote horizontal transfer of AMR genes and increased resistance.[26]

AIMEE MURRAY

University of Exeter

Antibiotics administered to humans and animals are not fully metabolised in the body, which can result in up to 90% of an antibiotic being excreted in an active form.[27] Human waste passes through the wastewater treatment system, which is not designed to remove pharmaceuticals. Active antibiotics are therefore released into aquatic environments via wastewater effluents. Antibiotics bound to sewage sludge are spread onto agricultural land as fertiliser, alongside antibiotic-containing animal manure. Runoff from these lands can

result in antibiotics entering rivers and streams. Finally, combined sewer overflows or direct discharge of human, animal or antibiotic manufacturing waste can result in direct contamination of the environment with antibiotics.

It was originally thought that antibiotics in the environment pose no threat in terms of selection for AMR, because concentrations are too low. However, experimental determination of the lowest concentration of an antibiotic that can select for AMR (the minimal selective concentration) confirmed selection can occur at much lower antibiotic concentrations than the MIC.[21] Further research since shows that selection for AMR can occur at very low, environmentally relevant concentrations, in both single bacterial species [21, 28, 29] and bacterial communities.[12, 30, 31, 32] In some cases, the strength of selection has been shown to be the same at low, environmentally relevant concentrations and at high, clinically relevant antibiotic concentrations, suggesting the environment plays an underappreciated role in the emergence of AMR.[23] Despite this evidence base, target 'safe' thresholds or environmental quality standards based on potential to select for AMR are yet to be defined for antibiotics.

Environmental quality standards require data on risks posed by a compound in the environment. Current environmental risk assessment approaches for human medicines still use standard ecotoxicological assays to determine the concentration at which a pharmaceutical poses a risk to model aquatic organisms.[33] Only two of the required tests use microorganisms (the target organisms for antibiotics and antimicrobials). These tests measure standard ecotoxicological effects, such as growth inhibition of algal species or respiration inhibition of an activated sewage sludge bacterial community. As they do not measure increases in AMR directly, this can result in determination of thresholds that do not protect against AMR evolution.[32, 34, 35] Numerous different experimental and modelling approaches exist to determine minimal selective concentrations or lowest observed effect concentrations for AMR selection; these have been recently reviewed.[36] However, it has been recommended that the latter are used, rather than minimal selective concentrations, to streamline their incorporation into current environmental risk assessment guidelines.[36]

Encouragingly, there has been some recent movement towards recognition of AMR in environmental risk assessment. The European Commission's Water Framework Directive regularly reviews and updates a 'Watch List' of contaminants of concern, that could pose an environmental risk. For the first time, the most recent version of the Watch List has explicitly acknowledged that evidence relating to selection for AMR was considered during the compound selection process. As a result, five new antimicrobials (the antibiotics trimethoprim and sulfamethoxazole and the antifungals clotrimazole, fluconazole and miconazole) were recommended for addition to the Watch List.[37] Compounds on the Watch List need to be monitored by EU member states. This is crucial for understanding risk, as environmental monitoring data (particularly for antibiotics) are scarce.

Many aspects of AMR evolution in the aquatic environment remain understudied. Most research thus far has focused on selection for AMR, i.e. AMR that will increase over time. It is also possible to determine the lowest concentration at which AMR will persist, i.e. AMR will still be lost over time, but at a slower rate.[31] This is important for understanding risk, particularly human or animal exposure risk, as even transiently high levels of AMR could increase the risk of AMR colonisation or infection.[36, 31] There is some evidence that some chemical variations of antibiotics, such as antibiotic transformation products [38] and enantiomeric

antibiotics,[39] could also select for AMR. Co-selection for antibiotic resistance by other compounds with antimicrobial effects such as biocides, heavy metals and pesticides can occur; even non-antibiotic pharmaceuticals may select for AMR.[40, 41] Therefore, current environmental surveillance approaches that only quantify parent antibiotic compounds may not fully represent the AMR selection risk. Furthermore, mixtures of compounds may interact, increasing the selective potential of the mixture compared with the individual constituents. Environmental quality standards based on single compounds may therefore not be fully protective against AMR selection (though this issue applies to all environmental risk assessment, not just risk assessment of AMR).[42] How these compounds interact in complex, environmental mixtures and potentially select for AMR is an area requiring significantly more research.

MARIA ROSA DOMINGO SANANES

Nottingham Trent University

In my research group, we aim to investigate if there are significant differences in the capacity for evolving and maintaining AMR in bacteria adapted to the environment when compared with host-associated bacteria.

Environmental conditions and genetic background can influence the potential for evolution of resistance to antibiotics.[24, 43, 44, 45] For example, the same AMR-causing mutation in different genetic backgrounds can result in different levels of resistance,[44] while evolutionary divergent strains from the same species can differ in the levels of resistance that can evolve [46, 47] and in the mutational pathways to resistance.[48] Therefore, adaptation to different niches may lead to genetic differences that impact the evolution of AMR. These genetic differences could be the result of disparities in the levels and evolutionary timescales of exposure to antibiotics. For example, environmental bacteria, particularly soil dwellers, have evolved in the presence of natural antibiotics (likely at relatively low levels). Consistent with this, antibiotic resistance genes are present even in relatively pristine environments such as Arctic permafrost, Antarctic lakes and the deep ocean.[49, 50] In contrast, host-associated bacteria may have evolved for a long time in antibiotic-poor conditions but are now often exposed to high antibiotic concentrations. These differences may have important consequences for how AMR evolves and is maintained in strains from these different backgrounds.

If genetic backgrounds that allow bacteria to survive as free-living organisms have a high probability to evolve AMR, along with low associated fitness costs, there is potential for the emergence and maintenance of AMR in the environment. This could have important implications for environmental bacterial communities and for the transfer potential of AMR genetic determinants to host-associated and pathogenic bacteria.

