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Biogeography and diversity patterns of antibiotic resistome in the sediments of global lakes

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ARTICLE INFO

Article history:

Received 26 April 2022

Revised 11 June 2022

Accepted 15 June 2022

Available online 23 June 2022

Keywords:

Antibiotic resistance genes

Biogeography pattern

Resistome diversity

Ecological processes

Metagenomic assembly-based analysis

ABSTRACT

Lakes act as one of the reservoirs and dispersal routes of antibiotic resistance genes (ARGs) and pathogenic resistant bacteria in aquatic environments. Previous studies reported the occurrence and distribution of ARGs in lakes worldwide; however, few investigated the biogeography and diversity patterns of antibiotic resistome in the environment. To fill this gap, a large-scale data set of sediment metagenomes was collected from globally distributed lakes and characterized comprehensively using metagenomic assembly-based analysis, aiming to shed light on the biogeography and diversity patterns of ARGs in lake ecosystems from a global perspective. Our analyses showed that abundant and diverse ARGs were found in the global lake sediments, including a set of emerging ARGs such as *mcr*-type and carbapenem-resistant *Enterobacteriaceae* related genes. Most of the identified ARGs were generally associated with the commonly used antibiotics, suggesting the role of increasing antibiotic consumptions on the resistome prevalence. Spatially, the composition and diversity of ARGs varied across geographical distances and exhibited a scale-dependent distance-decay relationship. Notably, the composition of ARGs was largely shaped by bacterial community structure, and their diversities were co-governed by stochastic process (~48%) and deterministic process (~52%). Findings provide a valuable insight to better understand ecological mechanisms of ARGs in lake ecosystems and have important implication for the prevention and control of resistome risk.

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Introduction

The overuse and/or misuse of antibiotics for human medicines and veterinary therapies/promoters in the past decades have accelerated the widespread emergence and dissemination of antimicrobial resistance (AMR) which has

been one of the global issues to public health (Zhang et al., 2011; Pehrsson et al., 2016; Cassini et al., 2018). It is estimated that pathogenic resistant bacteria has caused more than 700,000 deaths yearly and, more importantly, the AMR challenge will get worse if we cannot efficiently control the growing occurrence and propagation of antibiotic resistome (O'Neill, 2016; Van Boeckel et al., 2019). To date, AMR has been addressed not only in medical settings but also extends to various natural ecosystems (i.e., rivers and lakes) since environmental systems contribute to the evolution, development

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and dissemination of AMR (Berendonk et al., 2015; Yang et al., 2019). In a report of United Nations Environment Program (UNEP, 2017), AMR in environments has been listed as the first of six emerging issues of environmental concern. Also, there has been an increasing consensus on the need to address AMR in environments from the perspective of “One Health and Global Health” (Hernando-Amado et al., 2019).

In terms of antibiotic resistome in environments, a major concern is the dissemination of ARGs across geographical distances given their emergences in a specific ecosystem (Martínez et al., 2015; Hernando-Amado et al., 2019). For instance, *mcr-1*, a plasmid-mediated mobile colistin gene, was first described in China in April 2011 and subsequently reported in numerous countries across five continents (Liu et al., 2016; Wang et al., 2018); *sul4*, a newly discovered fourth mobile sulfonamide resistance gene, was already widespread in Asia and Europe when it was reported (Razavi et al., 2017). In particular, to make effective strategies to reduce resistome risks, it is necessary to explore the biogeographical features of ARGs for better understanding their ecological processes and dispersal dynamics in environments (Hu et al., 2020). Recently, several researches have discussed the ecological processes involved in resistome assembly. Peng et al. (2020) found that deterministic processes played a more important role in ARG assembly in rivers with higher urbanization levels. Also, Chen et al. (2021) showed that homogenous diffusion, homogenous selection and ecological drift were the dominant factors of ARG profile in lake sediments.

Lakes are the typical freshwater ecosystems and play a critical role in the provision and management of surface water resources. However, they are also the main environmental receptors receiving millions of tons of treated and/or untreated wastewater from various pollution sources (Chen et al., 2019; Wang et al., 2020). Since lakes harbor natural assemblages of bacteria that may allow wastewater-derived ARGs to persist, they accordingly appear to be an ideal environment to aggregate and disseminate antibiotic-resistant bacteria (ARB) and ARGs (Czekalski et al., 2015). Particularly, long retention times of ARB in lakes may increase chances of horizontal gene transfer (HGT) of ARGs in bacterial communities, resulting in potential risk to ecosystem and public health (Yang et al., 2018; Chen et al., 2020).

Although previous studies reported the occurrence, abundance and distribution of ARGs in some local lakes (Bengtsson-Palme et al., 2014; Czekalski et al., 2015; Liu et al., 2018; Yang et al., 2018; Stange et al., 2019; Wang et al., 2020), few investigated the biogeography and diversity patterns of ARGs in lake ecosystems from a global perspective. Until now, little is known about whether the large-scale distribution of ARGs in lakes presents a spatial biogeography distinction, and what ecological mechanisms (i.e., deterministic and stochastic processes) determine the ecology process of ARGs in lake ecosystems. In the present study, a meta-analysis has been implemented using a large-scale data set of sediment metagenomes collected from globally distributed lakes, aiming to advance our understanding on the biogeography and diversity patterns of antibiotic resistome in lake ecosystems by addressing a set of questions: (i) whether the composition and diversity of ARGs vary spatially across geographical distances? (ii) are the biogeography patterns observed for

ARGs? (iii) what are the ecological processes underlying the diversity of ARGs? Additionally, we also characterized the bacterial communities and their associations with ARGs in the environment.

1. Materials and methods

1.1. Data collection

The metagenome data was obtained by using the search term “lake sediment” and “metagenome” to retrieve the required data from the National Center for Biotechnology Information (NCBI) Sequence Read Archive. We referred to Li et al. (2018) to filter the data set and totally collected 123 qualified metagenomes of lake sediments (Appendix A Table S1). These sediment samples were from globally distributed lakes covering 14 countries and spanning 6 continents (Fig. 1a), including Africa ($n = 5$), Asia ($n = 59$), Europe ($n = 13$), North America ($n = 78$), South America ($n = 7$) and Oceania ($n = 4$). Since limited datasets were found in China, one of the world’s largest producers and consumers of antibiotics, two sampling events were conducted from one southern lake (Lake Tai) and one northern lake (Lake Baiyang) and 43 sediment metagenomes were obtained. Lake Tai is the third largest freshwater lake in China, while Lake Baiyang is the largest shallow fresh water lake in northern China. Details on the sample collection, DNA extraction and metagenomic sequencing can be found in our previous studies (Chen et al., 2019, 2020). In all, a total of 166 lake sediment metagenomes were used for subsequent analyses (Appendix A Table S2) and they were both generated using shotgun metagenomic sequencing by Illumina technology.

