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A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance

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ABSTRACT

Environmental surveillance of antibiotics and antibiotic resistance could contribute toward the protection of human, animal and ecosystem health. However, justification for the choice of markers and sampling sites that informs about different risk scenarios is often lacking. Here, we define five fundamentally different objectives for surveillance of antibiotics and antibiotic resistance in the environment. The first objective is (1) to address the risk of transmission of already antibiotic-resistant bacteria to humans via environmental routes. The second is (2) to address the risk for accelerating the evolution of antibiotic resistance in pathogens through pollution with selective agents and bacteria of human or animal origin. The third objective is (3) to address the risks antibiotics pose for aquatic and terrestrial ecosystem health, including the effects on ecosystem functions and services. The two final objectives overlap with those of traditional clinical surveillance, namely, to identify (4) the population-level resistance prevalence and (5) population-level antibiotic use. The latter two environmental surveillance objectives have particular potential in countries where traditional clinical surveillance data and antibiotic consumption data are scarce or absent. For each objective, the levels of evidence provided by different phenotypic and genotypic microbial surveillance markers, as well as antibiotic residues, are discussed and evaluated on a conceptual level. Furthermore, sites where monitoring would be particularly informative are identified. The proposed framework could be one of the starting points for guiding environmental monitoring and surveillance of antibiotics and antibiotic resistance on various spatiotemporal scales, as well as for harmonizing such activities with existing human and animal surveillance systems.

1. Introduction

The systematic surveillance of antibiotic use and antibiotic resistance prevalence in humans and animals is imperative for managing bacterial infectious disease (JPIAMR, 2019; WHO, 2015). Many low-income countries currently face substantial challenges in building national surveillance systems due to a lack of infrastructure and resources, resulting in a shortage of systematic data (FAO/OIE/WHO, 2018). In addition to the need for expanding systematic surveillance to all parts of the world, it has been increasingly recognized that surveillance requires an integrated approach, a so-called “One Health Perspective” (Berendonk et al., 2015; JPIAMR, 2019; Larsson et al., 2018; Matheu et al., 2017; O'Neill and The Review on Antimicrobial Resistance, 2015; Robinson et al., 2016; WHO, 2015). This approach refers to the complex interconnections between the health and well-being of animals, people, plants and their shared environment (OHC (One Health Commission), 2019). In the context of antibiotic resistance, One Health refers particularly to the flow of bacteria and genes between these compartments (Larsson et al., 2018). While surveillance in humans and animals has been developed for over two decades in some countries, inclusion of the environment in such efforts is currently in its infancy. ‘Environment’ is defined here as any location that is not within or on the human or domestic animal body and is thus covered by human and animal surveillance efforts. Accordingly, the environment includes but is not limited to water, soil and ambient air. While the need to include the environment in surveillance efforts is acknowledged, a conceptual framework that systematically clarifies the why, what and where questions is currently lacking.

Defining surveillance objectives is critical, as the different ways in which the environment contributes to human, animal and environmental health risks would likely be best informed by different types of surveillance data. Here, we define five fundamentally different objectives for the surveillance of antibiotics and antibiotic resistance in the environment. We outline the characteristics of these objectives and

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discuss the differential value of selected surveillance markers and sites. Our intention is not to provide a detailed blueprint but rather to give an indication of the range of surveillance objectives and their associated markers and sites to be considered during the design of environmental surveillance systems. Although each objective is considered separately, it should be noted that a surveillance effort would ideally meet several of these in parallel. In most cases, where a reference is made to the human situation, domestic animals are also implied.

2. Environmental surveillance objectives

2.1. Transmission of already antibiotic-resistant pathogens between humans, animals and the environment

Human and animal fecal waste are the main sources of antibiotic-resistant bacteria (ARB), both pathogenic and nonpathogenic, in the environment (Ahmed et al., 2018; Heuer et al., 2011; Karkman et al., 2019; Pruden et al., 2013; Stachler et al., 2019). Although subject to various biotic and abiotic processes that affect their fate after discharge, ARB of enteric origin can eventually reach sites where human exposure may occur and subsequent uptake might lead to carriage, colonization or even disease (Ashbolt et al., 2013; Huijbers et al., 2015; Larsson et al., 2018). Environmental exposure routes include but are not limited to drinking water (e.g. Talukdar et al., 2013), beach sand (e.g. de Oliveira and Pinhata, 2008), ambient air (e.g. Friese et al., 2013; de Rooij et al., 2019), fresh produce meant for raw consumption (e.g. Fogler et al., 2019) and recreational water (e.g. Leonard et al., 2018; O'Flaherty et al., 2019; Schijven et al., 2015; Soraas et al., 2013). The presence of already resistant pathogens of enteric origin in such matrixes indicates that the environment plays a role in human exposure, although its relative contribution remains unclear (Huijbers et al., 2015). Similar to bacteria of enteric origin, humans might also be exposed to opportunistic pathogens that can survive and multiply in the environment, such as *Pseudomonas aeruginosa* (Falkinham et al., 2015), and their uptake could lead to carriage, colonization or disease. The first environmental surveillance objective therefore addresses the risk of transmission of already resistant pathogens to humans via environmental routes.

