Meta-analysis reveals the global picture of antibiotic resistance gene prevalence across environments

Anna Abramova^{1,2}, Thomas U. Berendonk³, Johan Bengtsson-Palme^{1,2*}

¹ Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Guldhedsgatan 10A, SE-413 46 Gothenburg, Sweden

² Centre for Antibiotic Resistance research (CARE) at University of Gothenburg, Gothenburg, Sweden

³ Institute for Hydrobiology, Technische Universität Dresden, Dresden 01062, Germany

*Corresponding author: johan.bengtsson-palme@gu.se

Abstract

The environment is an important component in the emergence and transmission of antimicrobial resistance (AMR). Despite that, current AMR monitoring initiatives lack comprehensive reference data for the vast majority of environments. To enable monitoring to detect deviations from the normal background resistance levels in the environment, it is necessary to establish the typical baseline of AMR in a variety of settings. In an attempt to establish this baseline level, we here performed a comprehensive literature survey across 2516 scientific papers, 150 of which contained relevant qPCR data on antibiotic resistance genes (ARGs) in environments associated with potential routes of AMR dissemination. The collected data include 1487 samples distributed across 30 different countries and 17 environmental types, in a time span from 2005 to 2018. More than 330 different genes were identified from the collected studies. Most surveyed environments contained a diverse set of ARGs, but generally at low abundances. We used linear mixed models and

overrepresentation analysis to identify time trends and associations between ARGs and specific environments. Altogether these data represent a comprehensive overview of the occurrence and levels of ARGs in different environments, providing background data for risk assessment models within current and future AMR monitoring frameworks.

Keywords: antimicrobial resistance, AMR, qPCR, monitoring, surveillance

Introduction

According to the World Health Organization the increasing level of antibiotic resistance over the last decades is a considerable threat to global health¹. Already, current estimates suggest that hundreds of thousands of deaths are caused by antibiotic resistant bacteria each year worldwide, and this number is expected to reach several millions annually within the next 30 years².

Antibiotic resistance has been mainly investigated in clinical and agricultural settings.

However, the role of the environment as a source and dissemination route of resistance has been widely recognized in recent years³⁻⁵. Antibiotic resistance is widespread in nature and not limited to human-associated activities as evidenced by antibiotic resistance genes (ARGs) found in glaciers, permafrost and isolated caves which have virtually never been visited by humans⁶⁻⁸. It is even likely that resistance may be maintained independent of human activities in e.g. soil systems due to the presence of antibiotic-producing microorganisms, including certain fungal taxa⁹. Due to the extraordinary biological diversity in nature, the external environment can serve as a source of novel ARGs not yet present in clinically relevant pathogens¹⁰. Furthermore, the environment constitutes a dissemination route for resistant bacteria between humans and animals¹¹. However, it is not yet clear to what extent the resistant environmental bacteria and the presence of ARGs in the environment contribute to acquisition and spread of resistance in clinically relevant bacteria. To address these questions, it is essential to identify and quantify resistance in the environment. This in turn would allow pinpointing which environments possess the highest risks to human and domestic animal health and would subsequently enable us to devise measures to prevent – or at least delay – recruitment and dissemination of resistance factors from the environment.

Monitoring schemes for antibiotic resistance have been developed and put to use in clinical and agricultural settings, but systematic monitoring for antimicrobial resistance (AMR) in the environment is still lacking¹². Existing monitoring systems largely rely on culturing of pathogens and bacterial indicator species of interest¹³, and these approaches may not be directly applicable to the monitoring of resistance in the environment. For example, the majority of environmental bacteria are not culturable using standard laboratory methods and the breakpoints for resistance are adapted for clinically relevant pathogens. Circumventing culturing drawbacks, quantitative real-time PCR (qPCR) allows detection and quantification of individual ARGs. However, a major hurdle for using qPCR for monitoring of AMR is the decision of which genes to select as targets in these qPCR assays. While large arrays that can measure the abundances of hundreds of ARGs in parallel exist¹⁴, they are still fairly expensive and may not yet be ready for implementation in routine monitoring¹⁵, and this would thus necessitate selection of a smaller number of highly relevant qPCR targets.

To address this problem, and to design efficient monitoring and surveillance strategies, there is a need for comprehensive background data on abundances and prevalence of ARGs occurring in both pristine and human-impacted environments. Establishing background levels of ARGs is a prerequisite for determining “safe levels” for ARGs naturally occurring in the environment and would also provide insights into to which extent an environment has been enriched with ARGs due to human activity. However, there is currently no, or very little, reference data available for the majority of environments and no agreed-upon set of genes to be used for environmental AMR monitoring.

The aim of this study was to perform a literature survey of studies containing qPCR data for ARGs in various environments and identify a set of genes and reference values for key environments for AMR transmission, which could be used for monitoring purposes. Through screening of more than 2500 scientific papers, we found that most surveyed environments contained a diverse set of ARGs, but generally at low abundances. However, it was clear

that the existing data on ARG abundances is unevenly distributed across the world and that there is a general lack of data for most environments and geographical areas. The study highlights the need for knowledge of environmental background levels of AMR to understand spatiotemporal changes in ARG abundances that can be associated with increased risks for adverse human health effects.

Materials and Methods

Data collection

Literature collection was performed by mining the PubMed database in April-May 2020, November 2020 and October 2021. The searches were performed using three strings: “antibiotic resistance genes” AND (monitoring OR surveillance) AND qPCR AND’, “antibiotic resistance genes” AND “high throughput” AND’ and “antibiotic resistance” AND (qPCR OR “quantitative PCR”) AND’ complemented by environmental type: “sewage OR wastewater”, “surface water”, sediment, soil, food, “drinking water”, “bathing water”, “swimming pools”, “bathing water”, water, airport, (“public transport” OR bus OR train OR airplane OR boat OR tram OR underground OR metro), (industrial OR pollution), abandoned, cleanroom, animal and “one health”. Environmental types were selected based on an assessment of potential transmission routes for antibiotic resistance^{4,11}. Paper suitability was initially assessed by screening the title, abstract, and methods section. Only papers using qPCR as a quantification method and containing extractable data were selected. Review papers were not included in this study.

For each paper the following information was extracted: first author, DOI, year, country, sampling location, treatment/type (including distance from a contamination source, season,

type of fertilizer/antibiotic applied, stage of wastewater treatment process, source; e.g. for “water” it would be “potable”, “reclaimed”, “river” etc.), sample type, and abundance of antibiotic resistance genes (ARGs). For several studies missing information about the exact year of data collection, we extrapolated the sampling year based on the average time required from the data collection to the publication inferred from the other studies, corresponding to an estimate of two years. The ARG abundances were collected from text, tables and figures. Information from figures was extracted using WebPlotDigitizer v4.3 (<https://automeris.io/WebPlotDigitizer>). Abundances were reported as log₁₀ ARG abundance per 16S rRNA. ARGs with abundance below detection limit were not included in the analysis.

Data analysis

Data were analyzed in RStudio v1.3.1073¹⁶ with the R package ggplot2 v3.3.5¹⁷ used to plot the graphs. To determine the abundance distributions for ARGs across environments, density plots of the ARG abundances were used, and the R summary function was used to find the quartiles for each ARG. To investigate if there were any changes in ARGs levels over time, we built a linear mixed model (LMM). LMM allows incorporation of not only fixed effects but also random effects to account for cluster-correlated data from distinct sources of variability¹⁸. The model was designed with ARG *Abundance* as a response variable and sampling *Year* as explanatory variable and included sampling *Country* as a covariate:

$$Abundance = Year + (1|Country) + \epsilon$$

Analyses were performed using lme4 v1.1-27.1¹⁹ and lmerTest v3.1-3²⁰ packages.

To identify genes associated with particular environments, we used the R package indicpecies v1.7.9. This analysis uses relative frequency and abundance of species (in our

case genes were treated as “species”) in defined groups, to identify those that are the most characteristic of each group²¹. Only genes found in more than 20 samples were included in this analysis. The function *multipatt* was run with 9999 permutations. The results were visualized as a network using Cytoscape (cytoscape.org).

To identify a set of suitable qPCR targets we investigated how well the data from collected genes could predict the abundance of the other genes in the data matrix by performing a correlation analysis. Only genes with more than 10 entries were included in the analysis. R package stats (version 3.6.2) *cor* function with “pairwise.complete.obs” was used to calculate Spearman correlations.

Results and Discussion

Current data on AMR in the environment is geographically biased

Mining of the PubMed database resulted in 2516 papers and revealed a bias in the existing literature towards already fairly well studied environments (Figure S1). Unsurprisingly, the majority of the identified papers matched environmental categories that have been already extensively studied as primary sources of antibiotics and/or antibiotic resistance to the environment. These include environments linked to wastewater treatment plants, hospitals and industrial facilities, as well as agriculture and livestock production. This also highlights the undersampled nature of the other environments. To bring an example, it is suggested that human mobility, in particular international travel, could contribute to dissemination of ARGs across the globe^{10,22-24}. Despite that, categories associated with travel activity, such as “public transport” and “airports”, are represented by just a handful of papers. Similarly, water associated with recreational activities is another poorly investigated environmental category,

despite that accidental ingestion of water during, for example, swimming is considered to be a potential route for resistant environmental bacteria into the human gut²⁵.

In total, we identified 150 studies (1487 samples) containing relevant information on levels of ARGs measured by qPCR. These studies covered 17 different types of environments with “water” and “effluent” being the most commonly investigated (covered by 46 and 33 studies, respectively). In contrast, “food” and “air” were mentioned in just five studies or less. The samples collected in these studies originated from 30 different countries (Figure 1A), with China being the most studied among them, both in terms of number of studies and samples. The distribution of studies highlights white spots on the map; in particular, there were no studies originating from South America and just a handful of studies from Africa. This is worrisome, as South America and Africa are among the regions with the highest rates of self-medication and supply of non-prescribed antibiotics in the world^{26,27}. In 2014, a WHO report outlined Africa’s lack of established AMR surveillance systems¹ and a review from 2017 reported that more than a third of the countries on the continent did not have recent AMR data published in the public domain, and that only little of the reported data were from surveillance²⁸. This scarcity of publicly available data on AMR is corroborated by the current literature survey. The majority of samples in the selected studies were collected between 2015-2018 with a gradual increase in the number of publications over the last years (Figure 1C).

Figure 1. Overview of the collected data. Studies distributed by A) country, B) sampled environment, and C) year.

