Identifying Novel Antibiotic Resistance Genes (ARGs): Important Aspect of Metagenomic Research

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Abstract

The rapid growth of antimicrobial resistance (also known as AMR) is a major reason for concern when it comes to public health around the world. The rise of AMR is a result of the overuse and misuse of antimicrobial agents, such as antibiotics, antivirals, antifungals and antiparasitics. India is indeed one of the world's top consumers of antibiotics and has its own unique set of constraints related to its large population and diverse cultural, social and economic landscape. Major obstacles to the application of antimicrobial resistance (AMR) containment strategies include self-medication, the use of antibiotics for growth promotion in animals and the development of residual antibiotics in the environment. The use of antibiotics in various sectors, including aquaculture, medicine, agriculture and the food industry, has contributed to the spread of antimicrobial resistance. The presence of antibiotic resistance genes (ARGs) in aquatic environments is of particular concern because it increases the risk of antibiotic

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resistance in human pathogens. ARGs can spread from aquatic environments to humans through contaminated seafood, and they can also spread from humans to aquatic environments through wastewater discharge. The pollution due to antibiotics and the prevalence of antibiotic resistance genes vary greatly between low-middle and high-income countries, as well as between different regions within a given country. The presence and spread of antibiotic resistance genes are also influenced by several factors, including the presence of antibiotic residues, microbial communities and environmental variables. The presence of antibiotics and antibiotic resistance genes (ARGs) in the environment can have significant impacts on microbial communities, biogeochemical cycles and both marine organisms and human health.

Keywords

Metagenomics · Antibiotic resistance genes · Resistome · Multidrug-resistant bacteria · Microbiome

12.1 Introduction

Antibiotic resistance in bacteria has evolved naturally and long before antibiotics were manufactured in large quantities for human use. Bacteria have evolved a variety of mechanisms to resist antibiotics, including changes in the structure of target proteins, the production of enzymes that break down antibiotics and the efflux of antibiotics out of the cell (Larsson and Flach 2021). The spread of ARGs in the environment is also a major concern, as it can increase the risk of antibiotic resistance in human and animal pathogens, making it more difficult to treat infections (Galera-Laporta and Garcia-Ojalvo 2020; Sun et al. 2020; Reverter et al. 2020). Antibiotic use and consumption have been increasing globally, and projections indicate that this trend is likely to continue. A recent study estimated that, under the scenario of no policy interventions, worldwide antibiotic consumption in 2030 could be as much as 200% higher than the level in 2015 (Klein et al. 2018). Anthropogenic, or human-caused, antibiotic residues in the environment can result in a number of serious environmental problems. Thiamphenicol is one example of an antibiotic that has been shown to have adverse environmental impacts. Research has indicated that thiamphenicol can interfere with nitrate reduction processes in soil, which can result in increased levels of nitrous oxide (N_2O) being released into the atmosphere (Yin et al. 2016). The widespread use of antibiotics can put significant selective pressure on microorganisms, leading to the enrichment of ARGs (Wang et al. 2019; Migliorini et al. 2019). Antibiotic resistance is a complex phenomenon that can occur through various mechanisms, namely, enzymatic destruction, efflux pumps, cellular protection and both target defence and antibiotic deactivation (Zhang et al. 2019; Chen et al. 2019; Wilson et al. 2020). Antibiotic resistance can significantly impair the efficacy of antibiotics against pathogens, making it difficult or impossible to treat infections effectively (Pärnänen et al.

2019). Horizontal gene transfer (HGT) is a key mechanism by which antibiotic resistance genes (ARGs) can spread and become more prevalent in the environment. Mobile genetic elements (MGEs) which include transposons, integrons and plasmids are self-contained pieces of DNA that can move from one bacterium to another, often across species boundaries. These MGEs can carry multiple ARGs, providing bacteria with a "toolkit" of resistance mechanisms (Wang et al. 2018; Pallares-Vega et al. 2019; Zhao et al. 2019a). ARGs and antibiotics have frequently been found in rivers (Rodriguez-Mozaz et al. 2015; Singh et al. 2019; Das et al. 2020), lakes (Tang et al. 2015; Yang et al. 2018a), groundwater (Tong et al. 2020; Zainab et al. 2020) and coastal and estuarine environments (Griffin et al. 2019; Zheng et al. 2021). The presence of pollutants, including antibiotics and antibiotic resistance genes (ARGs), in these environments can have negative impacts on the health of estuarine and coastal ecosystems (Ward et al. 2020). The presence of ARGs and antibiotics in coastal and estuarine environments can have far-reaching consequences for biogeochemical cycling, ecological security and human health (Leonard et al. 2015; Zhao et al. 2019b).

Antimicrobial resistance in bacteria is currently the most prevalent type of resistance in microbes and a significant public health issue. The overuse and misuse of antibiotics have led to the evolution of bacteria that are resistant to multiple antibiotics, making it difficult to treat bacterial infections.