APRIL HAYES

University of Exeter

When determining AMR via bacteria's ability to survive concentrations of antibiotics, concentrations of those applied antibiotics are typically high, but it has been demonstrated that lower concentrations of antibiotics can also select for AMR. The minimal selective concentration (MSC) is the concentration of antibiotic where the sensitive and resistant strain are of equal fitness, and any increase in concentration would actively facilitate selection for the resistant strain. This selection at lower antibiotic concentrations has been demonstrated in single species,[21] and in complex microbial community experiments.[23, 28]

Additionally, there is a concentration of antibiotics termed the minimal increased persistence concentration (MIPC), which facilitates the slower loss of resistance genes when bacteria are exposed to these concentrations of antibiotics.[31] These MSCs and MIPCs are similar to the concentrations of antibiotics that can be found in the aquatic environment.

We also know that other non-antibiotic compounds can select for antibiotic resistance, including metals and biocides [25, 51] and there is evidence that MSCs may also apply to these compounds.[28] Metals are used in agriculture, and are persistent in the environment for long periods of time,[53] during which they will exert a selection pressure.[41] Biocides are sometimes used at very high concentrations, particularly when used in clinical settings. These may allow for the selection for highly resistant strains, and may be of concern in clinical strains such as MRSA.[54] It is clear that many antimicrobials can select for antibiotic resistance, which can occur in different environments over varying concentrations.

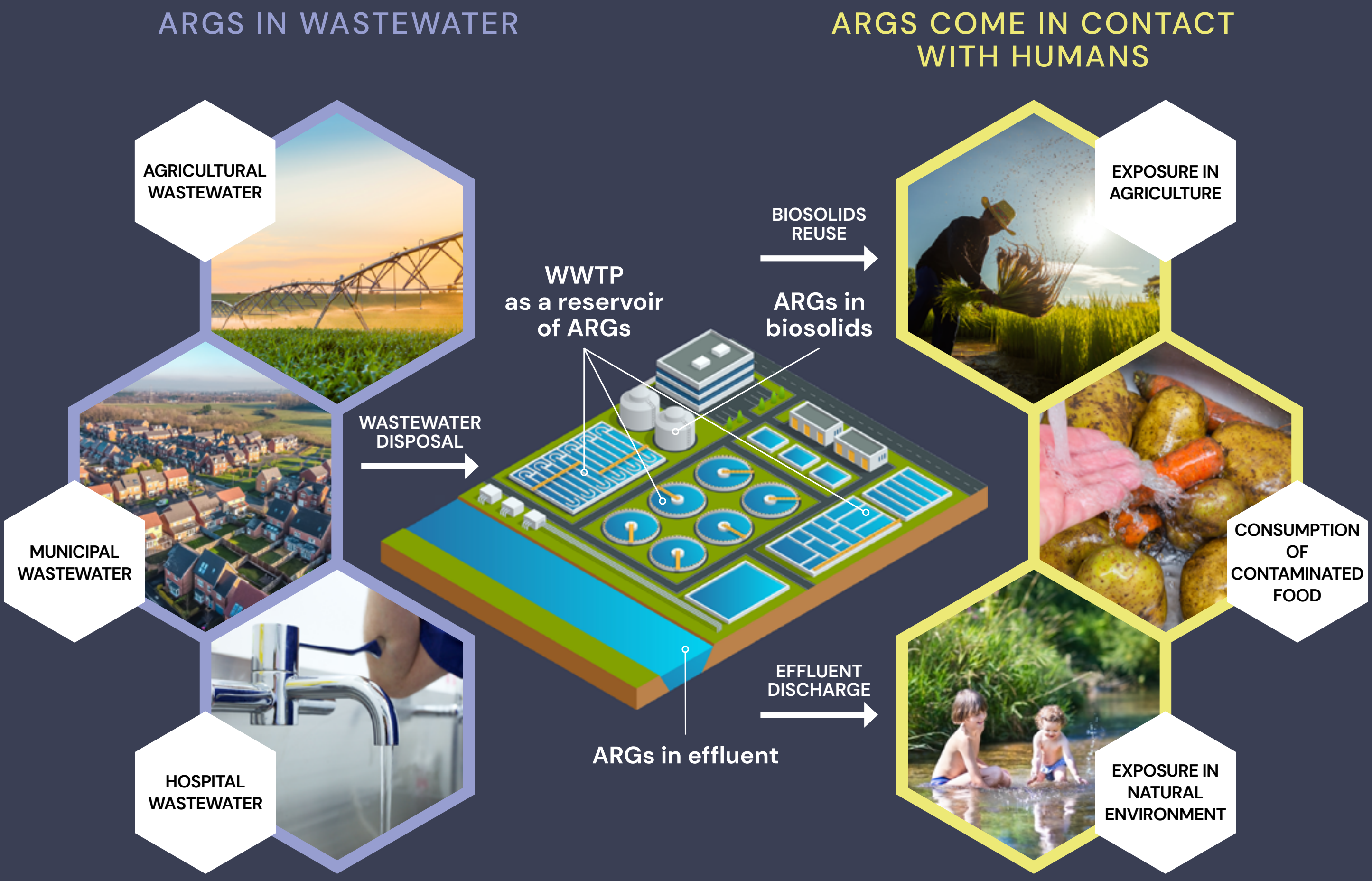
3 WASTEWATER & TREATMENT

DONALD MORRISON
Edinburgh Napier University

Wastewater plays a significant role in the distribution and proliferation of AMR. Wastewater discharges antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and residual concentrations of antibiotics into downstream aquatic and terrestrial environments. Effluent from agricultural, municipal, and healthcare wastewater reaches the human population as a direct result of its use in agriculture, as well as in contaminated food and water (**Figure 1**). Wastewater treatment plants (WWTPs) act as a reservoir for ARGs, which are subsequently released into the environment in the form of biosolids [55] – solid organic matter recovered from the sewage treatment process that are used as fertiliser. To quantify the impact of wastewater treatment on the dissemination of AMR, efficient surveillance programmes and strategies for both treatment and mitigation are critical.