1.2. Metagenomic assembly and open reading frame prediction

For each metagenome, the raw sequences were quality-filtered for removing the low-quality reads using FASTP (v0.19.5), and the clean reads were *de novo* assembled using MEGAHIT (v1.1.2) with default parameters. The assembled scaffolds (> 500 bp) were used to predict open reading frames (ORFs) using MetaProdigal (v2.6.3) with parameter ‘-p meta’ and the predicted ORFs were clustered to generate a non-redundant ORF set using CD-HIT (v4.6.8).

1.3. Annotations of resistome and microbiome

Information on the annotations can be found in our previous study (Chen et al., 2021). Briefly, to annotate ARGs, the predicted protein sequences of ORFs were searched against the ResFinder database using DIAMOND (v2.0.9) with an E-value of $1e-10$. For taxonomic annotation, Kaiju (v1.6.3) was used to search the ORFs against the proGenomes database with default parameters (Shoemaker et al., 2017).

1.4. Phylogenetic analysis of the shared ARGs

Phylogenetic analysis was conducted on the *sul4* gene which was shared by all lake sediments with high abundance. Firstly,

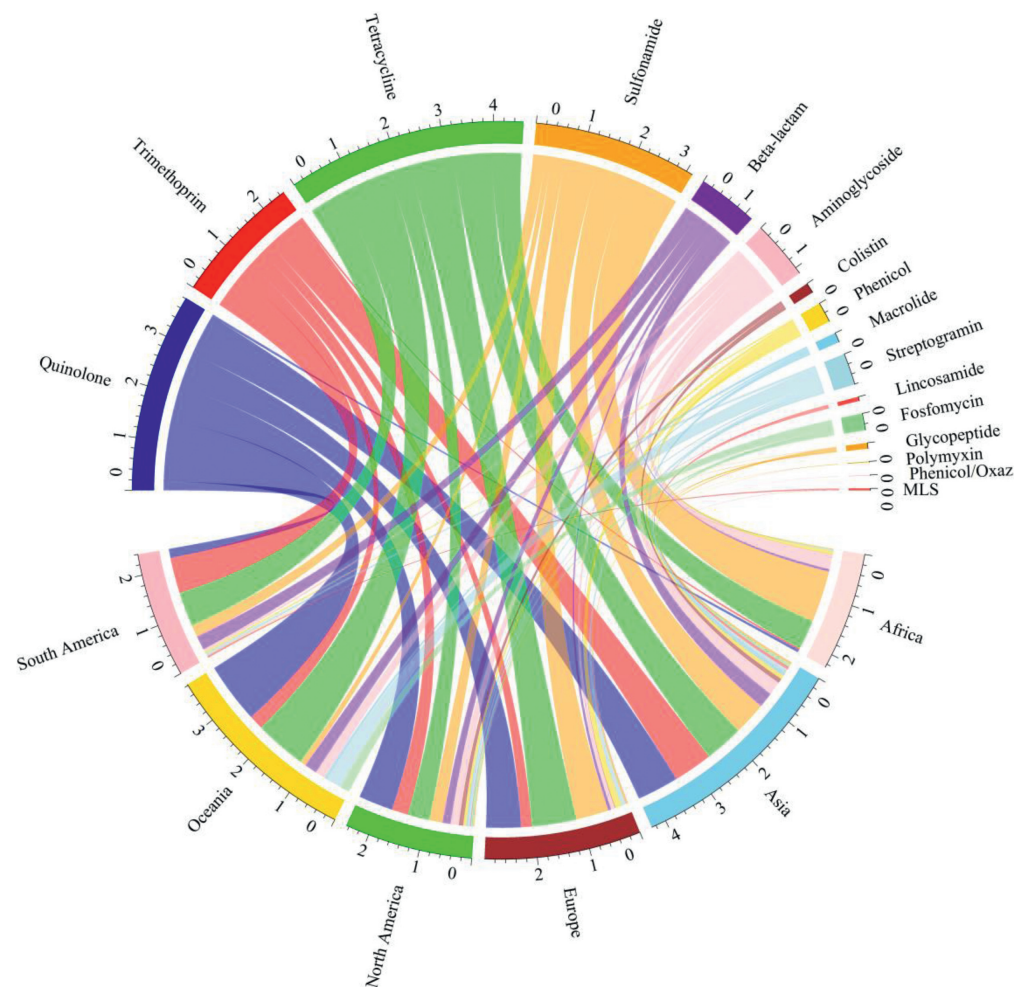


Fig. 1 – Chord diagram showing the profile of ARGs in the lake sediments; The numbers around the circumference represent the average coverage of the ARG type (x /Gb). Oxaz: Oxazolidinone; MLS: macrolide/lincosamide/streptogramin.

the predicted ORFs were searched against the reference *su14* sequences using the DIAMOND with a cutoff of $\geq 65\%$ similarity, and the resulting alignments were clustered using the CD-HIT with a threshold of 95% similarity and 90% coverage. Then, the representative sequences were aligned using MAFFT (v7.427) and trimmed with BMGE (v1.1). Finally, the phylogenetic tree was constructed based on the maximum-likelihood method using IQ-TREE (v1.6.8) with the best-fit model selected automatically by ModelFinder (Kalyaanamoorthy et al., 2017), and visualized using FigTree (v1.4.4).

1.5. Biogeography and diversity analyses of ARGs

1.5.1. Distance-decay relationship

To understand the biogeographical pattern of resistome in the lake ecosystems, we evaluated the distance-decay rate (DDR) of ARGs based on the Bray-Curtis similarity between samples and the geographic distance matrix, using linear least squares regression (Fierer and Jackson, 2006; Liu et al., 2018). Among them, the geographic distance matrix was constructed using Haversine formula with the latitudinal and longitudinal coordinates of sampling sites (Martiny et al., 2011). The matrix

permutation test (999 times) was used to check the statistical significance of the distance-decay curve slope by comparing the observed slope with the distribution of values in the permuted datasets, and the overall distance-decay slope was compared with the ratios of distance-decay curves at three spatial scales (local scale: 0-100 km, regional scale: 100-5,000 km and global scale: 5,000-25,000 km) (Wu et al., 2019).