12.2 Taxonomic Profiling

Understanding the taxonomic classifications of resistome components is crucial for locating the bacteria that produce a resistome. Taxonomic assignment analysis of resistome elements can provide information about the composition and relative abundance of the microbial community in a sample (Ruppé et al. 2018; Rice et al. 2020). There are two main approaches for identifying bacterial community composition from metagenomic data. The first approach is based on direct analysis of raw sequencing reads and does not require contig assembly. The second approach involves the assembly of contigs from the raw sequencing reads, followed by taxonomic assignment of the contigs (de Abreu et al. 2021). Taxonomic classification of metagenomic data without contig assembly can be a faster and more computationally efficient approach compared to contig assembly (Rodríguez-Brazzarola et al. 2018). The length and quality of sequences are crucial considerations during taxonomy classification analysis. Short reads or low-quality reads may have a higher error rate and may not provide enough information to accurately identify the bacterial taxa present in a sample (Breitwieser et al. 2019; Ye et al. 2019). The length of contigs generated by contig assembly is an advantage for taxonomic classification, as longer contigs provide more information to accurately identify the bacterial taxa present in a sample. Taxonomic classification and contig-based taxonomic classification rely heavily on the use of reference databases (Rodríguez-Brazzarola et al. 2018). Contig assembly can sometimes enable the reconstruction of partial genomes of previously unknown or uncultured bacterial organisms. In contig assembly, short reads from different bacterial taxa can be erroneously assembled together into a single contig, resulting in a chimeric contig. The quality of the assembled contigs and the accuracy of the taxonomic assignments made based on the assembled contigs can strongly influence the interpretation of the microbial community composition and function (Behera et al. 2020a, b, 2021a, b, 2022; Rout et al. 2022).

Furthermore, genome assembly can enable the identification of possible HGT regions, which are genomic regions that have been acquired by a bacterium from another organism through HGT mechanisms such as transduction, transformation or conjugation. In the context of antibiotic resistance genes, the size of gene sequences can have a substantial effect on gene annotation transfer and the investigation of biological mechanisms linked with resistance (ARGs). Taxonomic assignment by contig assembly can be a useful tool for identifying and understanding resistance mechanisms, particularly in the context of studying the structural relationships between microbiota and the resistome. The type of sample being worked with can influence the quality and quantity of DNA/RNA obtained, which can in turn impact the success of the assembly process. The sequences in the datasets are of good quality and long enough to be aligned directly to a reference database. The taxonomic classification can be performed using alignment-based methods, which are computationally less intensive than de novo assembly approaches (Rodríguez-Brazzarola et al. 2018). High-throughput sequencing can be used to study the taxonomic assignments of resistome elements in various environments, including water reservoirs to identifying antibiotic resistance genes (ARGs) in host-pathogen relationships such as hospitals (Chng et al. 2020), water reservoirs (Yu et al. 2020; Ekwanzala et al. 2020), soil (Chen et al. 2017), human faeces (Karkman et al. 2019), livestock wastewater and faeces (Jia et al. 2017), air (Yang et al. 2018b; Li et al. 2021) and biogeochemical and biogeographical processes (Kuang et al. 2016; Liu et al. 2018).

12.3 Functional Study and ARGs Database

The investigation of taxonomic signatures can assist us in gaining a deeper comprehension of the connections that exist among the various members of a microbial diversity. Functional metagenomics is an approach that purposes to identify functions in a microbial community by discovering new enzymes, biosynthetic gene clusters and antibiotic resistance genes (ARGs). Functional annotation typically involves several steps, including gene prediction, annotation transfer and functional assignment (Dong and Strous 2019). Metagenomics is a powerful tool for studying microbial communities and has the potential to provide important insights into microbial ecology, evolution and biotechnology (Zhang et al. 2011). There are numerous databases and methods available for identifying a microbial community's taxonomic diversity and undertaking functional assessments. These include databases of reference genomes, protein sequences and metabolic pathways, as well as bioinformatic tools for taxonomic classification, functional annotation and pathway analysis. Functional analysis of metagenomic data provides a wealth of information that can be used for various sub-analyses, depending on the sequencing depth and research question. These sub-analyses can include protein-protein interaction, pathway, functional category, gene ontology, protein family and subsystem analysis, among others. Each of these analyses provides different levels of detail and can be used to gain insights into the functional potential and metabolic activities of the microbial community. There are open-source software/applications, like Mothur (Schloss et al. 2009), MEGAN (Huson et al. 2007) and QIIME (Caporaso et al. 2010) for taxonomy and functional analysis. BLAST$^+$ is an important tool in genomic research and is widely used to annotate new genome sequences and to investigate the evolutionary relationships between different species (Altschul et al. 1997). DIAMOND is a powerful tool for annotating genomic data, particularly for the analysis of large datasets such as metagenomic samples. Its speed and accuracy make it an attractive option for researchers working with large amounts of sequence data (Buchfink et al. 2014). USEARCH is a useful tool for bioinformatic analysis, particularly for sequence searches and clustering (Edgar and Bateman 2010). RAPSearch2 is a powerful tool for sequence searching and is well suited for large-scale analyses (Zhao et al. 2012).