It is already known that resistance to five clinically important antibiotics (cefotaxime, imipenem, ciprofloxacin, vancomycin and methicillin) originated from environmental species of bacteria. In addition, recent studies confirm that the natural environment is a significant contributor to the emergence and transmission of resistant pathogens.[56, 57, 58]

Surveillance is key to both assess the AMR problem and inform public health actions and strategies. It is also one of the five strategic priorities of the WHO Global Action Plan on AMR.[59] However, although a number of surveillance schemes (e.g. EARS-Net and ESAC-Net) provide ongoing data on antibiotic resistance and antibiotic consumption in human and animal settings, no such data are available for the environment. Hence, there is a critical lack of data for supporting mitigation or intervention strategies.



Surveillance of environmental AMR is the focus of the work in my lab. We are presently partnering with two key environmental stakeholders in Scotland – Scottish Water and the Scottish Environment Protection Agency (SEPA). With Scottish Water we are undertaking a point prevalence survey of AMR and antibiotic residues in raw water sources that are used to provide drinking water. In partnership with SEPA we are commencing a 'catchment to coast' investigation of a river catchment that is impacted by multiple pollution sources. We have also compared AMR in wild birds in areas of high and low anthropogenic activity. In our approach we view the environment as divided into three areas – contamination sources (wastewater and manure), reservoir compartments (soil, water, air/dust, and wildlife) and human exposure-relevant sites (beach sand, recreational water, drinking water, ambient air, shellfish, and irrigation water).[60] Using culture-dependent methods [61] and strain typing techniques we focus primarily on three of five surveillance objectives [62] –

- assessment of the risk of transmission of already antibiotic-resistant bacteria to humans via environmental routes,
- assessment of the risk for accelerating the evolution of antibiotic resistance in pathogens through pollution with selective agents and bacteria of human or animal origin and
- identification of the population-level resistance prevalence.[62]

HOLLY TIPPER and ANDREW SINGER

UK Centre for Ecology & Hydrology

Sources of wastewater include: a) sewer systems and wastewater treatment plant (WWTP) effluent, b) industry effluent and c) aquaculture, agricultural and road runoff. Each of these transports antimicrobial-resistant bacteria (ARB) and AMR-driving chemicals (ARDCs; e.g. antibiotics, metals, biocides) to environmental compartments (soil, freshwater, saltwater and the air). Despite its treatment, treated wastewater contains an abundance of ARB, antimicrobial-resistance genes (ARGs) and ARDCs, and is primarily discharged into freshwaters where dilution is often limited – particularly in England. The problem this creates for AMR selection, dissemination and transmission is further exacerbated by the frequent discharge and disproportionate impact of combined sewer overflows (CSOs) on receiving rivers, which represent untreated sewage – containing as much as 100 to 1000 times more ARB, ARGs and ARDCs than treated sewage. The frequency of discharge from CSOs and their abundance (>15,000 compared with >5,000 WWTPs in England) demonstrates their disproportionate impact on environmental AMR.

Longitudinal monitoring of wastewater and rivers is inherently difficult, as: 'No man ever steps in the same river twice, for it's not the same river and he's not the same man', i.e. the river's flow and shape are always changing. This ever-changing nature of a river makes it exceedingly difficult to make valid comparisons of AMR prevalence or abundance over time – always comparing apples with pears. WWTPs are considerably less dynamic; however, they do have dynamics of operation, upgrades and of course the source population can change within the day, week, month, or year.

High-intensity, week-long periods of seasonal sampling can address some of these challenges at WWTPs. However, they are less able to address the dynamic nature of a river. One solution is to focus on monitoring the microbiome within ubiquitously found aquatic organisms with a small host range. The dynamics of a changing microbiome within endemic aquatic organisms offers an opportunity to capture the effects from chronic and intermittent pollution within a stretch of river. By focusing on the organism and not the physical environment (e.g. water, sediment), the longitudinal comparisons remain valid – comparing apples with apples. Our research has focused on this approach, which may offer an innovative method for future AMR surveillance.

4 SOIL

Antimicrobials can contaminate soils through several ways including the application of antibiotics to prevent diseases in crop production, antibiotic residues in livestock manures applied as crop fertilisers, and runoff from ground and surface water.[63] It is estimated that 75% to 90% of antibiotics administered to livestock animals are excreted, largely unmetabolised, in manure, which is then applied to crop land.[64] Absorption of AMR in crops then poses a risk to human and animal health via consumption of contaminated food products.

The diverse ecosystems and properties that comprise soil determine the proliferation and virulence of AMR in the environment. Soil's pH, organic matter content, ionic strength, microbial diversity, microbial alterations, and cation exchange capacity all impact the virulence of AMR as they are conditions that affect concentration and dispersal of resistant genes and bacteria.[65] Due to these numerous factors, scientific interventions for containing AMR in soils, including aerobic composting, anaerobic digesting, and genetic modification of crops, have had varying success without ever completely eradicating AMR from soil.[66]

MARCELA HERNÁNDEZ

University of East Anglia

Soil is one of the largest and most diverse habitats on earth. Soil microbiota represent a potential ancient evolutionary origin of antibiotic resistance. Soil is the most prominent reservoir of antibiotic resistance genes (ARGs) harbouring as much as 30% of all different known ARGs in sequence databases. The rise in use of antibiotics, including of third-generation extended-spectrum beta-lactams and fourth-generation carbapenems, is a global concern, and most importantly because carbapenems are among the last-resort antibiotics.

In our recent studies, we aimed to understand the potential of environmental reservoirs in members of autochthonous soil microbiomes potentially constituting the soil resistome. For this, we identified bacteria able to grow in the presence of several antibiotics in a British agricultural soil. We incubated soil with labelled water

and added high concentrations of several antibiotics including the ultra-broad-spectrum antibiotic meropenem. After incubation, we detected an increase of the 16S rRNA copy numbers in the active microorganisms incubated with labelled water compared with their controls. We found that some native soil bacteria (belonging to the phylum Acidobacteria) were highly abundant after two days of incubation with antibiotics. Our results also indicated the growth of several Proteobacteria including *Stenotrophomonas*, particularly after four days of incubation.

The indication that antibiotic-resistant non-pathogenic soil bacteria as well as potential clinical pathogens are present in British agricultural soil is of concern since AMR has the potential to spread within the environment and into human populations. These organisms and their ARGs might be further enriched by the application of antibiotics through antibiotic-contaminated livestock manure. Farmers could come into direct contact with these bacteria through soil or indirectly from their livestock. The wider community could also be exposed through contaminated water or the food chain. Therefore, it is of crucial importance to tackle AMR in the environment by actively promoting best practices to minimise the spread and development of AMR and by providing evidence to policymakers.