Environmental ARGs are diverse in almost all environments

We identified 331 different genes that were quantified in at least one of the collected studies. They belonged to several different ARG classes with β -lactamase and multi-drug resistance genes being the most diverse classes (54 and 46 unique ARGs, respectively) (Table S1). When ARG data were stratified by country (Figure 2), it was evident that overall the diversity of targeted and detected ARGs was fairly low (86% of the studies targeted less than 10 ARGs on average). Most of the studies used singleplex qPCR (119) and 29 used high throughput qPCR (HT-qPCR).

Most of the studied environments were characterized by a diverse set of investigated ARG classes (Figure S2), but despite that a limited but obvious bias was observed towards certain classes of ARGs. In particular, tetracycline and β -lactam resistance genes were among the

most commonly used targets for qPCR studies. Interestingly, studies investigating sewage targeted mostly β -lactamases in comparison to the other classes. This may be due to these genes being perceived to be associated with a relatively higher risk to human health, which would justify intensive research on their removal in sewage treatment plants. A range of macrolide-lincosamide-streptogramin B (MLSB) resistance genes were looked for and reported from all the investigated environments. Multidrug resistance genes showed a similar pattern with the exception of being absent from “feces” and “sewage” environments, which is most likely due to a limited number of studies targeting these particular ARG classes rather than this being their actual distribution. Colistin resistance genes were reported only from five environments in studies published starting from 2018. This class is an interesting case, since colistin belongs to the family of polymyxins which used to be one of the last classes of antibiotics for which resistance was not plasmid-mediated. In 2015, the first plasmid-borne colistin resistance gene was described²⁹, and since then it has been reported in many bacterial species and samples across the world, some predating the initial discovery^{30,31}, although it was not often targeted in the studies we analyzed. There were also several classes which were profiled consistently across all the environments, including sulfonamides (with the *sul1* and *sul2* genes reported from all the environments), vancomycin and aminoglycoside resistance genes.

Figure 2. Maximum diversity and maximum abundance of ARGs in sampled countries.

Around one in a thousand environmental bacteria carry clinical ARGs

Most of the ARGs present in more than 20 studies were characterized by higher than average abundance (Figure 3) and were, unsurprisingly, widespread across the studied environments (Figure 4). Among the 20 most reported genes, tetracycline resistance genes were the predominant class of ARGs. Sulfonamide resistance genes *sul1* and *sul2* were the two most reported genes, with *sul1* reported in 68% of studies and from all the environments. The *int11* gene was also reported from all the investigated environments and was characterized by the highest average abundance among all the genes in our data matrix. These genes are among the ones suggested as good monitoring targets by Berendonk et al.⁴. Genes encoding extended-spectrum β -lactamases, including *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV}, were among the common targets in qPCR studies, which is not surprising since they are ARGs commonly encountered in clinically relevant pathogens. The plasmid-mediated fluoroquinolone resistance gene *qnrS* has been reported from aquatic environments and wildlife^{4,32,33}. A study of the distribution of this gene in wildlife and water

reservoirs suggested that it can be horizontally transferred even in the absence of a selective pressure from antibiotics³⁴. The most commonly reported genes also included the erythromycin resistance genes *ermB* and *ermF*, and the aminoglycoside resistance genes *strB* and *aadA1*. Interestingly, there were several genes that were often highly abundant when detected, but which were not often reported, making them potentially suitable additional targets for AMR monitoring (*vanA*, *tnpA4*, *tnpA5*, *sul3*, *qacEdelta*, *mexF*, *IS613*, *floR*, *ereA* and *aadA2*).

For each ARG with more than a single observation, we estimated its typical environmental abundance levels (Figure S3). We found that for most ARGs, the typically reported abundances fell in an interval from 10^{-5} to 10^{-3} copies per 16S rRNA, roughly corresponding to one ARG copy in a thousand bacteria (Table S2). That said, some ARGs, such as the *mcr1* colistin resistance gene (mostly below 10^{-5} ARGs/16S), had lower typical abundances, and tetracycline resistance genes *tetO* and *tetQ* often occurred in abundances above 1 copy per 16S rRNA in fecal samples (Figure S3). Interestingly, overall *environmental* abundances of these two *tet* genes were in the typical 10^{-5} to 10^{-3} range, indicating that these genes may be good indicators of pollution with human fecal matter. They may even be more suitable than *int11*, which has been proposed as a marker of human anthropogenic impact in the past³⁵ but consistently appeared in 10^{-5} to 10^{-3} copies per 16S rRNA in most environments in our data (Figure S3).

Taken together, it seems normal to encounter most ARGs in abundances between 10^{-5} to 10^{-3} copies per 16S rRNA in a given environment, although we refer the reader to Figure S3 and Table S2 for specific values for individual ARGs. In general, we advise the use of 10 times the third quartile (3Q) values for a given ARG (Table S2) to determine the upper limit for what should be viewed as a deviation from normal background levels in the environment. This should be combined with studying the overall and environment-specific abundance distributions for the same gene (Figure S3). It is worth noting that this analysis does not

consider whether the detected ARG abundances are the result of anthropogenic activities^{36,37} or natural selection for ARGs in these environments^{9,38}.

Figure 3. Abundance of most reported ARGs. ARGs reported in more than 20 studies are represented in orange, and ARGs reported less but which were generally highly abundant are shown in blue. The line represents an average abundance across all data points.

Figure 4. Distribution of the most reported and abundant ARGs across environments.

Above the line are ARGs reported in more than 20 studies and below are the ARGs reported less but highly abundant.

It is unclear if ARG abundances have increased over time

To investigate if there were changes in ARG abundances over time, we used linear mixed models on the data for all of the ARGs in each of the environments, accounting for variability between countries (Figure S5). Due to the increased sensitivity of the qPCR methods and inclusion of a larger number of ARGs profiled in each study over time, we chose to use linear mixed models for the maximum values rather than all ARG values reported. Using this approach, we found positive trends for most of the environments (for cases where there was enough data to perform the estimates), except for in sediments, sludge, wastewater and treated wastewater where the trends were negative (Table S3). That said, the trends were significant only for soil (increase) and treated wastewater (decrease). Thus, the most prominent finding of this analysis might be that in most cases there was too little data to draw any conclusions on ARG abundance changes over time, highlighting the need for time series data to understand the long-term development of antibiotic resistance in the environment.

Most ARGs are not associated with specific environments

We performed an overrepresentation analysis showing which ARGs were associated with particular environments (Figure S6, Table S4). We found that a substantial number of ARGs were specifically associated with sludge, sewage, manure and soil, but not the other environments. However, comparably few ARGs were specifically associated with just a single environment. Some notable examples of such single-environment associations include *mcr1* in feces, *qnrD* in wastewater, *strA*, *strB*, *tnpA3* and *aphA3* in manure, and *aadA3* in sediments.

Abundance data for some ARGs provide redundant information

The set of most reported genes consists of potential candidates to be used as qPCR targets, since they were on average more abundant and were also found in most environments (Figures 3 and 4). To explore if they would be good predictors of overall ARG abundances,

we performed a correlation analysis of the most reported genes and the rest of the genes in the data matrix (Figure S4). Interestingly, this analysis revealed a certain degree of redundancy in terms of what information can be gained from the proposed monitoring targets. ARGs proposed as monitoring targets that strongly correlate with each other convey similar information on ARG abundance and diversity and may therefore not be very useful to use in the same panel for ARG monitoring in the environment. Genes that are overall redundant are the *tetO*, *tetX* and *tetA* genes, *strB* and *ermF*, *bla_{CTX-M}* and *bla_{SHV}*, *tetG* and *tetM*, as well as *int11* and *sul2* (Figure S4). If resources are constrained, it would seem wise not to use several genes in these smaller groups together in the same ARG panel for monitoring.

Priorities for environmental monitoring of AMR

It is well established that antibiotic resistance is an ancient phenomenon, and that the environment represents a rich reservoir of resistance factors which could be transferred to human pathogens under the right conditions^{9,38-40}. Therefore, environmental monitoring of antibiotic resistance is important in order to protect human and animal health. However, a systematic framework for monitoring in the environment is still lacking¹², and defining *what*, *where* and *how* to monitor is crucial for such efforts to contribute relevant data⁴. There are several main objectives for monitoring of AMR in the environment, including *i*) assessment of risks for transmission of already resistant pathogens to humans via environmental routes, *ii*) analysis of general levels of resistance in the human population, and *iii*) monitoring the evolution of resistance in the environment to identify emerging resistance threats⁴¹.

Risk ranking of ARGs

There have been several attempts to create a system for ranking ARGs by risk to human health. Two common criteria used to estimate risks include known presence of an ARG in human pathogens and whether it is located on a mobile genetic element, such as a plasmid. For instance, Martinez et al.⁴² proposed a ranking system which assigns the highest rank to

genes that have already been shown to be a clinical problem and the lowest rank to genes whose resistance function was inferred solely by homology. However, ARGs that are well-known and have been around for decades in clinical pathogens may be less of a concern than those that have recently emerged and that could potentially pose the next big threat, but are not yet widespread among pathogens¹⁰. Thus, ARGs which are not ubiquitous and not yet present in human pathogens must be carefully considered when assessing risks associated with environmental resistomes. A recent paper by Zhang et al.⁴³ presented another approach based on microbial ecology, with the decisive ranking criteria being enrichment of the ARG by anthropogenic activities. ARGs enriched by anthropogenic activities and correlated with antibiotics contamination should, by this line of thinking, pose a higher risk than those that are not enriched⁴³. This is, in theory, a sound approach; however, a major issue would be determining which ARGs that would be enriched by antibiotic exposure, as this is in most cases not known outside of ARGs commonly occurring in human pathogens. Another factor to consider in risk ranking could be the presence of an ARG in a variety of different environments⁴⁴ or in different bacterial species, including potential human pathogens, which would suggest it has a high capacity for horizontal gene transfer and transmission⁴⁵. From a human health risk perspective, it may be particularly relevant to target ARGs that confer resistance to last resort antibiotics, such as carbapenems, vancomycin or colistin⁴. Many of these genes are currently not abundant in environmental samples, which may present a challenge for environmental monitoring¹³. It has also been proposed to monitor the diversity of ARGs, as a high ARG diversity would be associated with a larger set of genes that could potentially be transferred to pathogens^{13,46}. Along the same lines, it is worth considering how well a certain ARG would predict the abundances of other ARGs in an environment, as it has been shown that some ARGs have better predictive power than others⁴⁶.