2.2. Accelerating the evolution of antibiotic resistance in pathogens through pollution with selective agents and bacteria of human or animal origin

In addition to the risk of transmission of already resistant pathogens, there is also the risk for mobilization, enrichment and transfer of novel emerging resistance factors to human pathogens. This may collectively be referred to as “the evolution of resistance” in the environment. Antibiotic resistance is an ancient, naturally occurring phenomenon that is widespread in the environment (D'Costa et al., 2011). There is a growing body of evidence that several clinically relevant antibiotic resistance genes (ARGs) originated in bacterial species thriving in the environment. For example, CTX-M extended-spectrum beta-lactamases (ESBLs) appear to have originated in the genus *Kluyvera* (Humeniuk et al., 2002; Poirel et al., 2002), while PER ESBLs were mobilized from the genus *Pararheinheimera* (Ebmeyer et al., 2019), both of which are primarily associated with water and soil. Furthermore, the diversity of the environmental bacterial gene pool is much larger than that normally associated with the human/domestic animal body, providing a rich ‘reservoir’ of different genetic traits, including resistance genes.

The use of antibiotics in humans and domestic animals plays a critical role in the evolution of resistance (ECDC/EFSA/EMA, 2017), but there is an increasing concern that antibiotics in the environment may also contribute to this process. Antibiotics in the environment may promote the evolution of resistance through different processes, such as increasing mutation frequencies, increasing recombination of DNA, favoring the mobilization of chromosomal resistance genes, increasing the likelihood for transfer by selecting for resistant donors, increasing the transfer frequency of mobile resistance factors between bacteria and, most importantly, selecting strains with (horizontally) acquired resistance (Bengtsson-Palme et al., 2018; Blazquez et al., 2018; Gillings and Stokes, 2012). In the environment, the primary focus is on horizontally acquired resistance, as mutation-based resistance in pathogens evolves independently of the diverse environmental bacterial gene pool. Additionally, mutation-based resistance likely has more opportunities to evolve in the human or domestic animal body during antibiotic treatment, where the selection pressure is high. The relative role of different mechanisms for horizontal gene transfer in antibiotic resistance evolution is still unknown (Volkova et al., 2014), but a selection pressure able to stably maintain emerging forms of resistance is a prerequisite for all.

Antibiotics and their metabolites can enter the environment through, for example, human and animal waste (e.g. Bengtsson-Palme et al., 2016; Karkman et al., 2016; Heuer et al., 2011), discharge from pharmaceutical manufacturing facilities (e.g. Kristiansson et al., 2011) and intensive aquaculture (e.g. Tamminen et al., 2011) where antibiotics may promote resistance if present at sufficiently high concentrations (Bengtsson-Palme and Larsson, 2016). In addition to evolution driven by the release of selective agents, it is plausible that the discharge of bacteria of human or domestic animal origin contributes to this process (Martínez, 2009). Interactions between these bacteria and the extensive environmental bacterial gene pool could increase the opportunities for gene exchange with pathogens, thus contributing to the evolution of resistance in clinically important bacteria (Forsberg et al., 2012; Klümper et al., 2015; Musovic et al., 2014). The transfer of resistance factors to pathogens may occur directly or indirectly via environmental bacteria or commensals (Ashbolt et al., 2013). The second objective is to address the risk for accelerating the evolution of antibiotic resistance in pathogens through pollution with selective agents and bacteria of human or animal origin.

2.3. Surveillance of the impact of antibiotics on ecosystem health

Antibiotics in the environment may also pose a risk for terrestrial and aquatic ecosystem health, including having effects on ecosystem functions and services (Brandt et al., 2015; Le Page et al., 2017; Le Page et al., 2018). Microbial communities play a key role in fundamental ecological processes, such as climate regulation (e.g., biological transformation of greenhouse gasses) and environmental quality regulation (e.g., sewage and waste treatment). Antibiotics and other selective agents released into the environment may result in changes in the function, tolerance and structure of microbial communities, thus impacting ecosystem health (Griffiths and Philippot, 2013; Roose-Amsaleg and Laverman, 2016). It has been shown, for example, that nitrification, denitrification and anaerobic ammonium oxidation, which are crucial for environmental quality regulation, could be partially or entirely inhibited by antibiotics at both therapeutic and subtherapeutic concentrations (Laverman et al., 2015; Roose-Amsaleg and Laverman, 2016). A site of particular concern is within wastewater treatment plants, where these processes are critical for the functionality of the treatment plants and where concentrations of antibiotics can be elevated. Under these and other field conditions, however, the role of antibiotics in impacting ecosystem health is elusive (Roose-Amsaleg and Laverman, 2016). The third objective addresses the effect of antibiotics on aquatic and terrestrial ecosystem health, including their effects on ecosystem functions and services.