5 AQUACULTURE

Aquaculture has been the fastest-growing food-producing industry over the last few decades,[67] with the amount of global protein production set to rise from 10% to 50% by 2030.[68] A shift to more intensive methods of farming has also led to increased reliance on the prophylactic use of antimicrobials.[69] Such aquaculture systems have previously been referred to as 'genetic reactors' for the recombination of AMR genes,[70] leading to the exposure of environmental bacteria to antimicrobials.[71] AMR genes from aquaculture systems enter the environment in the form of fish products themselves, waste products that are used as fertiliser and direct sediment runoff into the environment.[71]

Despite the reduction in the mg/kg use of antimicrobials in aquaculture in 'high-income countries' (HICs) in recent years, usage in middle- and low-income countries (MICs, LICs) continues to increase.[72] Effective environmental risk assessment (ERA) strategies are required for the monitoring of AMR dissemination as a direct result of aquaculture – each with their own strengths and weaknesses.[73]

KELLY THORNBER
University of Exeter

Aquatic food production (aquaculture) offers the potential for a more sustainable source of dietary protein than that of land animals, requiring less feed per kg of meat and reduced land usage. It is a huge international industry: in 2018 it was estimated to be worth USD 263 billion and to employ over 20 million people, predominantly in Asia where more than 85% of global production takes place.[67] As for land food sectors, aquaculture is rapidly intensifying to meet increasing demand; however, this development is largely unsustainable, with a lack of the required infrastructure needed to maintain good animal health and biosecurity whilst ensuring environmental protection. As a result, there is a substantial risk of driving AMR emergence and transmission, since the major aquaculture-producing countries are based in LICs and MICs, where typically there is a heavy burden of infectious disease, high and widely unregulated antibiotic usage, little or no waste water treatment, regular human exposure to aquaculture environments, and insufficient resources for adequate governance.[73, 74]

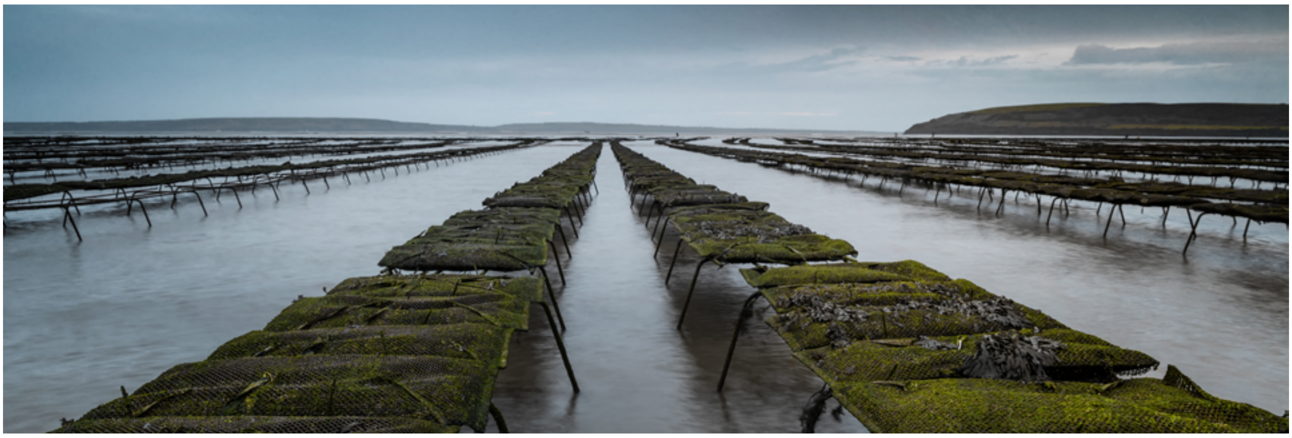
Despite the size and global importance of the industry, efforts to mitigate AMR risk in aquaculture are in their infancy compared with the livestock sector. An expanding body of literature is evidencing the presence of resistance and high levels of antibiotic residues in aquaculture environments and products aimed at human consumption.[73, 74, 75] What is now urgently required is greater understanding of the socioeconomic, cultural and political drivers that together create the conditions favouring AMR in aquaculture contexts. Understanding these drivers is crucial for identifying and developing socioeconomic and culturally appropriate interventions that can reduce AMR risk.[76] Efforts to address AMR in aquaculture also need to examine how AMR could be effectively embedded into sustainable development strategies such as improving sanitation and biosecurity.[77] It has been suggested that these so-called 'AMR-sensitive' interventions will be more cost-effective and potentially have wider efficacy than 'AMR-specific' approaches, such as directly regulating antibiotic usage,[77] and are therefore key areas for future research efforts.

PRABIN DAWADI

Nepal Academy of Science and Technology

Antibiotics are commonly used in aquaculture systems as feed supplementation or immersion baths to inhibit the growth of pathogenic bacteria as well as to promote growth of aquatic livestock.[78] Bacterial infections and subsequent antimicrobial therapy have led to a serious antibiotic resistance dilemma in aquaculture.[79] Drug residues are predominantly reported in aquaculture habitats, and they can also be delivered into open rivers by effluents from manufacturing plants.[80] Research also suggests that higher levels of antibiotic resistance are linked to warmer temperatures.[75]

Various mitigation solutions are crucial for overcoming AMR in aquaculture. Improving re-circulation amongst aquaculture systems can be a potential adaptation strategy against climate change.[81] Food supplements, such as probiotics, can also help aquatic farmed animals develop stronger immune systems to avoid antibiotic use.[82, 83] Proper management of agriculture, livestock production systems, and effluent can prevent cross-contamination between land and aquatic habitats.[75, 84, 85] As these mitigations suggest, limiting AMR requires an Ecological Approach to Aquaculture (EAA) and integration of agriculture and aquaculture.[86]



6 THE FUTURE OF AMR

As this brief exemplifies, reducing AMR in the environment will require a multifaceted approach as its environmental breadth and depth have far-reaching implications. Prevention, mitigation, and rectification measures will need to be developed and implemented at all stages of AMR proliferation in the environment, from identifying exposure sources to removing AMR from water sources and food products.