The development of new methods and tools for comprehensive metagenomic analyses is an ongoing process, and it is important for researchers to stay up-to-date with the latest advancements in the field in order to conduct the most effective and informative analyses. Resistome databases are crucial resources for understanding antimicrobial resistance and are constantly evolving as new information becomes available (Danko et al. 2021). The use of database for sequence analysis can introduce database bias that can affect the accuracy and relevance of the results, particularly for metagenomic analyses. The genomic surveillance of AMR is crucial for understanding the distribution and spread of resistance genes, as well as for identifying new resistance mechanisms (de Abreu et al. 2021). To support this effort, numerous annotation softwares and databases have been established to facilitate the analysis of antibiotic resistance gene (ARG) content in bacterial genomes or next-generation sequencing (NGS) metagenomic samples. These tools and databases provide valuable resources for researchers and practitioners to identify, annotate and compare ARGs in different bacterial genomes and metagenomes. Some of the most widely used annotation tools and well-known AMR databases are tabulated in Table 12.1.

The Comprehensive Antibiotic Resistance Database (CARD) (Alcock et al. 2020) is unique in that it combines sequence data with bioinformatic tools to aid in the detection and analysis of AMR genes. For example, the database includes curated detection models that can be used to identify AMR genes in sequenced bacterial genomes, as well as tools for visualising and analysing the genomic context of these genes. In addition to resistance genes, CARD also includes information on resistance mutations, which are genetic changes that can confer resistance to antibiotics. Like the resistance genes, the resistance mutations are organised by bacterial species. CARD focuses on delivering high-quality reference material and molecular

Table 12.1 Several bioinformatic databases available for studying antibiotic resistance genes (ARGs)

Database	Web link	References
CARD	https://card.mcmaster.ca/	Alcock et al. (2020)
ARDB	https://ardb.cbcb.umd.edu/	Liu and Pop (2009)
ResFinder	https://cge.cbs.dtu.dk/services/ResFinder/	Florensa et al. (2022)
SARG	https://smile.hku.hk/SARGs#	Yin et al. (2018)
ARGminer	https://bench.cs.vt.edu/argminer/#/home	Arango-Argoty et al. (2020)
NDARO	https://www.ncbi.nlm.nih.gov/pathogens/refgene/	Feldgarden et al. (2021)
MEGAres	https://megares.meglab.org/	Doster et al. (2020)
AMR++	https://github.com/Microbial-Ecology-Group/AMRplusplus	Bonin et al. (2023)
ARG-ANNOT	http://www.mediterranee-infection.com/article.php?laref=282&titre=arg-annot	Gupta et al. (2014)
ARG-database	https://smile.hku.hk/SARGs	Yang et al. (2016)

sequences that are arranged using the Antibiotic Resistance Ontology (ARO), a regulated vocabulary.

ARDB (Liu and Pop 2009) also known as the Antibiotic Resistance Genes Database tracks ARGs. It was first released in 2005 and is maintained by the Antibiotic Resistance Genes Reference Center at the University of Alberta, Canada. It includes information on a wide range of antibiotic resistance genes, including those found in both Gram-positive and Gram- negative bacteria. The database provides detailed annotations for each gene, including information on its function, location and associated resistance mechanisms.

Resfinder (Florensa et al. 2022) is a useful tool for the characterisation and identification of ARGs, and its use can help researchers better understand the distribution and prevalence of resistance genes in bacterial populations and metagenomic datasets. Resfinder also provides information about the sequence similarity and identity of the identified resistance genes, allowing researchers to compare the resistance genes to known reference sequences and assess the potential impact of any genetic variations or mutations. This can be important for understanding the mechanisms of resistance and for predicting the potential effectiveness of different antimicrobial therapies.

ARG-ANNOT (Gupta et al. 2014) is a web-based tool for both complete genomes and draft genomes and can be applied to both Gram-negative and Gram-positive bacteria. The tool provides detailed annotations for each detected AR gene, including information on its function, resistance mechanism and associated resistance phenotype. It also provides information on the genomic context of each gene, including its location on the chromosome and any associated mobile genetic elements.

ARG-database (Yang et al. 2016) ARGs-OAP (Antibiotic Resistance Genes Online Analysis Pipeline) is a bioinformatic tool designed to facilitate the analysis and finding of antibiotic resistance genes (ARGs) in metagenomic data. It includes a large and diverse database of reference sequences for comparison and can be applied to both short-read and long-read sequencing data. By providing a comprehensive and up-to-date database of reference sequences and detailed annotations for each gene, it helps to facilitate research on the molecular mechanisms of antibiotic resistance and on the development of new strategies for combating it.