1.5.2. Richness distribution pattern

Species abundance distribution (SAD), a universal tool in biodiversity theory to describe how many individuals of each species/genes are present in a community (Shoemaker et al., 2017), was employed to understand the richness distribution of sediment ARGs in lake ecosystems. In this study, five SAD models, including Broken-stick, niche preemption, Poisson lognormal, Zipf and Zipf-Mandelbrot, were tested to predict the SAD of each sample based on the rank-abundance distribution of ARGs. The percentages of variations in richness among genes explained by each SAD model were inferred by comparing the predicted and observed SADs with regression analysis (Wu et al., 2019).

1.5.3. Ecological processes underlying the diversity of ARGs

Recently, several biodiversity indices have been proposed to determine whether the variations of species/genes among different spatial sites are caused by deterministic or stochastic processes (Zhou et al., 2014), such as standard effect size, selection strength (SS), β_{RC} , RC_{Bray} , β_{NRI} and β_{NTI} (Stegen et al., 2012, 2013; Zhou and Ning, 2017; Ning et al., 2020). In the study, we evaluated the stochasticity in controlling the diversity of ARGs using a novel null-model-based stochasticity index (normalized stochasticity ratio, NST). Generally, null-model-based index is obtained with null-model algorithms based on the hypothesis that samples within each region are regarded to be sharing the same regional species pool (Martiny et al., 2011; Zhou et al., 2014; Wu et al., 2019). Study showed the novel NST index had considerably higher accuracy and precision than other indices (i.e. SS, standard effect size and stochasticity ratio) for the major similarity metrics (Ning et al., 2019).

1.6. Statistical analyses

Differences in the relative abundance and alpha-diversity of ARGs between regions were analyzed using Wilcoxon test and Kruskal-Wallis one-way analysis of variance. Non-parametric multivariate analysis of variance (Adonis) and principal coordinate's analysis (PCoA) were performed to investigate the differences in Bray-Curtis similarity of ARG profiles between sites. Mantel test and PCoA-based Procrustes analysis were used to reveal the association of ARGs with microbial communities. All statistical tests were significant at p -value < 0.05 and conducted in R (v3.6.0) with several packages, such as vegan, pheatmap, plyr, hmisc and gunifrac (<https://cran.r-project.org/>). In all analyses, the matrices of species/genes were Hellinger-transformed (Munk et al., 2018). The p -values were adjusted using Benjamini & Hochberg false discovery rate (FDR) procedure to reduce the false-positive results. All figures were drawn using ggplot (v 3.2.0) if not indicated.

2. Results

2.1. Characterization of antibiotic resistome in the lake sediments

In the study, a large-scale metagenomes were employed for characterizing the prevalence of antibiotic resistome in the sediments of globally distributed lakes (Appendix A Fig. S1a). Totally, more than 1.8 Tb metagenomic data was analyzed, obtaining 70.6 million non-redundant genes to annotate ARGs (Appendix A Table S2). Among them, about 0.024% sequences of the predicted ORFs were assigned to resistance genes, resulting in 326 unique acquired ARGs presumably conferring resistance to 16 antimicrobial classes across all samples (Appendix A Fig. S1b). Notably, the detectable genes represented almost all major mechanisms of antibiotic inactivation and efflux, including enzymatic inactivation (21.1%), enzymatic modification (9.5%), target modification (5.4%), target protection (6.9%) and efflux pumps (57.1%). When summing the coverage of ARGs to their corresponding antimicrobial class, quinolone, tetracycline, sulfonamide and trimethoprim resistant genes were identified as the top most domi-

nant ARG types, whereas aminoglycoside and beta-lactam resistant genes were relatively rare, followed by streptogramin, phenicol and other kinds of ARGs (Fig. 1). Correlation analysis showed the relative abundances for the top 3 classes (quinolone, tetracycline and sulfonamide) were significantly correlated with the total ARG coverage (Pearson's coefficient: 0.595~0.789, $p < 0.01$), approximately occupying 60% of the overall ARGs across all samples.

Of the detected 326 ARGs, 14 genes (~4.6%) were found across geographical regions (Appendix A Fig. S2). Among them, genes encoding *oqxB* (quinolone), *dfrA3* (trimethoprim), *sul4* (sulfonamide), *tetB(46)* and *tetB(60)* (tetracycline) were the top 5 most dominant ARGs and further identified as the core resistant genes since they were detected in at least 75% samples, accounting for 56.9%~70.0% to the total ARG coverage. Interestingly, the five core genes could also be used as the indicator to predict the prevalence of ARGs in the lake sediments because their relative abundances were strongly and significantly correlated with the total ARG coverage (Pearson's coefficient: 0.580~0.687, $p < 0.01$).

Additionally, the *sul4* gene, which was first reported on the integron amplifications of mobile gene cassettes in 2017 from river sediment in Indian (Razavi et al., 2017), was widely detected in the global lake sediments with high abundance (Appendix A Fig. S3). In particular, the gene showed significantly more abundant in the lakes from India, Romania, and Rwanda than other countries ($p < 0.05$, Kruskal-Wallis one-way ANOVA). Relatively, lakes from Indonesia and China had intermediate levels, whereas lakes from Oceania, South America and North America presented the lowest levels. Indeed, a complex phylogenetic relationship for the detected *sul4* sequences was found in the global lakes (Appendix A Fig. S4). A midpoint-rooted parsimony phylogenetic tree showed that there was a dominant type with subsequent diversification, implying the ancestral form of the gene, while the discernible clustering of *sul4* by sample sources in some clades suggested different evolution of the gene in different backgrounds.

2.2. Biogeographical pattern of antibiotic resistome in the lake sediments

To reduce the effect of sample evenness in this study, we mainly concerned the differences of ARGs between Asia ($n = 59$) and Normal America ($n = 78$). As shown in Fig. 2a-c, the total coverage and alpha diversity (Simpson index and subtype number) of ARGs from Asian samples were significantly higher than those from North American and other geographical locations ($p < 0.05$, Kruskal-Wallis one-way ANOVA), indicating that Asia had a higher coverage of ARGs but also a more abundant distribution of the different ARGs. Exception was shown in the high mountain Andean lakes from South America with low coverage of ARGs but high diversity.