Microbiologists play a crucial role in combatting AMR in the environment by investigating constant changes in the environment that precipitate and promote the evolution of AMR. They are also at the forefront of developing new techniques for monitoring, diagnosing, and mitigating AMR in all environmental sources.

For example, whole-genome sequencing (WGS) of bacterial pathogens is promising for epidemiological surveillance, outbreak detection, and infection control.[87] Researchers, including microbiologists, are using WGS to predict which strains of *Staphylococcus aureus* have a high likelihood of becoming resistant to antibiotics during treatment.[88] They have also harnessed this revolutionary tool to slow down or prevent resistance evolution by using antibiotic adjuvants.

Likewise, various applications of bacteriophages, which are viruses that infect bacteria, have potential for both animals (land and aquaculture livestock) and crop production.[89] They provide a promising alternative to antibiotics and growth hormones since they can specifically target bacteria without negatively effecting host microbiota.[90]

Over the last few decades, several molecular strategies and methods have been developed to combat AMR in the environment, including the development of sensitive and accurate diagnostics for both viral and bacterial diseases (to help reduce inappropriate antibiotic use), mRNA and DNA vaccines, gene-editing technology, and most recently, CRISPR technology.[89] However, all these developments have required multidisciplinary, multi-stakeholder collaboration and investment to ensure solutions are viable and long term. Due to the ever-evolving nature of bacteria, these solutions will need to keep apace or be replaced. It is therefore vital that public policy adequately recognises the significance of AMR in the environment and supports scientists' efforts to eradicate it.

RICHARD OMOLE

Obafemi Awolowo University

As humans are constantly developing technologies and innovations to ameliorate the impact of AMR, microbes are also constantly evolving strategies to evade these human technologies and innovations. Since these microbes will not stop evolving, researchers are continually developing novel tools that are essential for the rapid detection of these ever-evolving resistant microbes to guide their focused and timely reduction from the environment.

Nanotechnology is an emerging field that has found wide applications in many fields. Since this technology originated from physics and chemistry, nanomaterials used to be synthesised mainly by physical and chemical methods.[91] However, microbiologists have made commendable efforts in developing a more sustainable means (in terms of cost-effectiveness and eco-friendliness) of synthesising these nanomaterials using different microbes.[92, 93] Without sleeping on these efforts, microbiologists have advanced to utilising these

microbe-mediated nanomaterials to detect antimicrobial-resistant microbes in the environment, particularly water and food items,[94] which has further helped in devising means to completely delete these antimicrobial-resistant microbes from the environment.[95] Interestingly, some of these microbes used for the synthesis of nanoparticles were isolated from the environment.[96] Compared with conventional antibiotics, microbe-mediated nanomaterials have a unique modes of action as they target multiple sites in these resistant microbes,[97] which presents them as a promising tool that could lead to the end of AMR in the environment, if properly harnessed.

Therefore, it is recommended that great energy should be channelled towards the scale-up of this promising and sustainable innovation/technology in addressing the global menace of AMR in our environment.

PAVLOS TRUS

Newcastle University

Environmental pollutants such as heavy metals, crude oil, antimicrobials, insecticides and microplastics have been found to be major causative agents in the proliferation of AMR profiles amongst bacterial populations. For example, microplastics can absorb contaminants such as antibiotics and heavy metals used in aquaculture ponds. As bacterial populations are exposed to new environmental pressures (e.g. antibiotics) on the surface of the microplastics, mutations transpire at the chromosomal level leading to an increase in resistance. Furthermore, bacteria can form complex multi-species biofilms on microplastics, which facilitates the acquisition of AMR genes via vertical or horizontal transfer, with the latter implicating mobile genetic elements such as class 1 integrons. This is of major significance as bacteria harbouring extensive AMR profiles can be introduced to new ecosystems with the transport of microplastics by the water flow.[98] One approach that can be implemented to reduce the presence of microplastics in the environment is for synthetic polymers used in industrial and agricultural applications to be replaced with natural filtration systems and biodegradable polymers. It has been demonstrated that the application of tertiary sand filtration can reduce up to 79% of microplastics entering aquatic environments.[99] By reducing microplastics from entering aquatic environments the dissemination of AMR profiles between different niches can be effectively decreased.

Contributors

Applied Microbiology International (AMI) would like to wholeheartedly thank the following experts for their contribution to the development of this briefing:

Sophia Bakon
University College London

Nihal Bandara
University of Bristol

Prabin Dawadi
Nepal Academy of Science and Technology

James Hamuel Doughari
Modibbo Adama University

Hazel Hatton
Solent University

Suzy Clare Moody
Kingston University London

April Hayes
University of Exeter

Marcela Hernández
University of East Anglia

Donald Morrison
Edinburgh Napier University

Aimee Murray
University of Exeter

Richard Omole
Obafemi Awolowo University

Diane Purchase
Middlesex University London

Maria Rosa Domingo Sananes
Nottingham Trent University

Kelly Thornber
University of Exeter

Holly Tipper
UK Centre for Ecology & Hydrology

Andrew Singer
UK Centre for Ecology & Hydrology

Pavlos Trus
Newcastle University

Joy Watts
University of Portsmouth

REFERENCE

1 HM Government. UK One Health Report: Joint report on antibiotic use and antibiotic resistance, 2013–2017. **https://www.gov.uk/government/publications/uk-one-health-report-antibiotic-use-and-antibiotic-resistance-in-animals-and-humans**

2 O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations. **https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf**

3 Lord Trees. Environment Bill Volume 812 debated Monday 7 June 2021. **https://hansard.parliament.uk/Lords/2021-06-07/debates/6E1FE4FF-613D-44D6-8668-C8468E87D916/EnvironmentBill#main-content** (accessed 24 August 2021)