2.4. Surveillance of antibiotic resistance prevalence in human or animal populations through environmental samples

In contrast to the objectives discussed thus far, which relate to the active contribution of the environment to the risk for human, animal and environmental health, it is also plausible that environmental surveillance could be used to generate health-related data at the

population level in humans and animals. A single environmental sample taken from sites impacted by feces contains information about the microbiome of many individuals. Several recent studies have shown that analyses of enteric bacteria from environmental samples, such as sewage, manure and surface water, can resolve geographical and temporal antibiotic resistance trends in human and animal populations at local, regional and global scales (Ahammad et al., 2014; Hendriksen et al., 2019; Huijbers et al., In Review; Hutinel et al., 2019; Kwak et al., 2015; Munk et al., 2017; Reinthaler et al., 2013; Su et al., 2017; Walsh et al., 2011). After appropriate validation, such environmental surveillance data could potentially inform about the prevalence of intestinal carriage or perhaps more importantly, infections with resistant bacteria. In the latter case, the purpose of environmental surveillance would be similar to that of traditional clinical surveillance, i.e., identification of clinical resistance prevalence, serving as an early warning of emerging forms of resistance, evaluation of the effect of interventions, and in some cases where clinical surveillance is lacking or insufficient, potentially guiding empirical therapy (WHO, 2015). The fourth objective is therefore to analyze the current local resistance prevalence in human (or animal) populations by determining the resistance patterns of bacteria from environmental samples receiving (fecal) bacteria from human (or animal) sources.

2.5. Surveillance of antibiotic use in human and animal populations through environmental samples

Reducing the unnecessary or inappropriate use of antibiotics is an important step in the control of antibiotic resistance, both in the hospital, general population and agricultural settings (O'Neill and The Review on Antimicrobial Resistance, 2015). The analysis of spatio-temporal trends in antibiotic use can inform prescribing policy and trigger interventions for resistance containment at national and international levels. Antibiotic use data are lacking in many parts of the world, and the collection of these data is hampered by unregistered use (e.g., over-the-counter and internet sales). Furthermore, where antibiotic use data are available, the methods for antibiotic classification and measurement of use (e.g., prescription, sales and reimbursement) often differ, rendering data difficult to compare (WHO, 2018). Environmental matrixes impacted by human and animal excretions could provide information about antibiotic use in contributing populations. This possibility is supported by studies in which both legal and illicit drugs were monitored in wastewater, and the estimated mass flows were compared to independent sources of data estimating their usage in humans (Lindberg et al., 2005; ter Laak et al., 2010; Zuccato et al., 2008). Although less extensively studied, there is also evidence that antibiotic use in domestic animals can be monitored through feces (Berendsen et al., 2015). The fifth objective is therefore to analyze local antibiotic use in human (or domestic animal) populations by determining antibiotic concentrations in environmental samples.

3. Markers for environmental surveillance

Depending on the objective to be addressed, different types of surveillance markers will be most relevant. We therefore discuss the differential value of selected markers for addressing the five identified objectives and propose qualitative indexes of evidence of risk for each to facilitate an overview (Table 1). This qualitative index is divided into three categories: 'marker provides no or little evidence for risk (-), 'marker provides some evidence for risk' (+) and 'marker provides stronger evidence for risk' (++). As there are no strictly defined divisions between these, the indexes should be interpreted together with the accompanying text.

3.1. Selective agents

Selection pressure is, at least initially, key for newly acquired resistance traits to be maintained and spread in a microbial community, while over time, compensatory mechanisms can lead to maintenance also in the absence of selection pressure (Andersson and Hughes, 2010). Data on exposure levels to antibiotics in the environment can therefore inform about the risks of evolution of resistance (++). Environmental concentrations of antibiotics can be compared to concentrations at which different evolutionary processes, primarily selection, are expected to be promoted. Such effect (or no effect) levels are mainly generated from controlled experiments. For example, laboratory-based competition experiments between two closely related strains (Gullberg et al., 2011) or studies of complex microbial communities (Klümper et al., 2019; Kraupner et al., 2018; Lundström et al., 2016; Murray et al., 2018) have been used to estimate minimal selective concentrations or lowest and no observed effect concentrations for resistance selection. Bengtsson-Palme and Larsson (2016) also predicted no effect concentrations for resistance selection for 111 antibiotics. They based their prediction on the lowest minimum inhibitory concentration (MIC) values for each antibiotic found in the public database of the European Committee on Antimicrobial Susceptibility Testing. As a concentration that completely inhibits growth must also select for resistance, at least in some bacterial communities, MIC values were used to derive the upper boundaries for the concentrations selecting for resistance. It is challenging to take into account the combined effect of many selective agents, as antibiotics acting via the same mechanism can have an additive effect and one class of antibiotics or antibacterial agents may co-select for another (Gullberg et al., 2014; Pal et al., 2015; Ye et al., 2017). Antibacterial biocides and several metals are co-selective agents of particular concern (Brandt et al., 2015; Pal et al., 2017; SCENIHR, 2009; Song et al., 2017). Another challenge is that bioavailability may differ radically depending on the matrix as well as speciation of the selective agents (Song et al., 2017; Zhang et al., 2014).