SARG (Yin et al. 2018) database was designed to help researchers identify ARGs in metagenomic samples, which are complex mixtures of DNA from different microorganisms. SARG v2.0 builds on this concept by incorporating a much larger number of reference sequences for ARGs, which allows for more comprehensive coverage of the resistance gene landscape in different environments. ARGs-OAP v2.0 is designed to help researchers analyse and annotate high-throughput sequencing data for the presence of antibiotic resistance genes (ARGs). The database includes a large number of reference sequences for ARGs, which can be used to identify similar sequences in raw sequencing data using a similarity search strategy.

ARGminer (Arango-Argoty et al. 2020) able to capture a broader range of ARGs and provide more comprehensive information about each gene, including its sequence, function and resistance profile. It looks like the sequences from the different databases that were used to build ARGminer were processed to get rid of duplicates and mark them also with highest similarity from each of the databases. Overall, the use of an ensemble database like ARGminer can be a powerful tool for understanding the genetics and mechanisms of antibiotic resistance, which is a critical public health concern.

NDARO (Feldgarden et al. 2021) is the central hub for scholars to access data related to antimicrobial resistance (AMR) in infective organisms. The purpose of real-time AMR surveillance is that it keeps an up-to-date database of AMR genes and a combination of genetic and antibiotic susceptibility data. The Reference Gene Catalog, which is maintained by the NDARO, is a curated collection of antimicrobial resistance (AMR) genes from various bacterial pathogens. NDARO has expanded its focus beyond just antimicrobial resistance (AMR) genes to include other genetic elements that are important for understanding the biology of clinically important pathogens.

MEGAres (Doster et al. 2020) is an updated version of the MEGARes database, which is a comprehensive resource for studying antimicrobial resistance genes. It contains sequence data for thousands of hand-curated antimicrobial resistance genes, but it also includes additional features that are designed to aid in the analysis of metagenomic sequencing data. A crucial part of preventing AMR is identifying its genetic causes in the fight against this global public health threat. By using MEGARes to analyse metagenomic data, researchers can identify the presence and abundance of AMR genes in various environments and track their distribution and evolution over time.

AMR^{++} (Bonin et al. 2023) is a powerful and flexible bioinformatic pipeline that is designed to aid in the analysis of antimicrobial resistance genes. In the context of

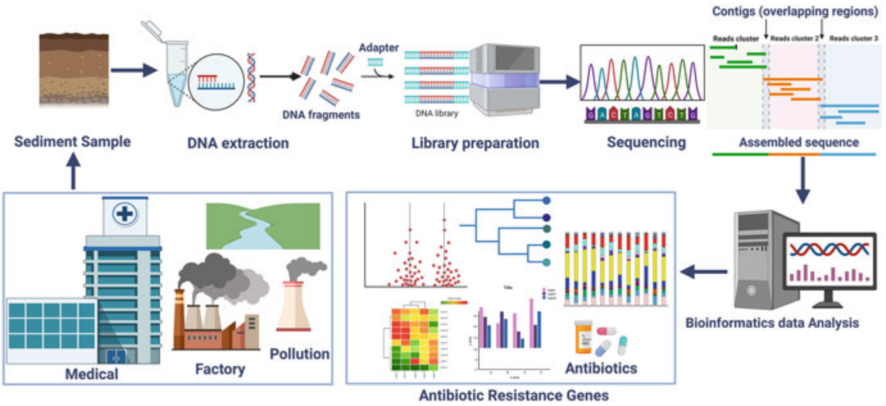

Fig. 12.1 Antimicrobial resistance genes showing the sources, and drivers of antimicrobial resistance in the aquatic environment

metagenomics, the DNA of a given sample or set of samples is sequenced using high-throughput sequencing technologies. The resulting data can be quite complex, with millions or even billions of short DNA sequences that need to be sorted and analysed. By providing a comprehensive view of the resistome in a given bacterial population, the pipeline can help researchers to better understand the mechanisms and evolution of antibiotic resistance and develop more effective strategies for combatting this important public health. The sources and drivers of AMR in the aquatic environment is crucial for developing effective strategies to mitigate its impact. These strategies may involve improved wastewater treatment processes, responsible antimicrobial use in agriculture and aquaculture, and enhanced surveillance and monitoring programs. Additionally, promoting public awareness and education regarding the proper disposal of pharmaceuticals and reducing unnecessary antimicrobial use are important steps towards combating AMR in the aquatic environment (Fig.12.1).