4 Kümmerer K. Resistance in the environment. *Journal of Antimicrobial Chemotherapy* 2011; 54(2), 311–320

5 Yang Y, Ashworth AJ, Willett C, Cook K, Upadhyay A, Owens PR *et al*. Review of antibiotic resistance, ecology, dissemination, and mitigation in U.S. broiler poultry systems. *Frontiers in Microbiology* 2019; 10, 2639

6 Nguyen AQ, Vu HP, Nguyen LN, Wang Q, Djordjevic SP, Donner E *et al*. Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. *Science of the Total Environment* 2021; 783, 146964

7 Berendonk TU, Manaia CM, Merlin C, Fatta-kassinos D, Cytryn E, Walsh F *et al*. Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology* 2015; 13, 310–317

8 Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A Review. *Frontiers in Microbiology* 2019; 10, 539

9 Collignon PJ, Conly JM, Andremont A, McEwen SC, Aidara-kane A, World Health Organisation Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance *et al*. World health organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. *Clinical Infectious Diseases* 2016; 63,1087–1093

10 Ritchie H, Roser M. Urbanization. Our World Data 2018; **https://ourworldindata.org/urbanization**

11 Sandifer PA, Sutton-Grier AE, Ward BP. Exploring connections among nature, biodiversity, ecosystem services, and human health and well-being: Opportunities to enhance health and biodiversity conservation, *Ecosystem Services* 2015; 12, 1–15 **https://doi.org/10.1016/j.ecoser.2014.12.007**

12 Charlop-Powers Z, Pregitzer CC, Lemetre C, Ternei MA, Maniko J, Hover BM *et al*. Urban park soil microbiomes are a rich reservoir of natural product biosynthetic diversity. *Proceedings of the National Academy of Sciences of the United States of America* 2016; 113, 14811–14816

13 Worsley-Tonks KEL, Miller EA, Gehrt SD, McKenzie SC, Travis DA, Johnson TJ *et al*. Characterization of antimicrobial resistance genes in Enterobacteriaceae carried by suburban mesocarnivores and locally owned and stray dogs. *Zoonoses Public Health* 2020; 67, 460–466

14 Mo SS, Urdahl AM, Madslien K, Sunde M, Nesse LL, Slettemeås JS *et al*. What does the fox say? Monitoring antimicrobial resistance in the environment using wild red foxes as an indicator. *PLoS One* 2018; 13, e0198019 **https://doi.org/10.1371/journal.pone.0198019**

15 Zhao H, Sun R, Yu P and Alvarez PJJ. High levels of antibiotic resistance genes and opportunistic pathogenic bacteria indicators in urban wild bird feces. *Environmental Pollution* 2020; 266,115200 **https://doi.org/10.1016/j.envpol.2020.115200**

16 Han XM, Hu HW, Shi XZ, Wang JT, Han LL, Chen D *et al*. Impacts of reclaimed water irrigation on soil antibiotic resistome in urban parks of Victoria, Australia. *Environmental Pollution* 2016; 211, 48–57 **https://doi.org/10.1016/j.envpol.2015.12.033**

17 Zhang Q, Lambert G, Liao D, Kim H, Robin K, Tung C *et al*. Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* 2011; 333(6050), 1764–1767

18 Wellington EMH, Boxall ABA, Cross P, Feil EJ, Gaze WH, Hawkey PM *et al*. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases* 2013; 13(2), 155–165

19 Drlica K. The mutant selection window and antimicrobial resistance. *Journal of Antimicrobial Chemotherapy* 2003; 52(1), 11–17

20 Andrews JM. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 2001; 48, 5–16

21 Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D *et al*. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens*, 2011; 7(7), e1002158

22 Andersson DI, Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resistance Updates* 2012; 15(3), 162–172

23 Murray KM, Zhang L, Yin X, Zhang T, Buckling A, Snape J *et al*. Novel insights into selection for antibiotic resistance in complex microbial communities. *ASM Journals* 2018; 9(4), e00969-18

24 Bottery MJ, Pitchford JW, Friman V. Ecology and evolution of antimicrobial resistance in bacterial communities. *The ISME Journal* 2020; 15, 939–948

25 Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. *Trends in Microbiology* 2006; 14(4), 176–182

26 Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology* 2012; 3, 399 **https://doi.org/10.3389/fmicb.2012.00399**

27 Kummerer K. Antibiotics in the aquatic environment – A review – Part 1. *Chemosphere* 2009; 75(4), 417–434

28 Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson D. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *mBio* 2014; 5(5), e01918-14

29 Kraupner N, Ebmeyer S, Hutinel M, Fick J, Flach C and Joakim Larsson DG. Selective concentrations for trimethoprim resistance in aquatic environments. *Environment International* 2020; 144, 106083

REFERENCES continued

30 Lundstrom SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T *et al*. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. *Science of the Total Environment* 2016; 553, 587–595

31 Stanton IC, Murray AK, Zhang L, Snape J, Gaze WH. Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. *Communications Biology* 2020; 3, 467

32 Murray AK, Stanton IC, Wright J, Zhang L, Snape J, Gaze WH. 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. *Environmental Health Perspectives* 2020; 128(10), 107007

33 EMA. Draft guideline on the environmental risk assessment of medicinal products for human use. 2018 **https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf**

34 Le Page G, Gunnarsson L, Snape J, Tyler CR. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environment International* 2017; 109,155–169

35 Tell J, Caldwell DJ, Häner A, Hellstern J, Hoeger B, Journel R *et al*. Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. *Integrated Environmental Assessment and Management* 2019; 15, 312–319

36 Murray AK, Stanton I, Gaze WH, Snape J. Dawning of a new ERA: Environmental Risk Assessment of antibiotics and their potential to select for antimicrobial resistance. *Water Research* 2021; 200, 117233

37 Gomez Cortes L, Marinov D, Sanseverino I, Cuenca AN, Niegowska M, Porcel E *et al*. Selection of substances for the 3rd Watch List under the Water Framework Directive. 2020: Luxembourg: Publications Office of the European Union 2020 **https://publications.jrc.ec.europa.eu/repository/handle/JRC121346**