With regard to the composition of ARGs, differences were also found across geographical regions (Appendix A Fig. S5). For example, the Asian samples were evenly distributed by the quinolone, tetracycline, sulfonamide and trimethoprim resistance genes, while the North America samples had a high ratio for the quinolone and tetracycline resistance genes. The Lake Kivu in Africa was dominated by sulfonamide resistance genes followed by tetracycline and aminoglycoside, while the

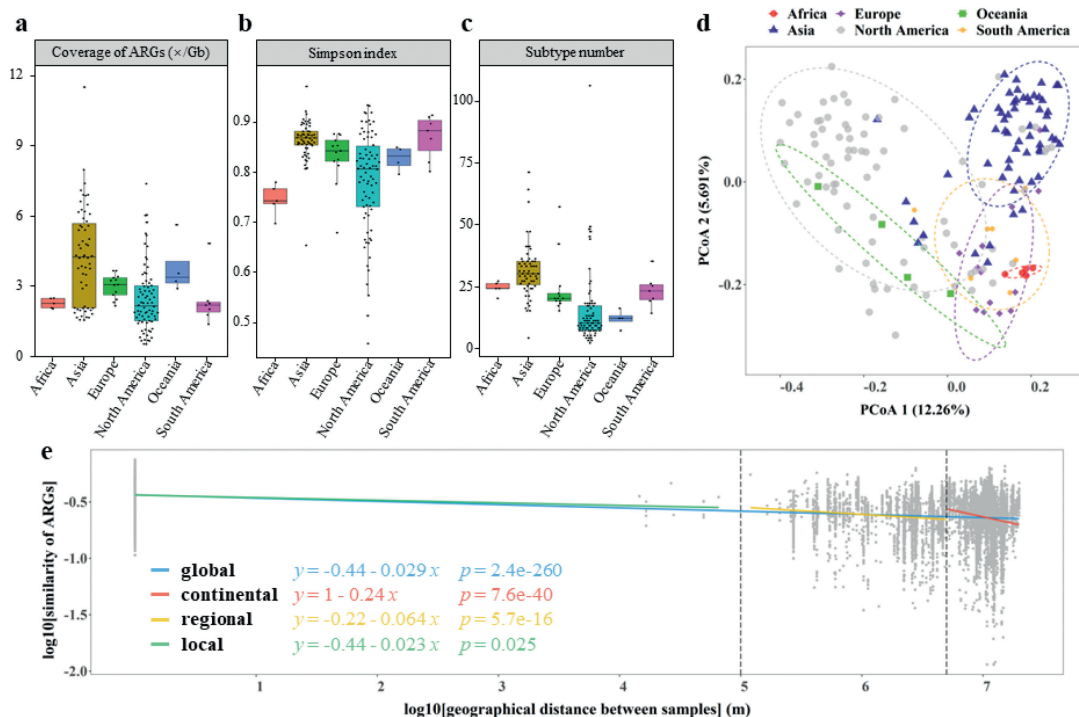


Fig. 2 – Spatial difference and biogeography pattern of ARGs in the lake sediments. a. Box plots showing the total ARG coverage per sample, stratified by region; b. ARG diversity across regions; c. ARG subtype number across regions. d. Resistome clustering in the sediment samples across regions using principal coordinate analysis (PCoA); e. Distance-decay relationship between log-transformed geographical distances vs log-transformed ARG similarity (Bray-Curtis).

high mountain Andean lakes in South America presented a large proportion of genes providing resistance to tetracycline and trimethoprim. In terms of beta-diversity, as shown in Fig. 2d, clustering of the ARGs was observed with a geographical separation despite that some samples from Oceania and other regions were included in the group of North America. Furthermore, we tested the geographical effect and found it to be significant (Adonis test, $\text{adj.}p = 0.001$) even though the geographical groupings only explained 24.7% of dissimilarity among the resistomes. Also, a clearer separation of all samples was found in the three groups (Africa/South America, Europe/North America/Oceania and Asia), which was confirmed by pairwise Adonis test ($p < 0.01$ for each pair) (Appendix A Fig. S6). The separation in the three groups was mainly associated with the composition of the ARGs in different regions, which might be related to the region/country-specific consumption pattern of antibiotics, and heterogeneous management effect such as antimicrobial use regulations and environmental policies (Klein et al., 2018; Munk et al., 2018).

Remarkably, a significant scale-dependent distance-decay relationship (DDR) was found in our analysis, that is, the similarity of ARGs decreased with increasing geographical distances (Fig. 2e), suggesting that bacterial ARGs had a biogeographical differences. In line with the results reported by previous investigations (Fierer and Jackson, 2006; Martiny et al., 2011; Wu et al., 2019), the slopes of DDRs in this study depended on spatial scale that they varied over local, regional and global scales. The significant negative DDRs (slope = -0.03 for Bray-Curtis, -0.038 for Sorensen and -0.044 for Can-

berra distance; $p < 0.01$) were observed across all spatial scales ($n = 13,695$ pairwise distances). Relatively, the slope of DDRs across continents (slope = -0.32 for Bray-Curtis, -0.38 for Sorensen and -0.28 for Canberra distance, $n = 8,525$ pairwise distances) was significantly steeper (more than six times) than the overall slopes for all similarity metrics.

2.3. Deterministic and stochastic processes underlying the resistome diversity

The richness distribution pattern of ARGs was checked by testing the five commonly used species abundance distributions (SAD) models. Fig. 3a shows the predicted abundance distribution of ARGs in the lake sediments for each model fitting to the observed SAD. It can be seen that the Poisson model, a log-normal model with Poisson-based sampling error, showed the best fit to the observed SAD and explained 93.3% of the variation of the abundance distributions of ARGs, compared with 90.0% for Broken-stick, 83.9% for Zipf-Mandelbrot, 88.7% for Zipf and 89.4% for the niche preemption models. The Poisson lognormal was also characterized by lower standard deviation than the other four SAD models.

Furthermore, we assessed the deterministic and stochastic mechanisms governing the diversity pattern of ARGs using a novel null model-based normalized stochasticity ratio (NST). Since the null-model algorithms generally require a high number of replicates, we mainly focused on the Asian and North American samples in the study. Herein, the NST values were calculated using the typical null-model algorithms