Monitoring targets identified in the current study

We compared the list of genes identified in the present literature survey with proposed candidates for environmental monitoring from earlier studies (Table S5). Some of the identified ARGs are already common in routine clinical monitoring (e.g. <https://www.oxfordbiosystems.com/AMR>, <https://www.opgen.com/acuitas/amr-gene-panel/>), including clinically relevant β -lactamases such as *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV}, the sulfonamide resistance gene *sul1*, the vancomycin resistance gene *vanA*, as well as *ermB* and *tetM* conferring resistance to macrolides and tetracyclines, respectively. These genes are already common both in the environment and among human pathogens, and therefore their usefulness in environmental monitoring is somewhat questionable. At the same time, they may function as a gauge of the total antibiotic resistance content in an environment, as some of them have been shown to be good predictors of the total resistome, particularly *tetM* and *bla*_{TEM}⁴⁶.

Considering the most common ARGs in our survey that are not yet included in routine clinical monitoring (Table 1) highlighted additional future monitoring targets, including the class 1 integrase *int11*, which is already a widely used marker for anthropogenic AMR dissemination³⁵. Despite that this gene is not a resistance gene itself, is often linked to ARGs and according to the existing literature it is widespread in the environment, found in both pathogenic and commensal bacteria, enriched by anthropogenic activities and correlates well with the abundances of other commonly occurring ARGs. This gene fulfills most of the criteria for being a suitable monitoring target, except perhaps that it is already so widespread (Figure 3; Figure S3) that it may not be able to sufficiently capture minor or long-term changes in the environment over time and space.

Similarly, the sulfonamide resistance gene *sul2* has also been suggested as a monitoring target by Berendonk et al. (2015)⁴ and was among the most reported in our data and is widespread across all the environments. However, *sul2* added little information compared to

int11 (Figure S4), so these two genes would be mostly redundant in AMR monitoring. In comparison, *sul3* was not so often reported despite being rather abundant in the cases when it was looked for. According to previous studies, *sul3* is typically less frequent and abundant than *sul1* and *sul2*^{47,48}. However, it has been shown to be associated with class 1 integrons and has been detected on a conjugative plasmid, suggesting its potential to be horizontally transferred and disseminated in the environment⁴⁹. Interestingly, *sul3* was first detected in an *E. coli* isolate from a pig, and it was later found in both healthy and diseased humans. Furthermore, it is enriched in polluted environments and is present in both commensal and pathogenic bacteria⁵⁰. Overall, these three *sul* genes are good indicators of mobile antibiotic resistance, but may suffer from not being that predictive for total ARG abundance and diversity⁴⁶, and their value for monitoring in addition to *int11* is somewhat doubtful.

The plasmid-mediated fluoroquinolone resistance gene *qnrS* was among the most reported genes in our study. It was found in all environments except manure and treated wastewater. As it is the only commonly reported fluoroquinolone ARG, it is an interesting target for monitoring. However, it has the drawback of generally not being able to induce clinically relevant levels of resistance without additional resistance mechanisms, which makes its clinical impact limited.

The list of most reported genes was dominated by tetracycline resistance genes. Among them, *tetM* was the third most often reported gene in the investigated studies, and has been previously proposed as a monitoring target and included in clinical screening^{4,46}. Tetracycline resistance genes are among the most powerful ARGs for predicting the diversity and abundance of other ARGs⁴⁶ and therefore, despite their widespread distribution, these genes can still be useful in monitoring to extrapolate other parts of the resistome.

Table 1. Characterization of genes identified in the current survey as potential targets for environmental AMR monitoring

ARG	Ecology	Association with MGEs	Presence in ESKAPE pathogens	Wide niche adaptation	Distribution across environments	Abundance variability (0,01-1,95)	Correlation coefficient and number of significant correlations
<i>intl1</i>	Enriched in polluted environments ³⁵	class 1 integron-integrase gene	yes ⁵¹	yes ⁵¹	All environments	1,61	0,64 (97)
<i>sul2</i>	Correlates with concentrations of antibiotics ^{52,53}	yes ⁵⁴	yes ⁵⁴	yes ⁵⁵	All environments	1,56	0,80 (150)
<i>sul3</i>	Correlates with heavy metals pollution ⁵⁶	yes ⁵⁷	?	yes ⁵⁸	6	1,54	0,71 (8)
<i>qnrS</i>	Enriched in fluoroquinolone contaminated environment ^{59,60}	yes ⁶¹	yes ⁶²	yes ⁶³	12	1,56	0,68 (23)
<i>strB</i>	Correlates with heavy metals pollution ⁶⁴	yes ⁶⁵	yes ⁶⁶	yes ⁶⁷	10	1,47	0,64 (83)
<i>ermF</i>	Correlates with antibiotics level ⁶⁸	yes ⁶⁹	?	yes ^{70, 71}	12	1,44	0,66 (68)
<i>tetB</i>	Correlates with antibiotics level ⁵²	yes ⁷²	yes ⁷³	yes ⁷⁴	12	0,29	0,73 (98)
<i>tetC</i>	Correlates with antibiotics levels ^{75,76}	yes ⁷⁷	yes ⁷⁸	yes ⁷⁹	12	1,49	0,70 (23)
<i>tetX</i>	Correlates with antibiotics ⁸⁰	yes ⁸¹	yes ⁸²	yes ⁸³	11	0,76	0,67 (83)
<i>tetQ</i>	Correlates with heavy metals ⁸⁴	yes ⁸⁵	?	yes ⁸⁶	11	1,35	0,72 (76)
<i>tetW</i>	Correlated with the concentrations of tetracycline residues ⁸⁷	yes ⁸⁸	?	yes	10	0,63	0,69 (60)
<i>aadA1</i>	Correlates with pollution levels ⁸⁹	yes	yes	yes	9	1,22	0,63 (97)
<i>aadA2</i>	Correlates with pollution levels ⁸⁹	yes	yes	yes	8	0,90	0,61 (88)
<i>vanA</i>	Correlates with antibiotic residues ⁹⁰	yes ⁹¹	yes ⁹²	yes ⁹¹	6	1,34	0,72 (3)
<i>floR</i>	Correlates with heavy metals ⁸⁹	yes	yes ⁹³	yes	8	1,62	0,70 (80)
<i>ereA</i>	Correlates with pollution ⁹⁴	yes ⁹⁵	yes	yes	7	1,36	0,63 (85)
<i>mexF</i>	Correlates with AB use ⁹⁶	yes ⁹⁷	yes ⁹⁸	yes ⁹⁹	8	1,10	0,77 (100)
<i>qacEdelta</i>	Correlates with heavy metals ¹⁰⁰	yes ¹⁰¹	yes ¹⁰²	?	7	1,46	0,80 (134)

There were also several genes that were not often included in qPCR studies, but when they were, they often appeared in relatively high abundances. Such genes could be interesting additional monitoring targets in future AMR monitoring schemes. Among these genes was the vancomycin resistance gene *vanA*, a previously suggested indicator of antibiotic resistance contamination of clinical origin which is thought to be uncommon in the environment⁹⁰. In our data, however, *vanA* has been about as abundant as other ARGs in the environment when it has been looked for. Another gene that seems to correlate with anthropogenic activities is *ereA*, a macrolide resistance gene which has previously been reported to be the most abundant in metal polluted soil¹⁰³ and is enriched by long-term application of manure⁹⁴. In contrast, the *mexF* gene has been reported from many different environments, including soil, sediments and water²² and is suggested to naturally occur in unaffected environments such as pristine Antarctic soils^{104, 105}, indicating that it might be a useful target for identifying enrichment of environmental ARGs.

Specific uses of qPCR as a monitoring tool

In this study, we have exclusively focused on the use of qPCR as a tool for monitoring the abundance of ARGs in the environment. There are, however, other ways of measuring the levels of AMR, including selective culturing and metagenomic sequencing approaches. In this context, it is worth considering not only the appropriate targets for monitoring, but also which methods that give the most informative output in a cost-efficient manner. In general, the qPCR technique has several benefits, but also some drawbacks as a surveillance tool. An important advantage is that it is a highly sensitive method that can detect much lower levels of ARGs than is currently possible using shotgun metagenomic sequencing¹⁰⁶. Furthermore, it can operate on very minute quantities of DNA, which makes it suitable also for low-biomass samples. In addition, as it operates on DNA, it will be able to detect ARGs in non-culturable bacteria and can function also on complex samples with many different species. On the other hand, qPCR does not provide any information about the genetic context of ARGs, such as whether they are chromosomal or mobile or if they are co-located

with other ARGs. Similarly, it does not indicate which hosts carry a given ARG. Crucially, it is also restricted to a predetermined set of target ARGs (or other types of genes) which limits the reusability of the data. For example, when the *mcr1* colistin resistance gene was discovered in 2015¹⁰⁷, it very quickly was detected in archived metagenomic sequence data²⁴, but such ‘retrospective monitoring’ would not be possible to perform using qPCR datasets. The limiting factor of predefined targets can be partially overcome by using qPCR arrays with hundreds of genes¹⁵, but this also comes with increased costs and may not be feasible for large-scale routine monitoring of environmental AMR. In addition, the technique would say close to nothing about emerging ARGs that may become the next generation resistance threats in human pathogens¹⁰, which makes qPCR-based monitoring practically useless as an early-warning system for emerging resistance, unless a panel of potential future ARGs is screened for. That said, qPCR clearly has its uses in monitoring for transmission of known high-risk ARGs via the environment, where it can provide relatively quick data on specific deviations from the normal background levels.

A more fundamental aspect of such deviations is whether an increase in the abundance of ARGs or mobile genetic elements in a particular environment or at a specific time point is a relevant indicator of a selective pressure for resistance. Importantly, an increase of ARG abundances without any context is more likely to be an indicator of human pollution³⁶, but it cannot be ruled out that such a change could be due to a specific selection pressure from antibiotics¹⁰⁸, by co-selection from other antibacterial compounds¹⁰⁹, or simply from taxonomic shifts that are unrelated to antibiotic resistance. In the end, the suitability of qPCR for environmental AMR surveillance comes down to the purpose of monitoring and what type of actions one might want to take based on the monitoring results. If transmission of known high-risk ARGs is the sole purpose of monitoring, qPCR is an excellent method thanks to its sensitivity. However, if identification of emergent resistance threats is the goal, qPCR is unlikely to give useful guidance; instead, shotgun metagenomics or selective culturing followed with genetic profiling would provide more useful information.