Antibiotic use enhances the transmission of ARB between people, e.g., at hospitals, as the highly selective environment in patients taking antibiotics provides opportunities for the proliferation of these bacteria (Almagor et al., 2018). Whether the much lower levels of antibiotics typically encountered in the external environment would enhance transmission is questionable. A subtle growth advantage for a resistant strain at concentrations well below the minimum inhibitory concentration could favor resistance evolution over time. However, a marginal selection pressure is highly unlikely to manifest in the rapid growth of resistant strains, partly because the external environment is hostile compared to the intestines. Accordingly, in the environment, the limiting factor for most resistant enteric pathogens to survive and subsequently colonize or infect a new host is likely not competition with their sensitive counterparts in water or soil, for example, but rather other biotic and abiotic factors (Bengtsson-Palme et al., 2018). Analyses of selective agents in the environment therefore provide very limited information on the risk of transmission of enteric pathogens (−). This might not be valid for pathogens that are able to survive and multiply well in the environment, such as *Aeromonas spp.,* where long-term, low selection pressures even slightly above the minimal selective concentration could lead to increased prevalence of resistant strains in the environment and hence an increased risk of transmission (Bengtsson-Palme et al., 2018).

For an assessment of the risk for ecological effects, antibiotic concentrations would provide the exposure estimate. With the hazard or effect level, which is usually derived from controlled experiments, antibiotic concentrations thus provide the foundation for an ecological risk assessment (++). Consistent with this observation, it has been proposed that no effect levels for resistance selection should be amended with other effect data to also be protective of ecosystem health (Bengtsson-Palme and Larsson, 2018; Le Page et al., 2017). Protection of both ecosystem health and a reduction in the risk for resistance selection comprised the foundation for the voluntary emission targets for antibiotics set by leading pharma industries in 2018 (AMR Industry Alliance, 2018).

Table 1
Level of evidence provided by individual markers according to different environmental surveillance objectives.[a]

	Risk of transmission	Risk for evolution	Risk for ecological effects	Population-level resistance prevalence	Population-level antibiotic use
Selective agents (antibiotics, metals, biocides)	−	++	++	−	++
Known ARGs and MGEs	+	+	−	+	−
Emerging ARGs	−	+	−	+	−
Fecal contamination	++	+	−	−	−
ARG-to-fecal contamination ratio	−	++	+	+	−
ARB Proportion (within species)	−	++	+	++	+
Absolute number	++	+	−	−	−
Functional community responses	−	+	++	−	−
Microbial diversity	−	−	+	−	−

Note: ARGs, antibiotic resistance genes; MGEs, mobile genetic elements; ARB, antibiotic-resistant bacteria; −, marker provides no or little evidence for risk; +, marker provides some evidence for risk; ++, marker provides stronger evidence for risk.

[a] The annotation of the level of evidence (−, + and ++) is simplified and should be interpreted with the accompanying main text.

For the estimation of antibiotic use in human or animal populations, it is apparent that concentrations of antibiotics in the environment could be an informative marker (++). Potentially valuable markers include the active substances themselves and, in contrast to estimating the selection pressure, inactive metabolites (Zuccato et al., 2008). It is important to note that estimates of use are likely to be more imprecise for those antibiotics that are rapidly degraded, such as many beta-lactam antibiotics, or where partitioning between particulate matter and the aqueous phase is difficult to predict.

3.2. Known antibiotic resistance genes and mobile genetic elements

While the phenotypic resistance of isolates is the most common endpoint studied in clinical settings, environmental microbial communities harbor complex mixtures of bacteria that often cannot be cultivated. Hence, culture-independent approaches that involve studying the genetic background of resistance in microbial communities are common in environmental research. A selection pressure that drives evolution would be expected to increase the relative number of ARGs corresponding to that selective agent and associated mobile genetic elements (MGEs) in exposed microbial communities. It is important to note that taxonomic shifts, independent of any antibiotic selection pressure, are also expected to result in changes in the abundance of individual ARGs (Bengtsson-Palme et al., 2016). A more consistent change of many ARGs conferring resistance to the same class of antibiotics therefore provides stronger evidence for a relevant on-site selection pressure (Bengtsson-Palme et al., 2016; Lundström et al., 2016). While the sum of all the analyzed ARGs can inform about selection pressure, this measure can be heavily influenced by a few inherently common ARGs. The dominance of a few ARGs might obscure selective effects restricted to more rare ones. Increases in ARGs and MGEs have been demonstrated in controlled exposure experiments with antibiotics (Lundström et al., 2016; Murray et al., 2018), but field studies are considerably less conclusive (Bengtsson-Palme et al., 2016; Karkman et al., 2016). One reason is that sites exposed to antibiotic residues are often also exposed to fecal material, both of which would likely increase the relative abundance of ARGs and MGEs. Karkman et al. (2019) showed that elevated levels of ARGs in the environment in most cases can be explained by increased exposure to fecal bacteria alone, with the exception of environments polluted with very high concentrations of antibiotics. Analyses of High Arctic soils also linked increased ARG abundance to the levels of phosphorus, in agreement with fecal transport as the main explanation, rather than the selection of ARGs that naturally reside in these soils (McCann et al., 2019). The finding of increased ARGs and MGEs in field samples therefore provides limited support for a selection pressure that drives the evolution of resistance (+).