12.4 Antimicrobial Resistance in Bacteria

Resistome is a term used to describe the total genetic potential of an ecosystem to resist antimicrobial agents (Martínez et al. 2014; Crofts et al. 2017). The resistome of an ecosystem is shaped by a variety of factors, including the presence of anthropogenic stressors, such as the release of antibiotics and other toxic compounds into the environment, and the presence of natural stressors, such as heavy metal ions and other toxic substances (Kraemer et al. 2019). Microorganisms have evolved the ability to detect, interact with and digest tiny compounds that control the antibiotics (Berendonk et al. 2015). The development of novel antibiotics that can avoid resistance can be influenced by knowledge about the evolution of resistance (McGarvey et al. 2012). The genomic era has led to new insights into the biology of bacteria, which could potentially lead to the discovery of new antibiotics, but the process of drug development is complex and time-consuming (Li et al. 2015). Soil microorganisms produce natural antimicrobial compounds, and these have been a rich source of antibiotics used in clinical medicine (Wrighton 2018; Crits-Christoph et al. 2018). Metagenomic mining has revealed that resistance genes have existed in microbial populations long before the modern "antibiotic era" (Yadav and Kapley 2021). The diversity and distribution of ARGs in the environment can help to inform the development of new antibiotic resistance methods, such as using natural chemicals from microbial communities to inhibit the propagation of resistance genes (Berendonk et al. 2015). In this study, antibiotic resistance in environmental bacteria can provide important insights into the natural history of resistance and the mechanisms that underlie the development and spread of resistance in clinical settings (Aminov 2009). The fast growth of antibiotic-resistant infections has revealed our limited understanding of the environmental mechanisms occurring in microbial communities (Waseem et al. 2017). Multi-drug-resistant bacteria (MDR) have evolved the ability to metabolise antimicrobials and can transfer these properties to other bacterial species through horizontal gene transfer (Holt et al. 2015).

12.5 Hotspots for the Spread of Antibiotic Resistance

Antibiotic use in agriculture, veterinary medicine and human medicine can lead to the growth and spread of resistant microorganisms in the environment. Antibiotic-resistant bacteria have been found in various environmental sources, including soil, water and wildlife; there is no direct evidence that these bacteria have existed four million years ago, in caves (Bhullar et al. 2012). ARG bacteria have also been discovered in the gastrointestinal tracts of persons living in distant places who have never been exposed to antibiotics as well as in samples of permafrost that are thousands of years old (Kunhikannan et al. 2021). The use of antibiotics in agriculture and animal husbandry can lead to the selection and spread of ARGs in the gut microbiota of animals (Wichmann et al. 2014; Berendsen et al. 2015). Antibiotic-resistant bacteria can live in close proximity to each other in the soil, which can facilitate the transfer of antibiotic resistance genes through horizontal gene transfer (Christensen et al. 1998). Horizontal gene transfer is a major factor promoting the growth of ARGs in the environment, and it highlights the importance of responsible use of antibiotics to minimise the emergence and spread of antibiotic-resistant bacteria. The use of antibiotics in animals can contribute to the development of antibiotic resistance in humans. When antibiotics are used in animal agriculture, bacteria can become resistant to these drugs and may spread to humans through food or other environmental pathways. This can lead to the rise and spread of ARG infections in humans, which can be difficult to treat with traditional antibiotics. Therefore, reducing the use of antibiotics in animal agriculture and promoting responsible use of antibiotics in human medicine are important strategies for minimising the emergence and spread of antibiotic-resistant infections in humans

(Mann et al. 2021). Workers who handle and process meat or work in agriculture may be exposed to bacteria that are resistant to antibiotics, and this can increase the risk of developing antibiotic-resistant infections (Manyi-Loh et al. 2018). It is critical to investigate the various environmental hotspots that contribute to the spread of antibiotic resistance in both pathogenic and non-pathogenic bacteria. Hotspots for antibiotic-resistant bacteria can be found in various environmental sources, with pharmaceutical manufacturing sites, wastewater systems and aquaculture, food and animal production and hospitals (Berendonk et al. 2015).

It is important to find the major drivers that contribute to the development and spread of antimicrobial resistance. Some of the major drivers include the following:

- Overuse and misuse of antibiotics: Antibiotic-resistant bacteria can arise and spread due to the overuse and improper use of antibiotics in both animals and human beings.
- Poor infection prevention and control practices: Poor infection control and prevention procedures in healthcare settings can facilitate the spread of antibiotic-resistant bacteria among patients.
- Inadequate sanitation and hygiene: Inadequate sanitation and hygiene can lead to the spread of antibiotic-resistant bacteria in the environment, particularly in water and soil.
- Agricultural and animal husbandry practices: The use of antibiotics in agriculture and animal husbandry can lead to the development and spread of ARGs in animals and the environment.
- Global travel and trade: The global movement of people, animals and goods can facilitate the spread of antibiotic-resistant bacteria across borders.
- Insufficient funding for research and development: The lack of investment in research and development of new antibiotics and alternative treatments for infectious diseases can limit our ability to effectively treat antibiotic-resistant infections.

Identifying and addressing these drivers is critical to addressing the problem of antimicrobial resistance and preserving the effectiveness of antibiotics for future generations.