Outcomes and recommendations

In this study, we performed a literature survey to explore the abundance and prevalence of ARGs in various environments as quantified by qPCR. We found that, overall, previous suggestions for ARGs to be included in environmental AMR monitoring^{4,46} seem relevant. Particularly, inclusion of the *intI1*, *sul1*, *bla_{TEM}*, *bla_{CTX-M}* and *qnrS* genes in environmental monitoring seems essential, along with a selection of the tetracycline genes *tetM* (but not *tetG*), *tetQ*, *tetW*, *tetC*, and either of *tetA* or *tetO*. However, there are also genes that are not often looked for in qPCR surveys that perhaps should be, including *sul3*, *vanA*, *tetH*, *aadA2*, *floR*, *ereA* and *mexF*. These genes are abundant in some environments, but were not often included in qPCR studies of environmental AMR. We also provide environmental baseline levels for the ARGs studied through qPCR (Figure 5; Figure S3); for most ARGs the typical abundance falls in an interval from 10^{-5} to 10^{-3} . It should be noted that this is the range of normal abundances of ARGs, and should not be considered a maximum acceptable limit of these ARGs in any given environment. Such maximum acceptable levels need to be determined taking risks to human health (and potentially the environment) into account^{5,110}, and would also need to consider transmission routes to humans¹¹. The absence of clear trends of increases or decreases in ARG abundances over time indicates a need for more systematic time series data on ARG abundances in a variety of environments. Our results also highlight the scarcity of AMR data from parts of the world, particularly from Africa and South America, and underscores the need for a concerted effort to quantify typical background levels of AMR in the environment more broadly to enable efficient environmental AMR surveillance schemes akin to those that exist in clinical settings.

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Supplementary Figures and Tables

Figure S1. Total number of collected studies per each environmental category.

Figure S2. Proportion of ARGs in each class reported in different environments.

847 **Figure S3.** The distribution of abundances for the ARGs with more than 100 observations

852 **Figure S4. Correlation analysis between the selected ARGs.**

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Figure S5. Relationship between ARG abundances and time. The data points are the abundances of ARGs in samples from each country and the lines are fitted to the maximum abundance values.

Table S1. Gene classes and the number of unique ARGs they contain

ARG Class	ARGs	Unique ARGs	Reported in studies
Beta lactams		55	78
	<i>blaampC</i>		
	<i>blaAAC</i>		
	<i>blaCMY2</i>		
	<i>blaEC</i>		
	<i>blaNDM</i>		
	<i>blaMOX</i>		
	<i>blaOCH</i>		
	<i>blaPAO</i>		
	<i>blaSM</i>		
	<i>blaROB</i>		
	<i>blaTEM</i>		
	<i>blaCTXM</i>		
	<i>blaOXY1</i>		
	<i>blaSHV2</i>		
	<i>blaPSE1</i>		
	<i>blaAXA1</i>		
	<i>blacepA</i>		
	<i>blacfxA</i>		
	<i>blacphA</i>		
	<i>blaIMP</i>		
	<i>blaVIM</i>		

	<i>blaOXA2</i>		
	<i>blaOXA48</i>		
	<i>blaOXA58</i>		
	<i>blaACT</i>		
	<i>blaL1</i>		
	<i>bla1</i>		
	<i>blaGES</i>		
	<i>blaCMY</i>		
	<i>blaI</i>		
	<i>blaKPC</i>		
	<i>blaLEN</i>		
	<i>blaM</i>		
	<i>blaMIR</i>		
	<i>blaOKP</i>		
	<i>blaOXA1</i>		
	<i>blaOXY2</i>		
	<i>blaPER</i>		
	<i>blaS1</i>		
	<i>blaSFO</i>		
	<i>blaSHV</i>		
	<i>blaSHV34</i>		
	<i>blaTLA</i>		
	<i>blaVEB</i>		
	<i>blaZ</i>		
	<i>carB</i>		

	<i>cepA</i>		
	<i>cfiA</i>		
	<i>cfxA</i>		
	<i>cphA</i>		
	<i>fox5</i>		
	<i>mecA</i>		
	<i>pbp</i>		
	<i>pbp5</i>		
	<i>penA</i>		
Multidrug		46	28
	<i>acrA1</i>		
	<i>acrA2</i>		
	<i>acrA3</i>		
	<i>acrA4</i>		
	<i>acrA5</i>		
	<i>acrB</i>		
	<i>acrF</i>		
	<i>acrR1</i>		
	<i>acrR2</i>		
	<i>adeA</i>		
	<i>adel</i>		
	<i>qacEdelta</i>		
	<i>ceoA</i>		
	<i>cmeA</i>		
	<i>cmeAF</i>		

	<i>cmle3</i>		
	<i>cmr</i>		
	<i>emrD</i>		
	<i>marR</i>		
	<i>mdtEyhIU</i>		
	<i>mdtg</i>		
	<i>mdth</i>		
	<i>mepA</i>		
	<i>mexA</i>		
	<i>mexB</i>		
	<i>mexE</i>		
	<i>mexF</i>		
	<i>mtrD</i>		
	<i>oprD</i>		
	<i>oprJ</i>		
	<i>oqxA</i>		
	<i>oqxB</i>		
	<i>pmrA</i>		
	<i>qacA</i>		
	<i>qacH</i>		
	<i>rarD</i>		
	<i>sdeB</i>		
	<i>tolC</i>		
	<i>ttgA</i>		
	<i>ttgB</i>		

	<i>yceEmdtG1</i>		
	<i>yceLmdtH1</i>		
	<i>yceLmdtH2</i>		
	<i>yceLmdtH3</i>		
	<i>yidYmdtL1</i>		
	<i>yidYmdtL2</i>		
MLSB		42	75
	<i>cfr</i>		
	<i>ereA</i>		
	<i>ereB</i>		
	<i>erm34</i>		
	<i>erm35</i>		
	<i>erm36</i>		
	<i>ermO</i>		
	<i>ermQ</i>		
	<i>ermA</i>		
	<i>ermB</i>		
	<i>ermC</i>		
	<i>ermF</i>		
	<i>ermJ/ermD</i>		
	<i>ermK</i>		
	<i>ermT</i>		
	<i>ermX</i>		
	<i>ermY</i>		
	<i>lmrA</i>		

	<i>InuB</i>		
	<i>ImrA</i>		
	<i>InuF</i>		
	<i>InuA</i>		
	<i>InuC</i>		
	<i>matA</i>		
	<i>mdet1</i>		
	<i>mdtA</i>		
	<i>mefA</i>		
	<i>mefB</i>		
	<i>mphA</i>		
	<i>mphB</i>		
	<i>mphC</i>		
	<i>msrE</i>		
	<i>msrA</i>		
	<i>msrC</i>		
	<i>oleC</i>		
	<i>pikR1</i>		
	<i>pikR2</i>		
	<i>vatA</i>		
	<i>vatB</i>		
	<i>vatC</i>		
	<i>vatD</i>		
	<i>vatE</i>		
	<i>vgaA</i>		

	<i>vgaB</i>		
MGE		40	92
	<i>cInt1</i>		
	<i>IncNkorA</i>		
	<i>IncNrep</i>		
	<i>IncPoriT</i>		
	<i>IncWrepA</i>		
	<i>intl1</i>		
	<i>intl2</i>		
	<i>intl3</i>		
	<i>IS1133</i>		
	<i>IS1247</i>		
	<i>IS2</i>		
	<i>IS21/ISAs29</i>		
	<i>IS256</i>		
	<i>IS26</i>		
	<i>IS3</i>		
	<i>IS6/257</i>		
	<i>IS61</i>		
	<i>IS613</i>		
	<i>ISAb3Acineto</i>		
	<i>ISEfm1Entero</i>		
	<i>ISPps1pseud</i>		
	<i>ISSm2Xanthob</i>		
	<i>korB</i>		

	<i>mobA</i>		
	<i>Tn3</i>		
	<i>TN543</i>		
	<i>Tn916/1545</i>		
	<i>tnpA1</i>		
	<i>tnpA2</i>		
	<i>tnpA3</i>		
	<i>tnpA4</i>		
	<i>tnpA5</i>		
	<i>tnpA6</i>		
	<i>tnpA7</i>		
	<i>tnpA24</i>		
	<i>tnpA25</i>		
	<i>tnpA26</i>		
	<i>tnpA27</i>		
	<i>Tp614</i>		
	<i>traN</i>		
Aminoglycoside		39	44
	<i>aac</i>		
	<i>aac6I</i>		
	<i>aac6Ib</i>		
	<i>aac6II</i>		
	<i>aac6Iy</i>		
	<i>aac6iz</i>		
	<i>aac2ic</i>		

	<i>aac3ia</i>		
	<i>aac3iv</i>		
	<i>aac3vi</i>		
	<i>aac6la</i>		
	<i>aacA</i>		
	<i>aacC</i>		
	<i>aacC1</i>		
	<i>aacC2</i>		
	<i>aacC4</i>		
	<i>aadA1</i>		
	<i>aadA2</i>		
	<i>aadA5</i>		
	<i>aadA9</i>		
	<i>aadD</i>		
	<i>aadE</i>		
	<i>acrD</i>		
	<i>ant2ia</i>		
	<i>ant3ia</i>		
	<i>aph</i>		
	<i>aph2Id</i>		
	<i>aph3ia</i>		
	<i>aph3III</i>		
	<i>aph3VIa</i>		
	<i>aph4Ib</i>		
	<i>aph6Ia</i>		

	<i>aphA1</i>		
	<i>aphA3</i>		
	<i>sat4</i>		
	<i>spcN</i>		
	<i>str</i>		
	<i>strA</i>		
	<i>strB</i>		
Tetracycline		31	116
	<i>tet32</i>		
	<i>tet34</i>		
	<i>tet35</i>		
	<i>tet36</i>		
	<i>tet37</i>		
	<i>tet38</i>		
	<i>tetA</i>		
	<i>tetB</i>		
	<i>tetD</i>		
	<i>tetE</i>		
	<i>tetG</i>		
	<i>tetH</i>		
	<i>tetJ</i>		
	<i>tetK</i>		
	<i>tetL</i>		
	<i>tetM</i>		
	<i>tetO</i>		

	<i>tetPB</i>		
	<i>tetQ</i>		
	<i>tetS</i>		
	<i>tetT</i>		
	<i>tetU</i>		
	<i>tetV</i>		
	<i>tetW</i>		
	<i>tetX</i>		
	<i>tetZ</i>		
	<i>tet39</i>		
	<i>tet44</i>		
	<i>tetC</i>		
	<i>tetPA</i>		
	<i>tetR</i>		
Vancomycin		21	18
	<i>vanA</i>		
	<i>vanB</i>		
	<i>vanC</i>		
	<i>vanG</i>		
	<i>vanHB</i>		
	<i>vanHD</i>		
	<i>vanRA</i>		
	<i>vanRB</i>		
	<i>vanRC</i>		
	<i>vanRC4</i>		