Fecal markers are widely used for the assessment of environmental transmission risks. While environmental ARGs and fecal markers often correlate (Ahmed et al., 2018; Karkman et al., 2019; Stachler et al., 2019), it does not imply that all or even most detected ARGs are present in enteric pathogens. Hence, the value of ARG abundance alone as a marker for the transmission risks of already resistant pathogens is limited (+). The supposition that ARGs in nonpathogens present a lower risk for human health than ARGs in pathogens necessitates identification of the host bacteria to the species or sometimes even strain level. We argue that nonpathogenic ARB in general constitute a lower risk than pathogenic ARB, as exposure to the former would also involve subsequent transfer of resistance to a pathogen coresiding in the human microbiome. Quantitative PCR of ARGs does not allow for host identification, and accurate assembly of contiguous sequences around ARGs from metagenomic shotgun data is often difficult (Bengtsson-Palme et al., 2017). Approaches that try to link ARGs to species by simple correlation of abundances may provide some information, but these approaches are generally not conclusive. Emulsion, paired isolation and concatenation PCR (epicPCR), a technique for linking ARGs to hosts in complex communities (Hultman et al., 2018), represents a step forward. However, this methodology does not yet allow for the differentiation between many genera, and even less so between species within certain genera, such as within the *Enterobacteriaceae* family. It is known that different ARGs have different host ranges. For those ARGs where the host range is restricted to certain pathogens, for example, the absolute abundance of such ARGs could indeed be informative about exposure and hence the transmission risk.

Similar difficulties in interpreting the increased abundance of ARGs for the risk for evolution could be applied to the class 1 integron-integrase gene. Since it is often genetically coupled to ARGs, the integrase gene is subjected to indirect selection pressure from antibiotics but often also has a fecal origin (Gillings et al., 2015). The class 1 integron-integrase gene might also be an alternative or complementary marker to ARG in the surveillance of transmission risk, as the counts correlate with antibiotic resistance gene abundance and anthropogenic pollution. Although the class 1 integron is widespread in human and animal microbiota, it is not associated with a particular bacterial species nor is it specific to pathogens (Gillings et al., 2015). An additional challenge with using gene abundance (ARGs or MGEs) to assess the transmission risk is that whether the genes represent dead or live cells is often unknown (Girones et al., 2010).

An increased relative abundance of ARGs can, as noted, reflect an environmental antibiotic selection pressure, primarily when potential contributions from the spread of enteric bacteria have been disentangled (Karkman et al., 2019). The reason is that enteric bacteria more often carry ARGs than do most other bacteria in the environment (Pal et al., 2016). In many cases, therefore, the increased prevalence of ARGs largely reflects fecal contamination rather than an environmental selection and enrichment of ARB (Karkman et al., 2019). Selective

pressure from antibiotics not only drives resistance evolution but also poses a risk for ecological effects. While this suggests that ARG abundance (when feces is excluded) could reflect a risk for ecological effects as well, it is a very indirect measure, and whether resistance per se has negative consequences for ecosystem health (−) is unknown.

The relative abundance of ARGs may inform about antibiotic resistance prevalence in human or animal populations (Hendriksen et al., 2019; Munk et al., 2017; Pehrsson et al., 2016). One strength of ARG abundance as a marker here is the broad taxonomic coverage, but the difficulty of assigning ARGs to pathogens in general or to one pathogen in particular is also a significant limitation. To identify spatiotemporal trends, resolution down to the species level may not be as necessary as it would be to inform empirical treatment. If samples with highly variable contributions from fecal material are compared, that should ideally be considered (see also Section 3.5).

3.3. Emerging antibiotic resistance genes

One may categorize ARGs into those that are known to be present in pathogens (and thereby can be more directly linked to transmission risks as well as the population-level resistance prevalence) and those ARGs that are not (yet) found in pathogens. Regardless of their association with pathogens, both can be informative about selection pressures and hence the risks for evolution and ecological effects. Martínez et al. (Martínez et al., 2015) proposed that the highest risk for genetic transfer of ARGs from environmental reservoirs to human pathogens is associated with the detection of mobile ARGs known to be present in pathogens. Bengtsson-Palme and Larsson (Bengtsson-Palme and Larsson, 2015) agreed that the *likelihood* may be greatest for such genes but not the *magnitude of the potential consequences*, which is an integrated aspect of the total risk that resistance development will occur and result in impaired health outcomes. Environmental detection of *already mobilized* resistance factors not yet found in pathogens would more directly reflect a risk for the emergence of resistance (evolution, +), with potentially much greater consequences for health than the transfer of a gene that is already widely circulating in pathogens and the human microbiota (Bengtsson-Palme and Larsson, 2015). Both functional screens (Allen et al., 2009; Forsberg et al., 2012; Razavi et al., 2017) and predictive approaches (Arango-Argoty et al., 2018; Berglund et al., 2019) have been applied to identify resistance factors with the potential to emerge as a threat to the effectiveness of antibiotic therapy. While such methods can be valuable to identify the genes of interest to monitor, they are probably not yet suitable for routine use in surveillance programs. For the surveillance of population-level antibiotic resistance prevalence, increases in the relative abundance of rare or even ARGs not yet identified in human pathogens might be informative as an early warning, particularly if they are already mobilized (+; Razavi et al., 2017).