38 Fatta-Kassinos D, Vasquez MI, Kuemmerer K. Transformation products of pharmaceuticals in surface waters and wastewater formed during photolysis and advanced oxidation processes – Degradation, elucidation of byproducts and assessment of their biological potency. *Chemosphere* 2011; 85(5), 693–709

39 Elder FCT, Feil EJ, Snape J, Gaze WH, Kasprzyk-Hordern B. The role of stereochemistry of antibiotic agents in the development of antibiotic resistance in the environment. *Environment International* 2020; 139, 105681

40 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 glyphosphate cause changes in antibiotic susceptibility in *Escherichia coli* and *Salmonella enterica* sewrovar Typhimurium. *mBio* 2015; 6(2), e0009-15

41 Dickinson AW, Power A, Hansen MG, Brandt KK, Piliiposian G, Appleby P et al. Heavy metal pollution and co-selection for antibiotic resistance: A microbial palaeontology approach. *Environment International* 2019; 132,105117

42 Backhaus T. Environmental risk assessment of pharmaceutical mixtures: demands, gaps, and possible bridges. *The AAPS Journal* 2016; 18(4), 804–813

43 Santos-Lopez A, Marshall CW, Scribner MR, Snyder DJ, Cooper VS. Evolutionary pathways to antibiotic resistance are dependent upon environmental structure and bacterial lifestyle. eLife 2019; 8, e47612

44 Hughes D, Andersson DI. Evolutionary trajectories to antibiotic resistance. *Annual Review of Microbiology* 2017; 71, 579–596

45 Durão P, Balbontín R, Gordo I. Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends in Microbiology* 2018; 26, 677–691

46 Card KJ, LaBar T, Gomez JB, Lenski RE. Historical contingency in the evolution of antibiotic resistance after decades of relaxed selection. *PLoS Biology* 2019; 17, e3000397

47 Lamrabet O, Martin M, Lenski RE, Schneider D. Changes in intrinsic antibiotic susceptibility during a long-term evolution experiment with *Escherichia coli*. *mBio* 2019; 10(2), e00189–19

48 Card KJ, Jordan JA, Lenski RE. Idiosyncratic variation in the fitness costs of tetracycline-resistance mutations in *Escherichia coli*. *Evolution* 2021; 75, 1230–1238

49 D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, *et al*. Antibiotic resistance is ancient. *Nature* 2011; 477(7365), 457–61

50 Nesme J, Cécillon S, O.Delmont T, Monier J, Vogel TM, Simonet P *et al*. Large-scale metagenomic-based study of antibiotic resistance in the environment. *Current Biology* 2014; 24, 1096–1100

51 Buffet-Bataillon S, Tattevin P, Bonnaure-Mallet M, Jolivet-Gougeon A. Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review. *International Journal of Antimicrobial Agents* 2012; 39(5), 381–389

52 Lu J, Jin M, Nguyen H, Mao L, Li J, Coin LJM *et al*. Non-antibiotic antimicrobial triclosan induces multiple antibiotic resistance through genetic mutation. *Environment International* 2018; 118, 257–265

53 Ali H, Khan E, Ilahi I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry* 2019; 6730305

54 Kampf G. Acquired resistance to chlorhexidine – is it time to establish an 'antiseptic stewardship' initiative? *Journal of Hospital Infection* 2016; 94(3), 213–227

55 Pallares-Vega R, Hernandez LL, Fletcher B, Vias-Torres E, van Loosdrecht MCM, Weissbrodt DG *et al*. Annual dynamics of antimicrobials and resistance determinants in flocculent and aerobic granular sludge treatment systems. *Water Research* 2021; 190, 116752

56 Bengtsson-Palme J, Kristiansson E, Joakim Larsson DG. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews* 2018; 42(1), fux053

57 Singer AC, Shaw H, Rhodes V, Hart A. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology* 2016; 7, 1728

58 Proia L, Anzil A, Subirats J, Borrego C, Farrè M, Llorca M *et al.* Antibiotic resistance along an urban river impacted by treated wastewaters. *Science of the Total Environment* 2018; 628–629, 453–466

REFERENCES continued

59 Global action plan on antimicrobial resistance. Geneva: World Health Organization; 2015 **http://www.who.int/antimicrobial-resistance/global-action-plan/en/**

60 Huijbers PM, Blaak H, de Jong MC, Graat EA, Vandenbroucke-Grauls CM, de Roda Husman AM. Role of the environment in the transmission of antimicrobial resistance to humans: a review. *Environmental Science & Technology* 2015; 49(20), 11993–12004

61 Marano RBM, Fernandes T, Manaia CM, Nunes O, Morrison D, Berendonk TU *et al*. A global multinational survey of cefotaxime-resistant coliforms in urban wastewater treatment plants. *Environment International* 2020; 144, 106035

62 Huijbers PMC, Flach CF, Larsson DGJ. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. *Environment International* 2019; 130, 104880

63 FAO. Antimicrobial Resistance in the Environment. **http://www.fao.org/antimicrobial-resistance/key-sectors/environment/en/** (accessed 13 September 2021)

64 Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews* 2011; 24(4), 718–733

65 Iwu CD, Korsten L, Okoh AI. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health. *MicrobiologyOpen* 2020; 9(9), e1035

66 Ferraresso J, Lawton B, Bayliss S, Sheppard S, Cardazzo B, Gaze W *et al*. Determining the prevalence, identity and possible origin of bacterial pathogens in soil. *Environmental Microbiology* 2020; 22(12), 5327–5340

67 The Food and Agriculture Organization of the United Nations. The state of world fisheries and aquaculture 2014; doi: 92-5-105177-1 **https://www.fao.org/3/5f6gge/5f6gge.pdf**

68 The World Bank. Fish to 2030: Prospects for fisheries and aquaculture. **https://www.fao.org/3/i3640e/i3640e.pdf**

69 Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology* 2006; 8, 1137–1144 **https://doi.org/10.1111/j.1462-2920.2006.01054.x**

70 Banquero F, Martínez JL, Canton R. Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* 2008; 19: 260–265 **https://doi.org/10.1016/j.copbio.2008.05.006**

71 Watts JEM, Schreier HJ, Lanska L, Hale MS. The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs* 2017; 15: 158. **https://doi.org/10.3390/md15060158**