	<i>vanSB</i>		
	<i>vanSC</i>		
	<i>vanTC</i>		
	<i>vanTE</i>		
	<i>vanTG</i>		
	<i>vanWB</i>		
	<i>vanWG</i>		
	<i>vanXA</i>		
	<i>vanXD</i>		
	<i>vanYB</i>		
	<i>vanYD</i>		
Biocide/Metal		16	16
	<i>arsA</i>		
	<i>cadA</i>		
	<i>chrA</i>		
	<i>copA</i>		
	<i>crcA</i>		
	<i>czcA</i>		
	<i>fabK</i>		
	<i>gadAB</i>		
	<i>merA</i>		
	<i>nimE</i>		
	<i>nisB</i>		
	<i>pcoA</i>		
	<i>pncA</i>		

	<i>qacF</i>		
	<i>rcnA</i>		
	<i>tcrB</i>		
Phenicol		10	16
	<i>catA1</i>		
	<i>catB3</i>		
	<i>catB8</i>		
	<i>catQ</i>		
	<i>cmIA1</i>		
	<i>cmIA5</i>		
	<i>cmIV</i>		
	<i>cmxA</i>		
	<i>fexB</i>		
	<i>floR</i>		
Trimethoprim		8	15
	<i>dfrA1</i>		
	<i>dfrA12</i>		
	<i>dfrA13</i>		
	<i>dfrA14</i>		
	<i>dfrA17</i>		
	<i>dfrA22</i>		
	<i>dfrC</i>		
	<i>folA</i>		
Quinolones		5	37

	<i>qepA</i>		
	<i>qnrA</i>		
	<i>qnrB</i>		
	<i>qnrD</i>		
	<i>qnrS</i>		
Sulfonamide		4	112
	<i>sul1</i>		
	<i>sul2</i>		
	<i>sul3</i>		
	<i>sulA</i>		
Bacitracin		2	8
	<i>bacA1</i>		
	<i>bacA2</i>		
Colistin		1	5
	<i>mcr1</i>		
Other		7	5
	<i>aerA3</i>		
	EAE5855		
	<i>gyr2</i>		
	Pe		
	<i>pica</i>		
	<i>trbC</i>		
	<i>uidA</i>		

Table S2. Abundance distribution for reported ARGs

Gene	Number of observations	Min	1Q	Median	Mean	3Q *	Max
aac	26	-4,9519212	-4,4757121	-4,2218488	-3,9104352	-3,8467018	-0,4484305
aac6I	13	-5,1149177	-4,457575	-4,3129996	-4,2296892	-3,7864876	-3,5455649
aac6Ib	112	-9,4	-3,7017708	-3,2348348	-3,5147957	-2,4674718	0,832994
aac6II	60	-9,34838	-4,4838731	-3,6406975	-3,9145249	-3,0994286	-1,4496602
aac6Iy	6	-5,5415792	-5,3246863	-5,2065132	-5,0372733	-4,9859138	-3,992576
aac2ic	8	-9,6499685	-8,4514287	-8,3158796	-8,3424101	-8,1023244	-7,4584276
aac3ia	7	-7,9318141	-7,7356733	-4,3870968	-4,6428478	-1,6451613	-1,4193548
aac3iv	6	-8,9194889	-8,1117715	-7,4712342	-7,6428411	-7,2043659	-6,5543958
aac3vi	8	-7,3165287	-6,1712173	-5,6683086	-5,6983329	-5,3215454	-3,9389987
aac6Ia	6	-8,7572714	-8,4378682	-8,0273174	-7,9450309	-7,4791277	-6,9871628
aacA	26	-4,3979487	-4,1868759	-3,7039003	-3,5162445	-3,1572828	-1,6575773
aacC	76	-5,9264869	-3,3427102	-2,4915878	-2,8260093	-1,9787251	-1,4243375
aacC1	8	-4,6478175	-4,4836352	-3,5679758	-3,7868803	-3,2467236	-2,9976637
aacC2	32	-5,126659	-4,0413708	-3,633275	-3,4912729	-2,9089268	-1,4367186
aacC4	42	-4,8354651	-3,8714078	-3,4859938	-3,2875111	-2,8651603	-0,4516129
aadA1	157	-8,4920128	-3,4800069	-2,8413476	-2,9429659	-2,0930233	-0,1503859
aadA2	109	-5,2146717	-3,4669575	-2,7242572	-2,7948976	-2,0982803	-0,8927934
aadA5	80	-8,2572749	-3,8354072	-3,3806947	-3,6959273	-2,7783093	-1,4546929
aadA9	52	-8,1272612	-5,4339749	-4,1509505	-4,5176717	-3,6642403	-1,9577465
aadD	17	-4,9144635	-4,242761	-3,7996539	-3,4780319	-2,4904596	-1,1873199
aadE	51	-4,853872	-3,9393305	-3,6289321	-3,5091075	-3,0525579	-1,6925396
acrA1	62	-9,7983176	-4,5694098	-3,6407291	-4,0731819	-3,2571287	-2,3896669
acrA2	36	-4,7581743	-3,8011755	-3,3906826	-3,3043091	-2,8582465	-1,2423775
acrA3	18	-5,5759434	-4,6322954	-4,2979081	-4,3284233	-3,9331025	-3,3575355
acrA4	56	-4,9871628	-3,42507	-3,13305	-3,1807451	-2,8515393	-1,3147428
acrA5	55	-4,8893869	-3,5638365	-3,1713401	-3,1925855	-2,8674122	-1,5985566
acrB	30	-9,377786	-5,0522255	-3,3723788	-4,0757612	-2,4374377	-0,14
acrD	2	-7,6194762	-7,5538031	-7,4881301	-7,4881301	-7,422457	-7,3567839
acrF	31	-8,6842965	-5,3889386	-4,1549196	-4,6841829	-3,2686842	-2,5925991
acrR1	45	-8,7878124	-4,6595559	-3,756962	-4,3560271	-3,4469199	-2,5100415
acrR2	22	-5,1726813	-4,320921	-3,3575616	-3,0495286	-1,5801535	-0,5542748
adeA	17	-8,4282912	-7,6126117	-4,4881166	-5,3356966	-3,6539834	-3,1357157
aerA3	2	-4,2564679	-4,0542826	-3,8520973	-3,8520973	-3,649912	-3,4477266
blaampC	92	-11	-4,0899574	-3,5369584	-3,7777532	-3,2555934	-0,6667569
ant2ia	5	-8,1396338	-7,8761484	-7,6252517	-7,4999105	-7,5451551	-6,3133637
ant3ia	6	-9,347983	-8,4464652	-8,1645803	-8,0269659	-7,8280046	-6,2284125
aph	5	-4,2218488	-3,8944898	-3,8461851	-3,015606	-1,7264574	-1,389049
aph2Id	5	-4,5015769	-2,6634215	-2,5751184	-2,8457754	-2,3498868	-2,1388734
aph6Ia	6	-8,1366771	-7,5961104	-5,3687942	-5,2353065	-2,77002	-2,2952624

aphA1	54	-5,8450704	-4,3147674	-3,494115	-3,5306465	-2,8955238	-1,2580645
aphA3	41	-5,7356828	-4,2218488	-3,3943662	-3,4935269	-3,056338	-0,3043478
bacA1	20	-7,8612914	-6,6266514	-4,4431466	-4,9368967	-3,6814638	-2,6289321
bacA2	28	-4,9691139	-4,13652	-3,4193046	-3,6168816	-3,2546558	-2,5050828
blaAAC	14	-9,2533658	-7,6850985	-4,4227994	-5,2930264	-4,275569	-1
blaCMY2	56	-8,5655485	-4,0731693	-3,6705772	-3,8480256	-3,2590396	-0,2798407
blaEC	7	-9,8694587	-8,5215603	-7,4412914	-7,9287559	-7,4050893	-6,3372422
blaNDM	47	-7,8613992	-6,5234999	-6,1733434	-5,8729964	-5,2381034	-3,3254902
blaMOX	19	-8,8134291	-4,3412505	-3,6845546	-3,8793275	-2,9517765	-2,5365646
blaOCH	24	-7,9439515	-4,6432774	-4,1915267	-4,5798147	-3,8035999	-3,3225839
blaPAO	8	-9,3338913	-7,8364708	-4,8828076	-5,6944753	-4,1300407	-2,5434821
blaSM	5	-6,9788179	-6,8961963	-6,8612914	-6,7511405	-6,7644716	-6,2549253
blaROB	21	-8,5214335	-7,4116928	-4,6005435	-5,5607359	-4,279847	-3,1817864
blaTEM	370	-9,8	-4,4157895	-3,4641077	-3,6297081	-2,6271632	1,97175361
blaCTXM	324	-9	-5,419205	-4,0809511	-4,3740689	-3,3140691	0,642455
blaOXY1	64	-7,9244534	-3,8213825	-3,2255231	-3,4123879	-2,6392158	-2,1257234
blaSHV2	16	-8,3458235	-6,8532003	-4,1709511	-4,9708162	-3,4322738	-3,1299724
blaPSE1	26	-9,7491569	-4,6517062	-3,9635711	-4,5068836	-3,4256515	-1,7
blaAXA1	2	-8,7585898	-8,6271005	-8,4956113	-8,4956113	-8,364122	-8,2326327
blacepA	24	-7,6675615	-5,375	-5,265625	-5,0293392	-4,8359375	-3,1870666
blacfxA	17	-8,2418818	-6,7423214	-3,9199687	-4,6323485	-2,9520865	-1,3710988
blacphA	21	-8,3936186	-6,391474	-3,7836514	-4,7724305	-3,4392422	-2,139662
blaIMP	32	-8,7721133	-4,1375602	-3,7746479	-3,9213569	-3,2409997	-2,5166415
blaVIM	21	-9,9136417	-6,872467	-4,761875	-5,4489683	-4,2856724	-2,853872
blaOXA2	14	-3,853872	-3,2468509	-2,6477416	-2,7427816	-2,3933399	-1,46
blaOXA48	18	-8,1419661	-6,8861805	-5,2053733	-5,6661858	-4,6885397	-2,8860566
blaOXA58	63	-8	-5,3794212	-3,0470588	-3,8263565	-2,401118	-0,8972332
blaL1	26	-4,9691139	-4,1896075	-3,8405332	-3,8223982	-3,5196756	-2,4629275
bla1	7	-4,3385827	-3,7626595	-3,5272436	-3,5523093	-3,1762427	-3,1225348
blaGES	52	-8,1349626	-4,4067049	-3,3018831	-3,5838719	-2,4912531	-1,1977569
blaCMY	18	-4,7429794	-4,2236942	-3,9066702	-3,8436073	-3,4414847	-2,7805278
blaI	9	-8,7996539	-8,3223935	-7,6289321	-7,5297612	-7,1992829	-4,3329287
blaKPC	35	-6,79588	-4,571941	-4,3451627	-4,2007091	-3,8617188	-1,4641784
blaM	24	-4,2422226	-3,4727048	-3,2069838	-2,7653714	-2,6125222	0,24891454
blaOKP	6	-5,2415834	-4,0908536	-3,8149419	-4,0017292	-3,7158567	-3,2732557
blaOXA1	99	-8,084507	-4	-3,3321838	-3,4596452	-2,6746968	-0,8552742
blaPER	24	-4,9767632	-4,3351809	-4,1740892	-3,9742154	-3,5960247	-3,1234935
blaS1	10	-3,5872892	-3,3580467	-3,1647432	-3,1425506	-2,8850679	-2,7453715
blaSFO	76	-6,3539482	-4,0139612	-3,154927	-3,5084731	-2,7850277	-1,6646986
blaSHV	128	-7,9	-5,2124389	-3,7363564	-4,0173508	-3,021704	0,05882353
blaSHV34	8	-2,6536473	-2,5598003	-2,429633	-2,1699697	-2,136826	-0,5421181
blaTLA	10	-4,6514926	-4,1030371	-3,5590519	-3,7143376	-3,3807557	-3,0352346
blaVEB	27	-4,7746972	-3,7551278	-3,3266218	-3,0462076	-2,2656466	-0,9753456