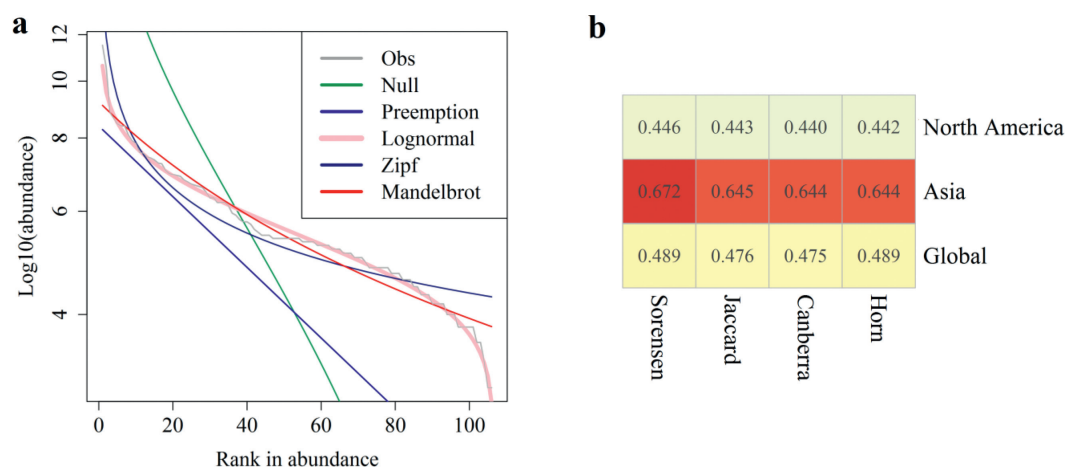


Fig. 3 – Ecological processes underlying the diversity of ARGs in the lake sediments. a. Predicted diversity distribution pattern of ARGs for five commonly used species abundance distribution (SAD) models fitting to the observed distribution in rank abundance form, that is, ordered from the most abundant genes to the least abundant on the x-axis; **b.** Average stochasticity ratio governing the diversity of ARGs, which is calculated with four incidence-based similarity metrics using a null model-based normalized stochasticity ratio.

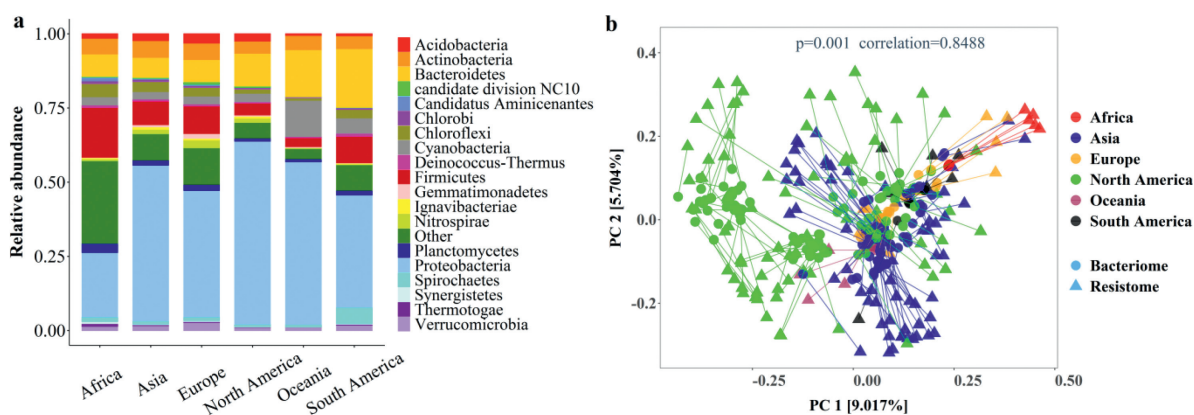


Fig. 4 – Distribution of bacterial community and its association with ARGs in the lake sediments. a. Phyla-level distributions. **b.** Procrustes analysis showing the correlation of bacterial community at genus level with ARGs.

with four incidence-based metrics (Jaccard, Sorensen, Canberra and Morisita-Horn) because the NST index had high accuracy and precision (>0.99) with incidence-based metrics (Ning et al., 2019). As shown in Fig. 3b, the average stochastic ratios in Asian samples were higher than those in North American samples. Notably, the stochastic ratios in Asian were higher than 0.5, resulting in stochastic factors dominating ARGs in the region. Regarding all samples, the average stochastic ratios with four incidence-based similarity metrics were close to 50%, indicating that deterministic and stochastic factors co-governed the ARG diversity at global scale.

2.4. Bacterial communities and their association with ARGs in the lake sediments

Based on the taxonomic annotations, a total of 49 bacterial phyla and 1370 genera have been detected across all samples. *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Cyanobacteria* were the top most bacterial phyla (Fig. 4a

and Appendix A Fig. S7). At lower taxonomic ranks, more than 4700 species were assigned, and the top most abundant species over-represented in the phylum of *Proteobacteria*. Comparatively, significant differences of the richness (α -diversity) and composition (β -diversity) at both high (phylum) and low (species) taxonomic ranks were found among different geographical regions (Adonis test, $\text{adj.}p=0.001$). Relatively, bacterial diversity was highest in Asia and lowest in North America. *Thiobacillus*, *Anaerolinea*, *Pseudomonas*, *Geobacter* and *Candidatus Accumilibacter* were the top 5 most abundant genera in Asian samples, whereas *Acidovorax*, *Methylobacter*, *Flavobacterium*, *Methylothera* and *Methylophilus* showed higher relative abundance in North America.

Then, Procrustes analysis was used to check the degree to which the bacterial structures dictate the ARG profiles. As expected, significant correlations were observed between the ARGs and the bacterial communities (correlation: 0.85, $p = 0.001$, Fig. 4b), suggesting the lake sediments with similar bacterial structure tended to have similar profile of ARGs.

The strength of associations between bacterial communities and ARGs were different between geographical regions, for example, the corresponding correlations were 0.91 and 0.86 for Asian and North America samples, respectively.

Further, we investigated the co-occurrence pattern of ARGs and bacterial communities based on the assembled contigs. Here, the taxonomies of contigs carrying ARGs (ACCs) were determined using a voting mechanism (Ma et al., 2017). At the species level, 2596 bacterial species (55% of the total annotated species) were assigned on the ACCs across all samples, predominantly affiliating with quinolone and tetracycline resistant genes (Appendix A Fig. S8). Among them, *Anaerolinea thermophila*, *Desulfobacca acetoxidans*, *Silanimonas lenta*, *Syntrophobacter fumaroxidans*, *Thiobacillus denitrificans*, *Nitrospira defluvia*, *Singulisphaera acidiphila*, and *bacterium JKG1* were the top most abundant species annotated on the ACCs. Relatively, quinolone resistance genes were found to be mainly carried by *Silanimonas lenta*, *Desulfobacca acetoxidans* and *Syntrophobacter fumaroxidans*, while *Anaerolinea thermophile* was identified as the major host of tetracycline-ARGs. Notably, 121 bacterial pathogen species were assigned on the ACCs, including a number of human bacterial pathogens (HBPs) listed as the WHO priority concerns and/or the top most frequently isolated strains of clinically relevant antibacterial resistance (Hu et al., 2018; Tacconelli et al., 2018), such as *Mycobacterium tuberculosis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Stenotrophomonas maltophilia*.