12.6 Conclusion

Metagenomics is an effective method for finding and investigating antibiotic resistance pathways utilising both sequence-based and function-based methods. It allows for the comprehensive analysis of complex microbial communities, providing insights into the diversity and distribution of ARGs. Antimicrobial resistance studies are commonly related to other components of the study being conducted, such as investigations of mutations, metabolic pathways, gene expression and infections. These other factors can affect the development and spread of antibiotic resistance, and understanding their interplay with resistance mechanisms is important for developing effective strategies to combat antimicrobial resistance. These studies involve large, complex datasets and require advanced computational and bioinformatic tools for their analysis. Proper data pre-processing, quality control and statistical analysis are essential to ensure the accuracy and reproducibility of results.

References

Alcock BP, Raphenya AR, Lau TTY et al (2020) CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/10.1093/NAR/GKZ935

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/NAR/25.17.3389

Aminov RI (2009) The role of antibiotics and antibiotic resistance in nature. Environ Microbiol 11: 2970–2988. https://doi.org/10.1111/J.1462-2920.2009.01972.X

Arango-Argoty GA, Guron GKP, Guron GKP et al (2020) ARGminer: a web platform for the crowdsourcing-based curation of antibiotic resistance genes. Bioinformatics 36:2966–2973. https://doi.org/10.1093/BIOINFORMATICS/BTAA095

Behera BK, Chakraborty HJ, Patra B et al (2020a) Metagenomic analysis reveals bacterial and fungal diversity and their bioremediation potential from sediments of river ganga and Yamuna in India. Front Microbiol 11:2531. https://doi.org/10.3389/FMICB.2020.556136/BIBTEX

Behera BK, Patra B, Chakraborty HJ et al (2020b) Metagenome analysis from the sediment of river ganga and Yamuna: in search of beneficial microbiome. PloS One 15:e0239594. https://doi.org/10.1371/JOURNAL.PONE.0239594

Behera BK, Dehury B, Rout AK et al (2021a) Metagenomics study in aquatic resource management: recent trends, applied methodologies and future needs. Gene Rep 25:101372. https://doi.org/10.1016/J.GENREP.2021.101372

Behera BK, Sahu P, Rout AK et al (2021b) Exploring microbiome from sediments of river ganga using a metagenomic approach. Aquat Ecosyst Health Manage 24:12–22. https://doi.org/10.14321/AEHM.024.04.04

Behera BK, Patra B, Chakraborty HJ et al (2022) Bacteriophages diversity in India's major river ganga: a repository to regulate pathogenic bacteria in the aquatic environment. Environ Sci Pollut Res 1:1–14. https://doi.org/10.1007/S11356-022-24637-7/FIGURES/5

Berendonk TU, Manaia CM, Merlin C et al (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13:310–317. https://doi.org/10.1038/nrmicro3439

Berendsen BJA, Wegh RS, Memelink J et al (2015) The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta 132:258–268. https://doi.org/10.1016/J.TALANTA.2014.09.022

Bhullar K, Waglechner N, Pawlowski A et al (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. PloS One 7:e34953. https://doi.org/10.1371/JOURNAL.PONE.0034953

Bonin N, Doster E, Worley H et al (2023) MEGARes and AMR++, v3.0: an updated comprehensive database of antimicrobial resistance determinants and an improved software pipeline for classification using high-throughput sequencing. Nucleic Acids Res 51:D744–D752. https://doi.org/10.1093/NAR/GKAC1047

Breitwieser FP, Lu J, Salzberg SL (2019) A review of methods and databases for metagenomic classification and assembly. Brief Bioinform 20:1125–1136. https://doi.org/10.1093/BIB/BBX120

Buchfink B, Xie C, Huson DH (2014) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60. https://doi.org/10.1038/nmeth.3176

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Chen QL, An XL, Zhu YG et al (2017) Application of struvite alters the antibiotic resistome in soil, rhizosphere, and phyllosphere. Environ Sci Technol 51:8149–8157. https://doi.org/10.1021/ACS.EST.7B01420/SUPPL_FILE/ES7B01420_SI_001.PDF

Chen H, Bai X, Li Y et al (2019) Characterization and source-tracking of antibiotic resistomes in the sediments of a peri-urban river. Sci Total Environ 679:88–96. https://doi.org/10.1016/J.SCITOTENV.2019.05.063

Chng KR, Li C, Bertrand D et al (2020) Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. Nat Med 26:941–951. https://doi.org/10.1038/s41591-020-0894-4

Christensen BB, Sternberg C, Andersen JB et al (1998) Establishment of new genetic traits in a microbial biofilm community. Appl Environ Microbiol 64:2247–2255. https://doi.org/10.1128/AEM.64.6.2247-2255.1998/ASSET/F56A4950-0A39-43DF-A058-BD6C277A7C2B/ASSETS/GRAPHIC/AM0680136006.JPEG

Crits-Christoph A, Diamond S, Butterfield CN et al (2018) Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis. Nature 558:440–444. https://doi.org/10.1038/s41586-018-0207-y