3.4. Fecal contamination

The risk of transmission is related to the absolute number of resistant bacteria, i.e., the colonization or infectious dose. Overall, this supports a simplified approach for measuring the concentration of fecal indicator bacteria (++), as performed under, for example, the bathing water directive in the European Union (Anonymous, 2006). One drawback of this simplified approach for assessing the risk of transmission is that it does not consider opportunistic pathogens of nonenteric origin that are able to survive and multiply well in the environment, such as *Legionella pneumophila*, *Mycobacterium avium* and *Pseudomonas aeruginosa*. The number of these species does not correlate with fecal coliform numbers, hence hampering the evaluation of the transmission risk through the concentration of fecal indicator bacteria (Falkinham et al., 2015). Fecal contamination might also provide some information about the risk for the evolution of resistance in pathogens (+). The reason is that enteric bacteria are already adapted to the mammalian intestine and could act as recipients for ARGs that have not yet emerged in human or animal pathogens.

3.5. Antibiotic resistance genes and fecal contamination ratio

A relationship between human illness and waterborn exposure to human fecal residues has been established, but such a relationship remains less clear for nonhuman sources (Boehm et al., 2009). The chosen fecal indicator(s) should therefore also preferably distinguish between human and nonhuman sources. crAssphage, a very common bacteriophage highly specific to the human intestinal microbiota, was recently used to analyze both ARGs and fecal contamination in environmental samples (Ahmed et al., 2018; Karkman et al., 2019; Stachler et al., 2019). The ratio of ARGs and fecal contamination could provide more information than each of these alone. The critical advantage of studying this ratio is that it helps to disentangle increases in resistance due to fecal contamination (transmission risk) from on-site selection (evolution risk, ++), where data on concentrations of selective agents can provide additional evidence. However, the ARG-to-fecal contamination ratio alone provides little evidence for the risk of transmission (−) because it is the absolute number of pathogens a human or domestic animal is exposed to that determines the risk of colonization or infection, not the ratio between resistant and nonresistant pathogens. As the ratio can help identify places with environmental selection pressure, it could also provide some information about the risks for ecological effects (+). For surveillance of population-level resistance prevalence, the ARG-to-fecal contamination ratio can be valuable (+). This is particularly true for samples with low or moderate contributions of feces where the contribution of ARGs from nonhuman sources can add noise to the analysis.

3.6. Antibiotic-resistant bacteria

Changes in the relative abundance of ARB in studies of microbial communities can be the result of simple taxonomic shifts, regardless of fecal contamination or direct antibiotic selection pressure that would favor the evolution of resistance (Kraupner et al., 2018). Analyses of the proportion of bacterial colonies on media with or without antibiotics, without determining the species, are therefore inconclusive in terms of within species selection (Flach et al., 2017; Guardabassi et al., 2002; Lundström et al., 2016; Silva et al., 2006). A more relevant indicator for a selection pressure driving evolution would therefore be to measure the selection of ARB separately for individual species (++; Flach et al., 2018; Kraupner et al., 2018). One drawback of such studies is that they must, by necessity, be limited in scope to a small subset of the microbial communities, and the effect (or lack thereof) may not be extrapolated to other species in the community. When technologies that can place ARGs into context (e.g. epicPCR) become sufficiently quantitative and allow for species resolution, an assessment of the proportion of bacteria carrying specific resistance determinants in parallel for a large range of species can be performed, providing valuable information about selection. As an increase in the proportion of ARB can be indicative of selection pressure (when controlling for fecal contamination, see also Section 3.2), it would also provide relevant information on exposure for assessing ecological effects (+). With regard to the risk of transmission, analyses of pathogenic ARB are informative. In contrast to understanding the risks for evolution, it is not the proportion of ARB (−) that is most informative but rather their absolute numbers (++).

Clinical surveillance programs that currently guide empirical treatment are primarily based on the proportion of resistant isolates for each bacterial pathogen in clinical samples (ECDC, 2018). The proportion of ARB within species is therefore probably the most informative measure for environmental surveillance of population-level antibiotic resistance prevalence, particularly if the purpose is to provide guidance for empirical treatment (++). For practical reasons, the species studied should be present in sufficient numbers in feces, show

sufficient survival in the studied environmental compartment, and allow easy and accurate isolation. Furthermore, the contribution of these species from sources other than humans or domestic animals needs to be limited. The selection of pathogen-antibiotic combinations that are evaluated should be based on clinical relevance.