3. Discussion

Metagenomic assembly analysis of a large-scale shotgun sequencing data set was conducted to investigate the biogeography and diversity patterns of ARGs in global lake sediments. Totally, 326 acquired ARGs conferring major types of commonly used antibiotics (i.e., sulfonamides, tetracyclines and quinolones) were found across all samples collected from worldwide, indicating the selection driven by the globally increasing antibiotic consumptions (Klein et al., 2018). In relative, the highest levels of ARGs were found in Lake Lonar and Lake Kazipally from India. Not strangely, the Lake Kazipally was reported to be polluted by exceptionally high levels of antibiotics from pharmaceutical production (Karkman et al., 2019), and the high abundance of ARGs in this environment suggested the role of human activities for resistance development and evolution, since the residual antibiotics could exert continuously selective pressure on microorganisms to evolve into resistance via mutation and/or acquire ARGs through horizontal gene transfer (Bengtsson-Palme et al., 2014). Similarly, several studies also showed human activities had altered the distribution and magnitude of ARGs in environments due to the increasing discharge of wastewater containing a diverse mixture of antibiotics and other co-selective agents (i.e., metals and biocides) especially in the developing countries (Jones et al., 2008; Marti et al., 2014; Zhu et al., 2017).

Remarkably, a set of emerging ARGs were found in the global lakes, including *optrA*, *tet(X)*, *mcr-type* and their variants, and an array of carbapenem-resistant *Enterobacteriaceae* related genes (i.e. *KPC* and *OXA-type*), as well as *sul4* mentioned above. The *optrA*, a newly characterized enterococcal

resistant gene conferring resistance to both phenicol and oxazolidinone, was found in a subset of samples from Europe and Asia. Not strangely, high veterinary amphenicol usage was reported in European countries, resulting in high abundance of chloramphenicol resistant genes in animal feces (Munk et al., 2018). The *tet(X)* gene, which confers resistance to tetracycline and tigecycline, was mainly found in Lake Erie (Canada). The gene was reported to mediate mobile tigecycline resistance identified in pathogens due to its association with different mobile genetic elements (Leski et al., 2013). Meanwhile, the carbapenemases *KPC* and *OXA-type* were identified in Lake Clifton (Australia), as well as in a subset of Chinese, American, Finnish and Canadian samples. Detection of *tet(X)* and an array of carbapenemases in environments was likely attributed to the pollution of feces from both municipal sewage and hospital effluents (Marathe et al., 2017). Of special interest was the *mcr-type* genes which were recently discovered in the clinical isolates from animal origins (Marathe et al., 2017). The mobilized colistin gene (i.e., *mcr-1*) can spread rapidly by horizontal gene transfer, entailing a lower fitness cost. In this study, they were also found widely in Asia and North America, implying that genetic exchange might occur between clinical and environmental microbiota. More importantly, many of these recently reported genes can make bacteria resistant to the last-resort drugs (i.e., colistin and carbapenems) (Queenan et al., 2007; Liu et al., 2016), contributing to the increased challenge for treating infections with some resistant pathogens (Martínez et al., 2015; Cerqueira et al., 2017).

Notably, the profiles of ARGs in global lakes presented spatial differences between geographical regions and exhibited a scale-dependent biogeography pattern. Relatively, the lakes from Asia showed significantly higher levels of ARGs than those from North America and other regions. Consistent with the findings, previous investigations also reported the high occurrences of ARGs in many Asian countries, such as China and India (Zhu et al., 2017; Van Boeckel et al., 2019). Likewise, similar trend was reported by a recent report on the global distribution of ARGs in sewage that a strong systematic separation of regions across the world was found based on the abundance of ARGs between the high-income countries and low-income countries (Hendriksen et al., 2019). Interestingly, the lower ARG levels were found in the lake sediments from Africa and South America, while the study on the resistome of urban sewage showed that the ARG levels in Africa and South America were higher than other regions (Hendriksen et al., 2019), suggesting the resistome changes occurring in environments and the distinctive fate of ARGs in different regions (Martínez et al., 2015). Usually, the presence of ARGs in environments are determined by complex conditions and transport mechanisms, potentially including selector agents, physicochemical, spatial, and microbial factors (Davies et al., 2010; Berendonk et al., 2015; Chen et al., 2019). In especial, the changing dynamics in the environments such as the discharge of a variety of pollutants from various sources will further complicate the behavior and fate of ARGs (Lu et al., 2020). The lakes inside mountains and/or surrounded by countryside with less anthropogenic activity would have dramatically different profiles of ARGs compared to urban lakes.

The distance-decay relationship is a fundamental pattern in ecology (Zhou et al., 2017). Previously, the observation of distance-decay pattern had been made for microbial communities, but few studies have been conducted on ARGs at a large scale. In this study, a scale-dependent biogeography pattern was found for the ARG profiles in global lake sediments, indicating that they were most likely subjected to some ecological processes such as environmental filtering and dispersal limitation (Hanson et al., 2012). Due to low abundance in the environments, ARGs always have low dispersal chances to stride over spatially segregated ecosystems via host species dispersal, horizontal gene transfer and gene flow (Liu et al., 2018). Thus, the biogeography pattern indicated the profiles of ARGs were largely influenced by environmental conditions and regional human activities (Berendonk et al., 2015). Similarly, the distinct biogeography patterns of ARGs were also observed in the waterbodies of China at a continental scale and found to be predominately related to the regional human activity and intensity such as antibiotic residue emissions (Liu et al., 2018).

With regard to the driver forces underlying resistome ecology in the lake ecosystems, the compositions of ARGs were mainly determined by bacterial communities, and the ARG diversities were co-governed by the deterministic and stochastic processes. This result was consistent with what have been reported in soil habitats and animal faecal microbiota (Forsberg et al., 2014; Munk et al., 2018), implying the phylogenetic composition of microbial taxa governed the resistome structure in the lake ecosystems. Notably, the deterministic forces were compounded by multiple intricate abiotic and biotic processes (i.e., environmental filtering, competition and facilitation) which could predominate under certain conditions of stress. Otherwise, null model analysis may overestimate stochasticity (Zhou and Ning, 2017; Ning et al., 2019). On the other hand, the richness distribution pattern of ARGs was characterized by Poisson lognormal model. The result was in line with previous studies that the lognormal dynamics, generally including the stochastic nature of dynamics, the multiplicative nature of growth, and the energetic cost of dispersal across geographic distances, was more commonly observed in microbial communities and appeared to be a result of the central limit theorem and law of large communities (Mende et al., 2017; Wu et al., 2019).