Crofts TS, Gasparrini AJ, Dantas G (2017) Next-generation approaches to understand and combat the antibiotic resistome. Nat Rev Microbiol 15:422–434. https://doi.org/10.1038/nrmicro.2017.28

Danko D, Bezdan D, Afshin EE et al (2021) A global metagenomic map of urban microbiomes and antimicrobial resistance. Cell 184:3376–3393.e17. https://doi.org/10.1016/j.cell.2021.05.002

Das BK, Behera BK, Chakraborty HJ et al (2020) Metagenomic study focusing on antibiotic resistance genes from the sediments of river Yamuna. Gene 758:144951. https://doi.org/10.1016/J.GENE.2020.144951

de Abreu VAC, Perdigão J, Almeida S (2021) Metagenomic approaches to analyze antimicrobial resistance: an overview. Front Genet 11:1711. https://doi.org/10.3389/FGENE.2020.575592/XML/NLM

Dong X, Strous M (2019) An integrated pipeline for annotation and visualization of metagenomic Contigs. Front Genet 10:999. https://doi.org/10.3389/FGENE.2019.00999/XML/NLM

Doster E, Lakin SM, Dean CJ et al (2020) MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Res 48:D561–D569. https://doi.org/10.1093/NAR/GKZ1010

Edgar RC, Bateman A (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/BIOINFORMATICS/BTQ461

Ekwanzala MD, Dewar JB, Momba MNB (2020) Environmental resistome risks of wastewaters and aquatic environments deciphered by shotgun metagenomic assembly. Ecotoxicol Environ Saf 197:110612. https://doi.org/10.1016/J.ECOENV.2020.110612

Feldgarden M, Brover V, Gonzalez-Escalona N et al (2021) AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep 11:1–9. https://doi.org/10.1038/s41598-021-91456-0

Florensa AF, Kaas RS, Clausen PTLC et al (2022) ResFinder—an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb Genom 8:000748. https://doi.org/10.1099/MGEN.0.000748/CITE/REFWORKS

Galera-Laporta L, Garcia-Ojalvo J (2020) Antithetic population response to antibiotics in a polybacterial community. Sci Adv 6:eaaz5108. https://doi.org/10.1126/SCIADV.AAZ5108/SUPPL_FILE/AAZ5108_SM.PDF

Griffin DW, Benzel WM, Fisher SC et al (2019) The presence of antibiotic resistance genes in coastal soil and sediment samples from the eastern seaboard of the USA. Environ Monit Assess 191:1–17. https://doi.org/10.1007/S10661-019-7426-Z/METRICS

Gupta SK, Padmanabhan BR, Diene SM et al (2014) ARG-annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212–220. https://doi.org/10.1128/AAC.01310-13/SUPPL_FILE/ZAC001142457SO1.PDF

Holt KE, Wertheim H, Zadoks RN et al (2015) Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A 112:E3574–E3581. https://doi.org/10.1073/PNAS.1501049112/SUPPL_FILE/PNAS.1501049112.SD01.XLSX

Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. Genome Res 17:377–386. https://doi.org/10.1101/GR.5969107

Jia S, Zhang XX, Miao Y et al (2017) Fate of antibiotic resistance genes and their associations with bacterial community in livestock breeding wastewater and its receiving river water. Water Res 124:259–268. https://doi.org/10.1016/J.WATRES.2017.07.061

Karkman A, Pärnänen K, Larsson DGJ (2019) Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nat Commun 10:1–8. https://doi.org/10.1038/s41467-018-07992-3

Klein EY, van Boeckel TP, Martinez EM et al (2018) Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U S A 115:E3463–E3470. https://doi.org/10.1073/PNAS.1717295115/SUPPL_FILE/PNAS.1717295115.SAPP.PDF

Kraemer SA, Ramachandran A, Perron GG (2019) Antibiotic pollution in the environment: from microbial ecology to public policy. Microorganisms 7:180. https://doi.org/10.3390/MICROORGANISMS7060180

Kuang J, Huang L, He Z et al (2016) Predicting taxonomic and functional structure of microbial communities in acid mine drainage. ISME J 10:1527–1539. https://doi.org/10.1038/ismej.2015.201

Kunhikannan S, Thomas CJ, Franks AE et al (2021) Environmental hotspots for antibiotic resistance genes. Microbiology 10:e1197. https://doi.org/10.1002/MBO3.1197

Larsson DGJ, Flach CF (2021) Antibiotic resistance in the environment. Nat Rev Microbiol 20:257–269. https://doi.org/10.1038/s41579-021-00649-x

Leonard AFC, Zhang L, Balfour AJ et al (2015) Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environ Int 82:92–100. https://doi.org/10.1016/J.ENVINT.2015.02.013

Li B, Yang Y, Ma L et al (2015) Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J 9:2490–2502. https://doi.org/10.1038/ismej.2015.59