The proportion of ARB might also be informative for the surveillance of antibiotic use in human populations if the relationship between antibiotic use, resistance in feces and resistance in the environment is strong (+). Such relationships have been shown for both humans and domestic animals in the United States and Europe (Bell et al., 2014; ECDC/EFSA/EMA, 2017) but might not hold true for all parts of the world. Factors including water quality, sanitation level and infection control could play a more important role than antibiotic consumption volumes in determining antibiotic resistance levels in low- and middle-income countries (Collignon et al., 2018). In addition to geographic differences, observed relationships between antibiotic use and resistance prevalence might reflect accumulated historical use more than current antibiotic use, as a reduction in antibiotic use is not necessarily mirrored by reduced resistance prevalence (Aarestrup et al., 2001; Enne et al., 2001; Sundqvist et al., 2010). This might be related to the time between the decrease in antibiotic use and sampling, compensatory mutations minimizing the fitness cost, nonhuman use of antibiotics contributing to the pool of ARB potentially transferred to humans, or coresistance (Enne et al., 2001). In addition, changes in the resistance prevalence generally happen relatively slowly (e.g., resistance trends are calculated over five years within the European Antimicrobial Resistance Surveillance Network; ECDC, 2018), while the use of specific antibiotics can, at least locally, change rapidly following an intervention.

3.7. Functional community responses

For ecological effect markers, assays that directly assess the functionality or services of microbial ecosystems would be ideal (++). Such assays exist for certain functions and services and are applied in regulatory toxicology (OECD Guidelines for the Testing of Chemicals; Brandt et al., 2015). Nevertheless, microbial communities may simultaneously provide a multitude of functions and services, many of which cannot be tested or are even known. In practice, therefore, toxicity to individual microbial species is often used as a simplified proxy for studying the effects of stressors on ecosystem functions and services in a laboratory context. An approach that considers field exposure to toxic agents in monitoring is the concept of pollution-induced community tolerance (PICT; Blanck, 2002). Here, samples are taken from both exposed and nonexposed environments, and the communities are challenged in short-term laboratory tests to one or more stressors, such as antibiotics. The readout could include, for example, respiration, nitrogen transformation and biosynthesis of nucleic acids and proteins (Brandt et al., 2015). If a community does not respond to a certain stressor, it suggests that the community has already been shaped by this or a similarly acting stressor previously in the field. If the stressor applied in the test is an antibiotic, this could also provide information on selection pressure relevant for the evolution of resistance (+). However, it provides an indirect measure only as it cannot distinguish between taxonomic shifts, between-species selection and phenotypic adaptation of individuals (Blanck, 2002). Additionally, PICT may not be the most sensitive marker (Lundström et al., 2016).

3.8. Microbial diversity

Microbial diversity, including both taxonomic and functional genetic diversity, might be used as a marker for the assessment of the ecological effects of antibiotics since redundancy in microbial communities might ensure against the loss of function (+). One might expect that an environmental selection pressure from antibiotics would lead to reduced diversity (Laverman et al., 2015; Proia et al., 2013) and that reduced diversity would therefore provide some information about the evolution risk. In some studies of environments exposed to exceptionally high levels of antibiotics, however, the overall taxonomic diversity is only moderately affected, possibly because over time other species take over the niches previously occupied by the sensitive species (−; Kristiansson et al., 2011; Lundström et al., 2016). The link between changes in taxonomic profiles and ecosystem services may be more difficult to establish (Griffiths and Philippot, 2013), but there has been some success in linking the abundance of different functional gene categories in communities to their overall function (Yang et al., 2017). It has also been argued that microbial diversity ensures long-term ecosystem functions and services and might therefore form its own environmental protection goal (Brandt et al., 2015).

4. Sites for environmental surveillance

As previously indicated for surveillance markers, the relevance of different surveillance sites will also depend on the objective that is to be addressed. Sewage might be used for assessing the risk of transmission as it provides a reflection of the amount of resistant bacteria that enter the environment. Subsequent modeling of their environmental fate based on, for example, analyses of treated sewage, and considering dilution and inactivation during the transport of ARB can provide an overview of the concentrations of different ARB from sewage that could reach exposure-relevant sites. To use these data for estimates of exposure due to contact with, for example, bathing water, factors such as the swallowed volume of water per person per swimming event should also be taken into account (O'Flaherty et al., 2019; Schijven et al., 2015). The direct assessment of sites where human exposure is likely to occur would be most suitable for assessing the risk of transmission. Such exposure-relevant sites include recreational areas (beach sand and swimming water), drinking water, and ambient air. Fruits, vegetables and shellfish may also be contaminated with ARB during growth in contaminated soil and/or water. This contamination is especially relevant if the products are consumed lightly cooked or raw. Recreational water and ambient air might be especially relevant in the direct vicinity of wastewater treatment plants and aquaculture and livestock farms, as wastewater and manure are major sources of fecal contamination of the environment (Huijbers et al., 2015; Pruden et al., 2013).