Indeed, ARGs in the environments are a complex problem associating with multiple driving forces, such as the consumption of antibiotics, economic development, environmental policy, hygiene, education, travel, and trade in different countries/regions (Hendriksen et al., 2019). Previous study showed the ARGs in aquatic environments were closely related to both human activities and environment conditions (Peng et al., 2020). The microbial communities in lakes were also controlled by a number of physical and chemical parameters, that is, environmental factors would exert an important effect on ARB and ARGs by impacting the composition and structure of their host bacterial community (Su et al., 2015). Particularly, the sediment ARGs in the high mountain Andean lakes was possibly driven by the enrichment of metals due to volcano eruptions (Herrera et al., 2016). Furthermore, seasonal and hydrothermal differences in the composition and abundance of ARGs had occurred, since

the samples were usually collected at different seasons, locations (i.e., lake depth and sediment depth) and climate zones, likely resulting in different seasonal and hydrothermal conditions in the abundance and diversity of ARGs and host species. For example, the samples of Lake Tai were collected at September when cyanobacteria were being bloomed to affect the bacterial community so influencing the ARGs (Chen et al., 2019).

This is an attempt to explore the biogeography and diversity patterns of ARGs in lake ecosystems at a global scale using metagenomic assembly-based analysis. Compared to amplification-based approaches, the metagenomic-based analysis can reveal completely the metagenomic profiling of ARGs in the environments which harbor many nonculturable microorganisms. Notably, the metagenomic samples from each region are limited and uneven, which may cause a risk that the data set would not be representative for some regions. Addition of including more representative lakes and samples is necessary in future to explore comprehensively the global distribution of ARGs in the environment. In addition, the metagenomes used here were differed in size and sequencing depth, which might exert effect on the detection of ARGs and their subsequent analyses. In the present study, the ARGs were analyzed based on the assembled contigs rather than the raw reads, because longer sequences contain more information than short reads (Chen et al., 2019). Also, the coverages of ARGs were normalized for comparison with the total read count in the metagenome to obtain the relative abundance. Nevertheless, reasonable strategy to combine more normalization methods for metagenome data with advanced multiple methods (i.e., Nanopore sequencing) would be helpful to check the recovery of ARGs and their ecological processes. As a whole, further study including more samples, lake-specific environment conditions, limnological/ecological parameters and human activities will give a deeper insight on the driving forces of ARGs in the environments.

4. Conclusions

This study conducted a large-scale metagenomics analysis for better understanding of the biogeography and diversity patterns of ARGs in lake ecosystems. Results showed abundant and diverse ARGs were found in the sediments of global lakes, including some emerging ARGs such as *optrA*, *tet(X)*, *mcr* and *carbapenemases* types. Spatially, the composition and diversity of ARGs varied across geographical regions and exhibited a scale-dependent distance-decay relationship. The profile of ARGs was mainly determined by bacterial communities and co-governed by deterministic and stochastic processes. Notably, a number of bacterial pathogen species were identified on the ARG-carrying contigs across all lake sediment samples, affiliating with the commonly used antibiotics. To reduce the global burden of ARGs, effective strategies should be adopted to preferentially control the significant human activities closely related to the reasonable usage of antibiotics, as well as sanitation improvement and infection control, access to clean water and food.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Beijing Natural Science Foundation of China (No. 8222059), the Major Science and Technology Program for Water Pollution Control and Treatment of China (No. 2017ZX07302), the 111 Project of China (No. B18006) and Beijing Advanced Innovation Program for Land Surface Science.

Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.06.024.

REFERENCES

- Bengtsson-Palme, J., Boulund, F., Fick, J., Kristiansson, E., Larsson, D.G.J., 2014. Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. *Front. Microbiol.* 5, e648.
- Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., et al., 2015. Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol.* 13, 310–317.
- Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., et al., 2018. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect. Dis.* 5, 1–11.
- Cerqueira, G.C., Earl, A.M., Ernst, C.M., Grad, Y.H., Dekker, J.P., Feldgarden, M., et al., 2017. Multi-institute analysis of carbapenem resistance reveals remarkable diversity, unexplained mechanisms, and limited clonal outbreaks. *Proc. Natl. Acad. Sci. USA.* 114, 1135–1140.
- Chen, H., Li, Y., Sun, W., Song, L., Zuo, R., Teng, Y., 2020. Characterization and source identification of antibiotic resistance genes in the sediments of an interconnected river-lake system. *Environ. Int.* 137, 105538.
- Chen, H.Y., Jing, L.J., Yao, Z.P., Meng, F.S., Teng, Y.G., 2019. Prevalence, source and risk of antibiotic resistance genes in the sediments of Lake Tai (China) deciphered by metagenomic assembly: a comparison with other global lakes. *Environ. Int.* 127, 267–275.
- Chen, H.Y., Liu, C., Teng, Y.G., Zhang, Z.L., Chen, Y.H., Yang, Y.Y., 2021. Environmental risk characterization and ecological process determination of bacterial antibiotic resistome in lake sediments. *Environ. Int.* 147, 106345.
- Czekalski, N., Sigdel, R., Birtel, J., Matthews, B., Bürgmann, H., 2015. Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 Swiss lakes. *Environ. Int.* 81, 45–55.
- Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74, 417–433.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA.* 103, 626–631.
- Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., et al., 2014. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 509, 612–616.
- Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., Martiny, J.B.H., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10, 497–506.
- Hendriksen, R.S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., et al., 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* 10, 1124.
- Hernando-Amado, S., Coque, T.M., Baquero, F., Martínez, J.L., 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nat. Microbiol.* 4, 1432–1442.
- Herrera, C., Custodio, E., Chong, G., Lamban, L.J., Riquelme, R., Wilke, H., et al., 2016. Groundwater flow in a closed basin with a saline shallow lake in a volcanic area: Laguna Tuyajto, northern Chilean Altiplano of the Andes. *Sci. Total Environ.* 541 (15), 303–318.
- Hu, A., Wang, H., Li, J., Mulla, S.I., Qiu, Q., Tang, L., et al., 2020. Homogeneous selection drives antibiotic resistome in two adjacent sub-watersheds, China. *J. Hazard. Mater.* 398, 122820.
- Hu, F., Zhu, D., Wang, F., Wang, M., 2018. Current status and trends of antibacterial resistance in China. *Clin. Infect. Dis.* S128, 67.
- Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., et al., 2008. Global trends in emerging infectious diseases. *Nature* 451, 990–993.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14 (6), 587.
- Karkman, A., Pärnänen, K., Larsson, D.G.J., 2019. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. *Nat. Commun.* 10, 80.
- Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., et al., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. USA* 115, E3463–E3470.
- Leski, T.A., Bangura, U., Jimmy, D.H., Ansumana, R., Lizewski, S.E., Stenger, D.A., et al., 2013. Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone. *Int. J. Antimicrob. Agents* 42, 83–86.
- Li, L.G., Yin, X., Zhang, T., 2018. Tracking antibiotic resistance gene pollution from different sources using machine-learning classification. *Microbiome* 6, 93.
- Liu, L., Su, J.Q., Guo, Y., Wilkinson, D.M., Liu, Z., Zhu, Y.G., et al., 2018. Large-scale biogeographical patterns of bacterial antibiotic resistome in the waterbodies of China. *Environ. Int.* 117, 292–299.
- Liu, Y.Y., Wang, Y., Walsh Dsc, T., Yi, L.X., Zhang, R., Spencer, J., et al., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16, 161–168.
- Lu, J., Wang, Y., Jin, M., Yuan, Z., Pond, P., Guo, J., et al., 2020. Both silver ions and silver nanoparticles facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes. *Water Res.* 169, 115229.
- Ma, L.P., Li, B., Jiang, X.T., Wang, Y.L., Xia, Y., Li, A.D., et al., 2017. Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey. *Microbiome* 5, 154.
- Marathe, N.P., Pal, C., Gaikwad, S.S., Jonsson, V., Kristiansson, E., Larsson, D.G.J., 2017. Untreated urban waste contaminates Indian river sediments with resistance genes to last resort antibiotics. *Water Res.* 124, 388–397.
- Marti, E., Variatza, E., Balcazar, J.L., 2014. The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends Microbiol.* 22, 36–41.