blaZ	8	-5,5818133	-5,2320882	-5,0186688	-5,0601195	-4,8226441	-4,6738916
cadA	2	-7,6194762	-7,5538031	-7,4881301	-7,4881301	-7,422457	-7,3567839
carBR	7	-9,1294125	-8,8951746	-8,5467512	-8,4301032	-8,3431604	-6,8578887
catA1	19	-4,5957915	-4,2092942	-3,9691139	-3,9051943	-3,6972801	-2,682459
catB3	39	-9,5724772	-3,8905281	-3,5148865	-3,9456811	-2,9506131	-1,338237
catB8	26	-8,7121983	-3,8974557	-3,4971436	-3,8394211	-3,0643817	-2,464276
qacEdelta	120	-8,844664	-3,3187531	-2,4819809	-2,7126507	-1,7741203	-0,0140845
ceoA	60	-8,2395775	-3,9456964	-3,6016247	-3,9255556	-3,2327188	-2,7734948
cepA	9	-4,5666736	-4	-3,7528496	-3,805982	-3,5168945	-3,3898928
cfiA	12	-8,9443978	-5,7524547	-4,5586412	-5,3652273	-4,0057294	-3,6989743
cfr	19	-5,045256	-1,6	-0,0762763	-1,1162645	0,05913301	0,38512222
cfxA	39	-5,5147436	-3,8264397	-3,5649401	-3,4376749	-3,0915908	-0,8
chrA	2	-7,1758794	-6,9949507	-6,814022	-6,814022	-6,6330932	-6,4521645
clntI1	7	-1,7706635	-1,4015458	-1,2364901	-1,1948657	-0,9866868	-0,5804411
cmeA	2	-5,4793796	-5,0273253	-4,5752709	-4,5752709	-4,1232166	-3,6711623
cmeAF	1	-3,8632794	-3,8632794	-3,8632794	-3,8632794	-3,8632794	-3,8632794
cmle3	7	-9,4958677	-7,8539813	-6,6345122	-7,2505934	-6,559965	-5,7958817
cmlA1	87	-8,4736672	-3,9396562	-2,9912759	-2,9963404	-1,9332355	0,38512222
cmr	15	-6,7	-6,1	-5,3	-4,9172566	-4,2	-1,7
cmxA	53	-4,883213	-4,3564978	-3,7759852	-3,7303478	-3,3593656	-1,394695
copA	1	-0,25451	-0,25451	-0,25451	-0,25451	-0,25451	-0,25451
cphA	68	-4,928582	-3,6220746	-3,2670164	-3,2503907	-2,8053226	-1,1818139
crcA	1	-0,1414851	-0,1414851	-0,1414851	-0,1414851	-0,1414851	-0,1414851
dfrA1	74	-9,3488328	-4,2182613	-3,3287419	-3,63422	-2,4831123	-1,4221643
dfrA12	18	-4,7281584	-4,3055341	-4,04875	-3,9910516	-3,772736	-2,9535735
dfrA14	8	-3	-2,395	-2,01	-1,9675	-1,6	-0,98
dfrA22	9	-5,1545455	-5	-3,8534483	-4,0760152	-3,2829651	-3,173913
emrD	57	-9,2741984	-4,7713457	-3,3129996	-3,7671441	-2,568688	-1,7287489
ereA	93	-5,7536257	-3,6298389	-3,1898844	-2,8672118	-2	1
ereB	37	-8,8477117	-4,4841262	-3,8356471	-4,2853293	-3,3791447	-2,443281
erm34	13	-8,7987667	-7,6695862	-4,5373519	-5,4458531	-4,1769623	-2,8896839
erm35	21	-5,5425699	-4,4019682	-3,8632794	-3,9117773	-3,3212662	-2,5269969
erm36	52	-8,2211255	-5,1621572	-3,8102132	-4,2993815	-3,2722702	-1,565947
ermA	29	-8,799559	-4,543634	-3,7659168	-4,1245782	-2,8454918	-2,2745551
ermB	329	-9	-3,4977625	-2,6594954	-2,8298469	-1,8526316	0,96214585
ermC	69	-8	-4,7372665	-3,0988142	-3,7588676	-2,4953826	-1,2235294
ermF	132	-7,7695518	-3,3183742	-2,3476248	-2,4713145	-1,348239	-0,0462403
ermJ.ermD	13	-3,9685915	-3,4720877	-3,2616159	-3,3138558	-3,1643943	-2,7264254
ermK	11	-9,8865665	-6,0348983	-4,4682944	-5,2750132	-4,0539917	-2,9943914
ermT	34	-4,8927934	-4,2388007	-3,9867639	-3,8542724	-3,58861	-1,9691138
ermX	67	-7,2684112	-4,2037123	-3,3592194	-3,4562063	-2,4078085	-1,5799236
ermY	8	-4,3467875	-4,1446568	-3,9642989	-4,011612	-3,8691077	-3,7695518
fabK	5	-9,2641377	-8,5128616	-8,3882767	-7,0378448	-4,6259991	-4,3979487

fexB	14	-3,8249515	-0,4682028	-0,2982323	-0,9201291	-0,1521647	0,62546438
floR	100	-6,1943918	-3,9093381	-3,2617171	-2,8596675	-2,0702723	0,6213178
folA	10	-5,61	-4,9325	-4,2723214	-4,3894643	-3,7975	-3,36
fox5	76	-9,3747383	-4,7305809	-3,1970383	-3,692453	-2,7072836	-1,7189666
gadAB	5	-4,7	-4,5	-3,8	-4,06	-3,7	-3,6
gyr2	5	-4,4259687	-3,6989743	-3,2218488	-3,0091532	-2,6989743	-1
lmrA	4	-4,3233639	-4,2472275	-3,9447051	-3,9515585	-3,6490361	-3,5934598
lncWrepA	17	-6,5211268	-4,8136364	-4,4347826	-4,452097	-3,5016894	-2,7935261
intl1	558	-9	-3,3	-2,3186645	-2,4605839	-1,3984615	1,6
intl2	30	-6,8831169	-4,796847	-3,9961965	-3,736675	-2,735369	-0,5
intl3	17	-6,9264693	-5,1445934	-3,4588161	-3,6255432	-2,6338028	-0,6901408
lnuB	41	-8,3979487	-3,7996539	-3,3494623	-3,4781035	-2,8223531	-1,9439515
IS613	44	-5,7117124	-4,3112874	-3,5553119	-3,3845149	-2,4690811	-0,7144427
korB	15	-4,24	-3,545	-3,29	-3,1670795	-2,8631237	-1,8441558
lmrA	1	-8,2313619	-8,2313619	-8,2313619	-8,2313619	-8,2313619	-8,2313619
lnuA	26	-6	-4,3776291	-3,8476912	-3,9238597	-3,5159762	-2,2783521
lnuC	24	-8,263635	-6,4175808	-4,6070531	-5,0548177	-4,0725676	-2,4221643
m1	33	-9,3364361	-4,6736641	-2,977268	-3,7881121	-2,5697474	-0,5493375
marR	24	-8,4377714	-5,8865797	-4,4831167	-5,1111517	-4,0721631	-2,9318141
matA	63	-8,9661145	-3,7730543	-3,4424928	-3,6549946	-2,6801942	-1,6185579
mdet11	8	-4,8354651	-4,0866969	-3,8986526	-3,9785206	-3,7261774	-3,4958677
mdtA	9	-9,7695518	-6,7114692	-4,8447377	-5,6509908	-3,8827287	-3,1463179
mdtEyhU	56	-7,4596753	-4,4101237	-3,7267907	-4,0854895	-3,2921318	-2,4638156
mecA	31	-7,920904	-6,5329057	-4,5228787	-4,7377975	-3,3734982	-0,2218488
mefA	120	-9,9251478	-3,7135486	-2,748603	-2,9429358	-1,5392058	-0,254625
mepA	63	-7,8865665	-3,8365972	-3,5199931	-3,7904286	-3,2034977	-1,4254439
merA	2	-7,2727273	-7,2485153	-7,2243033	-7,2243033	-7,2000914	-7,1758794
mexA	17	-8,7262964	-7,455932	-5,1824591	-5,470074	-3,7986288	-1,3
mexB	2	-6,6934673	-6,6672652	-6,6410631	-6,6410631	-6,614861	-6,5886589
mexE	38	-8,2629615	-5,5317143	-4,367695	-4,9469165	-3,9138445	-3,4596753
mexF	84	-6,1438756	-2,8493666	-2,220371	-2,5306468	-1,7572611	-1,2328441
mcr1	154	-8,4945434	-6,81646	-5,6887561	-5,6048708	-4,7296834	-2,0627351
mphA	108	-6,9657831	-4,3380631	-2,868606	-3,3589138	-2,4028707	-1,6193818
mphB	28	-8,9194889	-4,9600395	-4,4399778	-4,90916	-3,7739709	-2,5196337
mphC	32	-9,2153828	-4,4817915	-4,1935496	-4,2751543	-3,5788722	-2,4526788
msrE	44	-4,7859922	-3,4839494	-3,0471681	-2,9622431	-2,5808547	-0,1
msrA	19	-4,8624149	-4,5154085	-4,1549196	-4,0907898	-3,6648042	-3,3396151
msrC	30	-8,7746972	-5,6977763	-4,2770997	-4,8745868	-3,8229581	-1,2
mtrD	53	-9,7983176	-5,2757241	-3,7825723	-4,4779232	-3,3724661	-2,2848479
nimE	8	-4,8687542	-4,4689075	-4,3103563	-4,2391671	-4,0574653	-3,5767541
nisB	10	-5,9782568	-4,6195616	-4,2391418	-4,3495379	-3,7407879	-3,5342916
oleC	41	-7,6733467	-4,2424731	-3,329453	-3,9782998	-3,1356379	-2,5316527
oprD	78	-8,3272758	-4,9150927	-3,2420128	-3,7458889	-2,53695	-1,1296789