The risk for mobilization and enrichment of novel ARG in a bacterial community is related to the strength of the selection pressure to which they are subjected (Bengtsson-Palme et al., 2018). Furthermore, the risk for the transfer of ARGs to pathogens is enhanced in places where there are high concentrations of fecal bacteria. Additionally, a high diversity of gene donors increases the likelihood that rare transfer events will occur. Considering all these points, it can be argued that wastewater treatment plants, raw sewage overflow, treated wastewater discharge sites, manure impacted soils, pharmaceutical manufacturing discharge sites, and surface waters in proximity to intensive agriculture and aquaculture constitute good starting points for trying to understand the risks for emergence of resistance. For evolution in the environment to play a role in human and animal health, it is also important that there are opportunities for transmission of ARB to humans or animals, such as in recreational swimming areas, via drinking water and in agricultural settings (Leonard et al., 2018; O'Flaherty et al., 2019; Schijven et al., 2015). Recommended surveillance sites to assess the impact of antibiotics and ARB on ecosystem health will be similar to environmental surveillance for evolution, i.e., those with anticipated high selection pressures but with an additional emphasis on those where concerns about ecosystem functions and services are high.

Surveillance of population-level antibiotic resistance prevalence would ideally be conducted as close as possible to the population of interest, i.e., the human or animal population for which antibiotic resistance data should be generated. The reason is that the relative contribution of microbiota to an environmental sample, as well as the distance between the sampling site and the population under

surveillance, may affect how well antibiotic resistance in associated human and animal populations can be estimated (Pehrsson et al., 2016). Differential survival in the environment between resistant and nonresistant strains, negative or positive, as well as the high proportion of fecal bacteria, makes untreated sewage a more attractive option than treated wastewater or surface water. Theoretically, hospital sewage could provide a better estimate of the resistance prevalence for that particular hospital than urban wastewater taken downstream at a municipal treatment plant. When a smaller population contributes to the sample, such as with hospital effluents, the collection of composite samples can be critical (Colque Navarro et al., 2014; Kwak et al., 2015). Single grab samples might contain bacteria from only a few individuals, whereas many samples taken over several hours would represent more individuals. Another factor to be considered is the connection of populations to converging sewage networks. This is different for high-, middle- and low-income countries but also in rural and urban settings (WHO/UNICEF, 2017); therefore, each setting needs a tailor-made sampling strategy.

The surveillance of population-level antibiotic use through environmental samples should also be conducted as close as possible to the population of interest. In this case, the environmental persistence of antibiotics plays an important role, and the manner in which samples are taken arguably merits more consideration due to systematic hourly changes in the concentrations of many pharmaceuticals entering wastewater treatment plants (Ort et al., 2010). The further from the population of interest that samples are taken, the more factors affect antibiotic concentrations, such as sorption to particles, degradation and, in some cases, the restoration of parent molecules from their metabolites (Dinh et al., 2017; Pikkemaat et al., 2016). For each antibiotic, the percentage and form of the excreted compound, as well as its persistence, must be considered through calibration with independently derived usage data, ideally from a range of sites and time points. For antibiotics that are prone to rapid degradation, such as many beta-lactam antibiotics, it would likely be difficult to acquire precision in the usage estimates from environmental concentration data (Berendsen et al., 2015; Dinh et al., 2017; Lindberg et al., 2005).

5. Final remarks

The current paper defines objectives for environmental monitoring or the surveillance of antibiotics and antibiotic resistance. It discusses the levels of evidence provided by different markers and identifies sites where monitoring can be particularly informative for each objective. For the establishment of any environmental surveillance framework, one needs to be very explicit about its objective(s). Only then can the most informative markers and sites be selected, as outlined in this paper. Overall, a combination of markers for fecal contamination and antibiotic resistance, together with measurement of selective agents would be informative for all five objectives. Considering all surveillance sites that were addressed, the in- and outlets of wastewater treatment plants, or any location where waste from human or animal populations comes together, could provide starting points for the standardization of sampling around the world.

Some of the objectives may be better approached by directed research efforts rather than broad systematic, long-term surveillance. Additionally, some markers may at this point in time be too costly or otherwise challenging to apply in large-scale surveys, noting that these factors may differ between countries and change over time. Analyses of the costs associated with the analysis of different markers and sites should therefore also precede any decisions about implementation. Finally, we think it would be beneficial if specific objectives as well as limitations associated with applying different markers would be more explicit in future scientific literature. The framework presented here could be one of the starting points for harmonizing environmental surveillance with human and animal surveillance systems for antibiotic resistance, e.g., in the design and implementation of action plans at the national (India, 2017; Sweden, 2017; United Kingdom, 2019) and global level (FAO/OIE/WHO, 2018).

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Conflict of interest

The authors declare that they have no conflicts of interest.

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