- Martínez, J.L., Coque, T.M., Baquero, F., 2015. What is a resistance gene? Ranking risk in resistomes. *Nat. Rev. Microbiol.* 13, 116.
- Martiny, J.B., Eisen, J.A., Penn, K., Allison, S.D., Horner-Devine, M.C., 2011. Drivers of bacterial beta-diversity depend on spatial scale. *Proc. Natl. Acad. Sci. USA* 108, 7850–7854.
- Mende, D.R., Letunic, I., Huerta-Cepas, J., Li, S.S., Forslund, K., Sunagawa, S., et al., 2017. proGenomes: a resource for consistent functional and taxonomic annotations of prokaryotic genomes. *Nucleic Acids Res* 45, D529–D534.
- Munk, P., Knudsen, B.E., Lukjancenko, O., Duarte, A.S.R., Gompel, L.V., Luiken, R.E.C., et al., 2018. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nat. Microbiol.* 3, 898–908.
- Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. *Proc. Natl. Acad. Sci. USA* 116, 16892–16898.
- Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., et al., 2020. A quantitative framework reveals ecological drivers of grassland soil microbial community assembly in response to warming. *Nat. Comm.* 11, 4717.
- O'Neill, 2016. Tracking Drug-Resistant Infections Globally: Final Report And Recommendations.
- Pehrsson, E.C., Tsukayama, P., Patel, S., Mejía-Bautista, M., Sosa-Soto, G., Navarrete, K.M., et al., 2016. Interconnected microbiomes and resistomes in low-income human habitats. *Nature* 533, 212–216.
- Peng, F., Guo, Y.Y., Isabwe, A., Chen, H.H., Wang, Y.M., Zhang, Y.P., et al., 2020. Urbanization drives riverine bacterial antibiotic resistome more than taxonomic community at watershed scale. *Environ. Int.* 137.
- Queenan, A.M., Bush, K., 2007. Carbapenemases: the versatile beta-lactamases. *Clin. Microbiol. Rev.* 20, 440–458.
- Razavi, M., Marathe, N.P., Gillings, M.R., Flach, C.F., Kristiansson, E., Joakim Larsson, D.G., 2017. Discovery of the fourth mobile sulfonamide resistance gene. *Microbiome* 5, 160.
- Shoemaker, W.R., Locey, K.J., Lennon, J.T., 2017. A macroecological theory of microbial biodiversity. *Nat. Ecol. Evol.* 1, 107.
- Stange, C., Yin, D., Xu, T., Guo, X., Schäfer, C., Tiehm, A., 2019. Distribution of clinically relevant antibiotic resistance genes in Lake Tai, China. *Sci. Total Environ.* 655, 337–346.
- Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* 6, 1653–1664.
- Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., et al., 2013. Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7, 2069–2079.
- Su, J.Q., Wei, B., Ou-Yang, W.Y., Huang, F.Y., Zhao, Y., Xu, H.J., et al., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ. Sci. Technol.* 49, 7356–7363.
- Tacconelli, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., et al., 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* 18, 318–327.
- UNEP, 2017. Frontiers 2017 Emerging Issues of Environmental Concern. United Nations Environment Programme, Nairobi.
- Van Boeckel, T., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N.G., et al., 2019. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 365, 1266.
- Wang, Z., Han, M., Li, E., Liu, X., Wei, H., Yang, C., et al., 2020. Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: their links with microbial communities, antibiotics, and water quality. *J. Hazard. Mater.* 393, 122426.
- Wang, R., van Dorp, L., Shaw, L.P., Bradley, P., Wang, Q., Wang, X., et al., 2018. The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. *Nat. Commun.* 9, 1179.
- Wu, L., Ning, D., Zhang, B., Li, Y., Zhang, P., Shan, X., et al., 2019. Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nat. Microbiol.* 4, 1183–1195.
- Yang, Y., Li, Z., Song, W., Du, L., Ye, C., Zhao, B., et al., 2019. Metagenomic insights into the abundance and composition of resistance genes in aquatic environments: influence of stratification and geography. *Environ. Int.* 127, 371–380.
- Yang, Y.Y., Song, W.J., Lin, H., Wang, W.B., Du, L.N., Xing, W., 2018. Antibiotics and antibiotic resistance genes in global lakes: a review and meta-analysis. *Environ. Int.* 116, 60–73.
- Zhang, Q., Lambert, G., Liao, D., Kim, H., Robin, K., Tung, C.K., et al., 2011. Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* 333, 1764–1767.
- Zhou, J.Z., Deng, Y., Zhang, P., Xue, K., Liang, Y.T., Van Nostrand, J.D., et al., 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc. Natl. Acad. Sci. USA* 111, E836–E845.
- Zhou, J., Ning, D., 2017. Stochastic community assembly: does it matter in microbial ecology? *Microbiol. Mol. Biol. Rev.* 81, e00002–e00017.
- Zhu, Y.G., Zhao, Y., Li, B., Huang, C.L., Zhang, S.Y., Yu, S., et al., 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. *Nat. Microbiol.* 2, 16270.