oprJ	85	-9,7983176	-3,6838786	-2,826258	-3,3195944	-2,3838499	-1,6126117
oqxA	12	-8,5	-8,1	-7,7	-7,6666667	-7,475	-6,4
oqxB	15	-5,7865613	-4,4782204	-3,971831	-3,6008572	-2,9599385	0,197158
pbp	10	-5,3120301	-4,09698	-2,4304932	-3,1089294	-2,3268078	-2,1295969
pbp5	12	-4,8094445	-4,2508616	-3,9366504	-4,0107935	-3,6955019	-3,4762535
pcoA	1	-0,2848365	-0,2848365	-0,2848365	-0,2848365	-0,2848365	-0,2848365
Pe	10	-4,1837541	-3,5878742	-3,4025898	-3,4220243	-3,0575072	-2,9356766
penA	38	-7,7986288	-6,4075	-4,4403721	-4,8199716	-3,6153585	-2,7746907
pica	23	-5,7431367	-4,9770035	-4,4249577	-3,9392326	-2,7211733	-2,3728929
pikR1	13	-9,3526979	-7,8865665	-7,733349	-6,35675	-4	-3,251812
pikR2	48	-7,6615436	-3,9482714	-3,5450468	-3,9798384	-3,2520815	-2,8416375
pmrA	9	-5,4727296	-3,8312798	-3,7632196	-3,9266196	-3,5852665	-3,1374944
pncA	49	-7,756962	-3,8144471	-3,2567356	-3,6334207	-2,8514444	-2,1778319
putitive.multidr ug	11	-4,2411998	-3,9001858	-3,4436975	-3,4870363	-3,0346401	-2,5844252
qacA	7	-8,6798537	-6,8291018	-4,4042612	-5,5860711	-4,3309733	-3,6982323
qacF	2	-6,5367965	-6,3950597	-6,2533229	-6,2533229	-6,1115861	-5,9698492
qacH	69	-8,2321238	-4,5256628	-3,2422246	-3,8369603	-2,563812	-1,6454398
qepA	32	-5,9389987	-3,0297479	-2,5613262	-2,5493383	-1,9930486	0,197158
qnrA	142	-8,3687529	-6,845024	-4,9638346	-5,0229518	-3,4740046	-1,1764706
qnrB	26	-5,6526316	-4,3501921	-3,4238688	-3,5862685	-2,8243778	-1,9705882
qnrD	41	-7,7	-6,4	-4,7835655	-4,763266	-2,9963613	-1,8878924
qnrS	393	-9,1	-5,0435554	-4,1	-4,1631106	-3,038961	0,47058824
rarD	22	-8,7522673	-4,0337549	-3,6006563	-4,1338242	-3,1947056	-2,5725607
rcnA	2	-7,4891775	-7,4560791	-7,4229807	-7,4229807	-7,3898823	-7,3567839
sat4	30	-4,7996539	-4,2991914	-3,641241	-3,6646016	-2,9332013	-2,1237822
sdeB	17	-7,8134291	-3,9389987	-3,7269987	-4,1828991	-3,4328898	-3,1415634
spcN	25	-8,9665762	-7,26984	-5,2695474	-5,49099	-3,9685915	-3,1242878
str	18	-4,4271284	-3,9057186	-3,3187413	-3,2537543	-2,6871732	-2
strA	75	-9,1446281	-4,2287413	-3,7260682	-3,4447449	-2,0908467	-0,3625103
strB	141	-8,6193469	-3,7958817	-2,8124793	-3,085025	-2,0869565	-0,5647059
sul1	765	-8,9	-3,1249387	-2,1788618	-2,218761	-1,227451	4,8
sul2	590	-9,1146388	-3,520734	-2,6721964	-2,6869709	-1,6696442	1,8260748
sul3	39	-5,5855379	-3,3331239	-2,8111366	-2,7216294	-2,0587677	0,4557973
sulA	34	-8,8398976	-6,4318696	-5,27	-5,7033514	-4,753757	-3,2328441
tet32	60	-4,7524423	-3,3637245	-2,8587124	-3,0098629	-2,5860722	-1,8631433
tet34	44	-9,1649439	-4,3320343	-3,8832	-4,272518	-3,5286535	-1,2656578
tet35	1	-4,5883829	-4,5883829	-4,5883829	-4,5883829	-4,5883829	-4,5883829
tet36	18	-4,8469236	-4,0949094	-3,8865665	-3,9235627	-3,6828237	-3,2141929
tet37	13	-8,7619539	-7,9546772	-4,457575	-5,1143805	-2,7271419	-1,3281819
tet38	1	-4,4259687	-4,4259687	-4,4259687	-4,4259687	-4,4259687	-4,4259687
tetA	385	-8,9578198	-3,4343434	-1,7687854	-2,0825628	-0,374125	1,85847704
tetB	156	-8,7958817	-4,0110132	-3,4067165	-3,3829141	-2,495229	0,48482513

tetD	53	-8,8153857	-6,5	-4,6259991	-5,084205	-3,8167316	-1,8436364
tetE	72	-8,7798919	-5,5018888	-4,0798467	-4,3997362	-2,9836115	-1,161435
tetG	378	-8,3936186	-3,1787495	-2,3714722	-2,2879638	-1,1872499	2,2008505
tetH	183	-9,8275433	-3,2227397	-2,024844	-2,2259687	-0,7593561	1,03981055
tetJ	4	-5,0042759	-4,2351824	-3,9590698	-4,1992026	-3,92309	-3,8743951
tetK	19	-8,5345977	-3,2017457	-2,7226473	-3,238397	-2,6025475	-1,7383996
tetL	86	-9,5898576	-4,0605076	-3,0799566	-3,4240705	-2,3787083	-0,1526718
tetM	427	-9,1191865	-4,494399	-3,2	-3,3500243	-2,164459	1,09221902
tetO	353	-10	-3,5271433	-2,2344892	-1,918661	0,33041377	2,15014216
tetPB	60	-8,7258422	-4,7	-3,8531806	-4,2325497	-3,3592	-2,671624
tetQ	326	-8,0712121	-2,8427497	-1,4428991	-1,5486061	0,58103895	2,23502316
tetS	51	-4,9643519	-4,1353584	-3,3492696	-3,3915836	-2,8998718	-1,0906948
tetT	32	-5,116127	-4,435937	-3,8360699	-3,570502	-3,2013359	-0,8396947
tetU	4	-8,6326279	-7,7772651	-7,480988	-7,1504514	-6,8541743	-5,0072016
tetV	5	-4,8354651	-4,5666736	-4,279847	-4,2100269	-4,139662	-3,2284867
tetW	332	-8,1747368	-3,9514789	-2,8229127	-3,0437981	-2,063991	0,67759664
tetX	276	-9,6612946	-3,764144	-2,5372043	-2,8407957	-1,9196489	0,70141674
tetZ	24	-5,9	-5,4	-4,3	-4,0233388	-2,8344077	-0,9281875
tet39	7	-3,99	-3,015	-2,19	-2,0971429	-1,375	0,28
tetC	112	-5,48	-3,6292797	-2,7723529	-2,560247	-1,6196716	0,63992522
tetPA	27	-4,2999069	-3,8704301	-3,5697823	-3,514727	-3,2457368	-2,5844252
tetR	90	-7,4692391	-4,5936188	-3,2876524	-3,7874239	-2,9514343	-1,6757175
tnpA1	95	-6,6056338	-4,3687145	-3,2319752	-3,0276395	-2,2577933	-0,1135376
tnpA2	69	-5,9685294	-3,7695518	-3,3391345	-3,2671155	-2,5624647	-1,1
tnpA3	63	-5,8459139	-3,6570035	-3,3122538	-3,0986786	-2,4440694	-0,465575
tnpA4	81	-4,6124482	-3,3788237	-2,552842	-2,5805286	-1,7466162	-0,7717788
tnpA5	72	-5,3982669	-3,6565714	-2,8824055	-2,7650906	-1,7945112	-0,7697893
tnpA6	34	-5,6221894	-3,974026	-3,0085936	-3,1572054	-2,6565282	-1,1691273
tnpA7	65	-5,1379625	-3,5354436	-2,7475389	-2,912904	-2,2718196	-1,5397139
tolC	54	-8,1396338	-4,1796297	-3,7339183	-4,0883324	-3,3678387	-2,5974748
Tp614	43	-6,1852119	-4,382708	-3,4122212	-3,4033446	-2,3296932	-0,8700133
ttgA	32	-8,733349	-4,8931103	-4,1810699	-4,8715991	-3,9296981	-3,1366771
ttgB	38	-8,6499685	-5,6875218	-4,411502	-4,9822019	-3,9419088	-2,9756554
uidA	5	-4,9	-4,6	-3,6	-3,66	-3,6	-1,6
vanA	26	-4,9691139	-3,2970107	-2,3896854	-2,3162074	-1,2550536	0,26245109
vanB	64	-7,8694587	-3,9544912	-3,5363188	-3,9753566	-3,1820009	-2,1545424
vanC	67	-7,8239875	-3,6301961	-2,8413215	-3,327126	-2,6240706	-2,1667916
vanG	3	-4,4881166	-4,3379915	-4,1878664	-4,218267	-4,0833422	-3,9788179
vanHB	42	-7,7544873	-4,1762167	-3,6889915	-4,1342044	-3,3535687	-2,1954289
vanHD	5	-8,4221643	-8,2564924	-7,9151498	-6,6300255	-4,3129996	-4,2433216
vanRA	9	-4,6259991	-4,3467875	-4,1493538	-4,062933	-3,9546772	-2,5668187
vanRB	9	-8,3142583	-7,7399286	-5,4107448	-6,0590979	-4,457575	-4,116983
vanRC	2	-5,5868778	-5,5104379	-5,433998	-5,433998	-5,3575582	-5,2811183