72 Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA *et al*. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences of the United States of America* 2018; 115(15), E3463–3470

73 Thornber K, Verner-Jeffreys DW, Hinchliffe S, Rahman MM, Bass D, Tyler CR. Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture* 2020; 12(2), 966–986

74 Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in aquaculture. *Scientific Reports* 2020; 10, 21878 **https://doi.org/10.1038/s41598-020-78849-3**

75 Reverter M, Sarter S, Caruso D, Avarre JC, Combe M, Pepey E *et al*. Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature Communications* 2020; 11, 1870 **https://doi.org/10.1038/s41467-020-15735-6**

76 Hinchliffe S, Butcher A, Rahman MM. The AMR problem: demanding economies, biological margins, and co-producing alternative strategies. *Palgrave Communications* 2018; 4, 142 **https://doi.org/10.1057/s41599-018-0195-4**

77 World Bank. Pulling Together to Beat Superbugs 2019; **https://www.worldbank.org/en/topic/agriculture/publication/pulling-together-to-beat-superbugs-knowledge-and-implementation-gaps-in-addressing-antimicrobial-resistance**

78 Abu Bakar I, Khan Ayub M, Muhd Yatim A, Abdullah Sani N. Pesticide and antibiotic residues in freshwater aquaculture fish: chemical risk assessment from farm to table. *Asian Journal of Food and Agro-Industry* 2010; 3, 328–334

79 Preena PG, Arathi D, Raj NS, Arun Kumar TV, Arun Raja S, Reshma RN *et al*. Diversity of antimicrobial-resistant pathogens from a freshwater ornamental fish farm. *Letters in Applied Microbiology* 2020; 71, 108–116

80 Borella L, Salogni C, Vitale N, Scali F, Moretti VM, Pasquali P *et al*. Motile aeromonads from farmed and wild freshwater fish in northern Italy: An evaluation of antimicrobial activity and multidrug resistance during 2013 and 2016. *Acta Veterinaria Scandinavica* 2020; 62, 6

81 Ahmed N, Turchini GM. Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production* 2021; 297, 126604

82 Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC. Use of probiotics in aquaculture. *International Scholarly Research Notices* 2012; 916845

83 Hai NV. The use of probiotics in aquaculture. *Journal of Applied Microbiology* 2015; 119, 917–935

84 Bentzon-Tilia M, Sonnenschein EC, Gram L. Monitoring and managing microbes in aquaculture – Towards a sustainable industry. *Microbial Biotechnology* 2016; 9, 576–584

85 Aubin J, Callier M, Rey-Valette H, Mathé S, Wilfart A, Legendre M *et al*. Implementing ecological intensification in fish farming: definition and principles from contrasting experiences. *Reviews in Aquaculture* 2019; 11, 149–167

86 Willot PA, Aubin J, Salles JM, Wilfart A. Ecosystem service framework and typology for an ecosystem approach to aquaculture. *Aquaculture* 2019; 512, 734260

87 Oniciuc EA, Likotrafiti E, Alvarez-Molina A, Prieto M, Santos JA, Alvarez-Ordóñez. The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. *Genes* 2018, 9(5), 268

88 Papkou A, Hedge J, Kapel N, Young B, MacLean CR. Efflux pump activity potentiates the evolution of antibiotic resistance across S. aureus isolates. *Nature Communications* 2020; 11, 3970

REFERENCES continued

89 Mann A, Nehra K, Rana JS, Dahiya T. Antibiotic resistance in agriculture: Perspectives on upcoming strategies to overcome upsurge in resistance. *Current Research in Microbial Sciences* 2021; 2, 100030

90 Svircev A, Roach D and Castle A. Framing the future with bacteriophages in agriculture. *Viruses* 2018;10(5), 218

91 Sastry M, Patil V, Sainkar SR. Electrostatically controlled diffusion of carboxylic acid derivatized silver colloidal particles in thermally evaporated fatty amine films. *Journal of Physical Chemistry B* 1998; 102, 1404–1410

92 Silambarasan S, Abraham J. Biosynthesis of silver nanoparticles. *African Journal of Biotechnology* 2013; 12(21), 3088–3098

93 Omole RK, Torimiro N, Alayande SO, Ajenifuja E. Silver nanoparticles synthesized from *Bacillus subtilis* for detection of deterioration in the post-harvest spoilage of fruit. *Sustainable Chemistry and Pharmacy* 2018, 10: 33–40

94 Daramola OB, Torimiro N, Fadare TO, Omole RK. Functionalized inorganic nanoparticles for the detection of food and waterborne bacterial pathogens. *Nanoscience and Nanotechnology Research* 2020; 6(1), 1–14

95 Torimiro N, Daramola OB, Oshibanjo OD, Otuyelu FO, Akinsanola BA, Yusuf OO *et al*. Ecorestoration of heavy metals and toxic chemicals in polluted environment using microbe-mediated nanomaterials. *International Journal of Environmental Bioremediation and Biodegradation* 2021; 9(1), 8–21

96 Pourali P, Zadeh NR, Yahyaei B. Silver nanoparticles production by two soil isolated bacteria, *Bacillus thuringiensis* and *Enterobacter cloacae*, and assessment of their cytotoxicity and wound healing effect in rats. *Original Research-Basic Science* 2016; 24(5), 860–869

97 Lee NY, Ko WC, Hsueh PR. Nanoparticles in the treatment of infections caused by multidrug resistant organisms. *Frontiers in Pharmacology* 2019; 10, 1153

98 Zhang Y, Lu J, Wu J, Wang J, Luo, Y. Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. *Ecotoxicology and Environmental Safety* 2020; 187, 109852

99 Funck M, Al-Azzawi M, Yildirim A, Knoop O, Schmidt T, Drewes J *et al*. Release of microplastic particles to the aquatic environment via wastewater treatment plants: The impact of sand filters as tertiary treatment. *Chemical Engineering Journal* 2021; 426, 130933

Applied
Microbiology
International

Phone: +44 (0)20 3880 2881
Email: info@appliedmicrobiology.org
Website: www.appliedmicrobiology.org