vanSB	25	-8,5917635	-4,6345122	-4,1943274	-4,8680501	-3,9625735	-2,3493354
vanSC	12	-9,5421181	-8,3015527	-4,77639	-5,9996954	-4,1147686	-3,65267
vanTC	24	-8,4156688	-7,3559786	-4,0727908	-5,233389	-3,8530716	-3,4528488
vanTE	2	-8,6736641	-7,4949164	-6,3161686	-6,3161686	-5,1374209	-3,9586731
vanTG	4	-8,6439741	-5,7072625	-4,6306355	-5,5545612	-4,4779342	-4,3129996
vanWB	4	-9,6675615	-5,8418956	-4,2529976	-5,3901932	-3,8012952	-3,3872161
vanWG	18	-4,9674841	-4,1820246	-3,7359799	-3,4458515	-2,7	-1,4
vanXA	2	-9,2276783	-9,0570475	-8,8864167	-8,8864167	-8,7157859	-8,5451551
vanXD	12	-5,6651271	-5,3954207	-5,0292321	-4,5799358	-3,6340027	-2,7159564
vanYB	10	-9,8413476	-9,0790905	-7,2620702	-6,9918029	-4,7254346	-4,1007987
vanYD	30	-8,4377714	-5,4297866	-4,0068331	-4,8102204	-3,676235	-3,2134935
vatB	2	-4,455932	-3,9143668	-3,3728017	-3,3728017	-2,8312366	-2,2896714
vatC	6	-4,6259991	-4,5489226	-4,3896479	-4,4143419	-4,275569	-4,2411998
vatD	2	-3,8312798	-3,6693995	-3,5075193	-3,5075193	-3,345639	-3,1837588
vatE	39	-9,7258422	-5,7627968	-4,279847	-4,7755884	-3,3557418	-1,3129996
vgaA	13	-9,9691139	-7,6989743	-4,6478175	-5,8281677	-4,1549196	-3,3952341
vgaB	23	-7,9318141	-6,3450359	-4,3129996	-4,8974519	-3,8535059	-2,6363882
yceEmdtG1	14	-4,9691139	-3,8268428	-3,7181892	-3,5742862	-2,9839538	-2,5474412
yceLmdtH1	38	-8,4449555	-4,1769623	-3,7194468	-4,3053937	-3,413565	-2,6516951
yceLmdtH2	17	-4,935294	-4,5666736	-4,123327	-3,9623303	-3,6458916	-2,649545
yceLmdtH3	24	-4,4881166	-4,2220775	-3,7596053	-3,7089243	-3,3075991	-2,6615435
yidYmdtL1	37	-8,9829667	-6,3891858	-4,1995384	-4,9366458	-3,756962	-2,7694444
yidYmdtL2	10	-4,4723799	-4,3190911	-3,7537676	-3,6491411	-3,2134537	-2,4334002

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Table S3. Results of the LMM models for all the data points and for maximum values only. Year was set as a fixed factor and Country as a random factor.

Environment	Effect size	p-value
biofilm	4,99E-01	
effluent	1,28E-01	0.0657
feces	1,06E+00	0.224
influent	1,16E-01	0.187
manure	3,90E-02	0.509
sediments	-1,10E-02	0.873
sludge	-3,20E-02	0.710
soil	1,66E-01	0.00566
treated wastewater	-3,96E-01	0.0427
wastewater	-2,60E-01	0.239
water	4,90E-02	0.641

Table S4. ARGs associated with certain environments (only results which are significant after FDR correction). A is the probability that a sample is in the target group (=environment) and B is the probability that you will find the gene in a sample that belongs to this specific environment. Stat is the Indicator value is maximum (i.e. 1) when all occurrences of a gene are found in a single environment and in all samples of this environment.

Multilevel pattern analysis						
Environment	ARG	A	B	stat	p.value	sign
air	ermX	0.6531	0.8929	0.764	0.000999	***
air	ermC	0.4964	0.7857	0.625	0.000999	***
air	vanB	0.5820	0.5714	0.577	0.000999	***
air	tet32	0.5739	0.5714	0.573	0.000999	***
air	erm36	0.6534	0.5000	0.572	0.000999	***
air	blaGES	0.5228	0.3571	0.432	0.001998	**
air	tetS	0.4605	0.3571	0.406	0.000999	***
air	erm35	0.5465	0.2143	0.342	0.005994	**
manure	aphA1	0.4921	0.3158	0.394	0.00200	**
manure	aadA9	0.4777	0.2895	0.372	0.00400	**
manure	aadA5	0.3539	0.3158	0.334	0.00899	**
sediments	yceLmdtH 1	0.6334	0.1429	0.301	0.017	*
sewage	mecA	0.7788	0.7500	0.764	0.000999	***
sewage	blaNDM	0.4203	0.2500	0.324	0.010989	*
air+manure	tnpA1	0.6198	0.5303	0.573	0.000999	***
air+manure	tetL	0.4769	0.2879	0.371	0.004995	**
air+sediments	mexF	0.5955	0.2258	0.367	0.004	**
air+sewage	blaCMY2	0.6082	0.3333	0.45	0.000999	***
air+treated ww	ermA	0.7223	0.3750	0.52	0.000999	***
biofilm+sewage	blaKPC	0.8598	0.4655	0.633	0.000999	***
biofilm+soil	mphC	0.89453	0.09898	0.298	0.017	*

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effluent+influent	cfxA	0.8121	0.1517	0.351	0.005	**
feces+sewage	mcr1	0.8190	0.4349	0.597	0.000999	***
influent+sediments	acrA5	0.76078	0.16974	0.359	0.004	**
influent+sediments	mepA	0.59926	0.15498	0.305	0.020	*
manure+sediments	tetR	0.6265	0.2159	0.368	0.003	**
manure+sediments	blaL1	0.9229	0.1057	0.312	0.011	*
manure+sewage	aphA3	0.6397	0.2609	0.409	0.002	**
manure+treated ww	strB	0.5107	0.4800	0.495	0.000999	***
manure+treated ww	strA	0.5850	0.3000	0.419	0.001998	**
manure+wastewater	intI2	0.7724	0.1471	0.337	0.012	*
sediments+sludge	aacC4	0.6827	0.1095	0.273	0.021	*
sediments+treated ww	aadA1	0.6307	0.4129	0.51	0.000999	***
treated ww+wastewater	qnrD	0.5958	0.2381	0.377	0.002	**
air+sediments+soil	oprD	0.8450	0.1543	0.361	0.00699	**
air+sewage+treated ww	ermF	0.6630	0.6458	0.654	0.000999	***
manure+sediments+soil	qacEdelta	0.7794	0.2000	0.395	0.003	**
manure+sediments+wastewater	aadA2	0.6632	0.2451	0.403	0.000999	***
manure+treated ww+wastewater	dfrA1	0.5565	0.2500	0.373	0.002	**
air+influent+sediments+soil	fox5	0.90445	0.12915	0.342	0.00999	**
air+influent+sediments+wastewater	aac6II	0.7777	0.1277	0.315	0.016	*
effluent+influent+manure+sludge	mefA	0.7200	0.2335	0.41	0.003	**
food+influent+sediments+sludge	acrA4	0.91595	0.11512	0.325	0.012	*
influent+manure+sediments+sludge	tnpA2	0.8072	0.1396	0.336	0.00999	**
influent+manure+sediments+sludge	blaOXY1	0.7582	0.1244	0.307	0.01598	*
influent+sediments+sludge+soil	mphA	0.8813	0.1636	0.380	0.00200	**
influent+sediments+sludge+soil	aacC	0.9006	0.1169	0.324	0.00599	**

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Global AMR Prevalence

influent+sediments+sludge+wastewater	tnpA7	0.8550	0.1399	0.346	0.00599	**
influent+sewage+treated ww+wastewater	blaOXA58	0.7708	0.1894	0.382	0.002	**
air+influent+sediments+sludge+soil	oprJ	0.9056	0.1228	0.333	0.00799	**
food+influent+manure+sediments+sludge	tnpA5	0.8363	0.1164	0.312	0.021	*
influent+manure+sediments+sludge+soil	blaSFO	0.9141	0.1115	0.319	0.013	*
air+influent+sediments+sewage+sludge+wastewater	blaOXA1	0.8080	0.1611	0.361	0.00799	**
air+biofilm+food+influent+manure+sediments+soil	blaampC	0.9005	0.1060	0.309	0.012	*
biofilm+effluent+feces+food+influent+sediments+wastewater	qnrA	0.8600	0.1293	0.333	0.013	*
biofilm+effluent+influent+sediments+sewage+treated ww+wastewater	blaCTXM	0.7535	0.3800	0.535	0.000999	***

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Table S5. Comparison of the top most reported and abundant ARGs to existing lists of suggested monitoring targets. Genes in red are already included into clinical monitoring schemes.

Antibiotic class	Berendonk et al., 2015	Bengtsson-Palme 2018	Most reported in the current data	Abundant but not often reported
Integrases	intl1		intl1	
Sulfonamide	sul1 , sul2		sul1 , sul2	sul3
Beta-lactamases	blaTEM , blaCTX-M , blaNDM-1 , blaVIM , blaKPC	blaTEM	blaTEM , blaCTX-M , blaSHV	
	mecA			
Fluoroquinolone	qnrS		qnrS	
Aminoglycoside	aac(6')-Ib-cr	aac(6')-Ib		
	aph	aph(6)-Id		
			strB	
			aadA1	aadA2
Vancomycin	vanA			vanA
MLSB	ermB , ermF	ermC	ermB , ermF	
				ereA
Tetracycline	tetM	tetM , tetQ, tetW, tetC, tetA, tetO,	tetM , tetQ, tetW, tetC, tetA, tetO, tetX, tetG, tetB	tetH
Transposase				tnpA4, tnpA5, IS613
Chloramphenicol				floR
Multidrug				mexF
				qacEdelta