Li X, Wu Z, Dang C et al (2021) A metagenomic-based method to study hospital air dust resistome. Chem Eng J 406:126854. https://doi.org/10.1016/J.CEJ.2020.126854

Liu B, Pop M (2009) ARDB—Antibiotic resistance genes database. Nucleic Acids Res 37:D443. https://doi.org/10.1093/NAR/GKN656

Liu L, Su JQ, Guo Y et al (2018) Large-scale biogeographical patterns of bacterial antibiotic resistome in the waterbodies of China. Environ Int 117:292–299. https://doi.org/10.1016/J.ENVINT.2018.05.023

Mann A, Nehra K, Rana JS, Dahiya T (2021) Antibiotic resistance in agriculture: perspectives on upcoming strategies to overcome upsurge in resistance. Curr Res Microb Sci 2:100030. https://doi.org/10.1016/J.CRMICR.2021.100030

Manyi-Loh C, Mamphweli S, Meyer E, Okoh A (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 23:795. https://doi.org/10.3390/MOLECULES23040795

Martínez JL, Coque TM, Baquero F (2014) What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol 13:116–123. https://doi.org/10.1038/nrmicro3399

McGarvey KM, Queitsch K, Fields S (2012) Wide variation in antibiotic resistance proteins identified by functional metagenomic screening of a soil DNA library. Appl Environ Microbiol 78:1708–1714. https://doi.org/10.1128/AEM.06759-11/SUPPL_FILE/AEM6759-11_SUPPLEMENTAL_MATERIAL.PDF

Migliorini LB, Brüggemann H, de Sales RO et al (2019) Mutagenesis induced by sub-lethal doses of ciprofloxacin: genotypic and phenotypic differences between the Pseudomonas aeruginosa strain PA14 and clinical isolates. Front Microbiol 10:1553. https://doi.org/10.3389/FMICB.2019.01553/XML/NLM

Pallares-Vega R, Blaak H, van der Plaats R et al (2019) Determinants of presence and removal of antibiotic resistance genes during WWTP treatment: a cross-sectional study. Water Res 161:319–328. https://doi.org/10.1016/J.WATRES.2019.05.100

Pärnänen KMM, Narciso-Da-Rocha C, Kneis D et al (2019) Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. Sci Adv 5:eaau9124. https://doi.org/10.1126/SCIADV.AAU9124/SUPPL_FILE/AAU9124_SM.PDF

Reverter M, Sarter S, Caruso D et al (2020) Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat Commun 11:1–8. https://doi.org/10.1038/s41467-020-15735-6

Rice EW, Wang P, Smith AL, Stadler LB (2020) Determining hosts of antibiotic resistance genes: a review of methodological advances. Environ Sci Technol Lett 7:282–291. https://doi.org/10.1021/ACS.ESTLETT.0C00202/SUPPL_FILE/EZ0C00202_SI_001.PDF

Rodríguez-Brazzarola P, Pérez-Wohlfeil E, Díaz-del-Pino S et al (2018) Analyzing the differences between reads and contigs when performing a taxonomic assignment comparison in metagenomics. In: Lecture notes in computer science (including subseries lecture notes in artificial intelligence and lecture notes in bioinformatics) 10813 LNBI, pp 450–460. https://doi.org/10.1007/978-3-319-78723-7_39/COVER

Rodriguez-Mozaz S, Chamorro S, Marti E et al (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 69:234–242. https://doi.org/10.1016/J.WATRES.2014.11.021

Rout AK, Dehury B, Parida PK et al (2022) Taxonomic profiling and functional gene annotation of microbial communities in sediment of river ganga at Kanpur, India: insights from whole-genome metagenomics study. Environ Sci Pollut Res 29:82309–82323. https://doi.org/10.1007/S11356-022-21644-6/METRICS

Ruppé E, Ghozlane A, Tap J et al (2018) Prediction of the intestinal resistome by a three-dimensional structure-based method. Nat Microbiol 4:112–123. https://doi.org/10.1038/s41564-018-0292-6

Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09/ASSET/91BD47E1-E1DA-4980-B8B3-5DFA9C4F1FE7/ASSETS/GRAPHIC/ZAM0230904840002.JPEG

Singh R, Singh AP, Kumar S et al (2019) Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. J Clean Prod 234:1484–1505. https://doi.org/10.1016/J.JCLEPRO.2019.06.243

Sun J, Liao XP, D'Souza AW et al (2020) Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. Nat Commun 11:1–11. https://doi.org/10.1038/s41467-020-15222-y

Tang J, Shi T, Wu X et al (2015) The occurrence and distribution of antibiotics in Lake Chaohu, China: seasonal variation, potential source and risk assessment. Chemosphere 122:154–161. https://doi.org/10.1016/J.CHEMOSPHERE.2014.11.032

Tong L, Qin L, Guan C et al (2020) Antibiotic resistance gene profiling in response to antibiotic usage and environmental factors in the surface water and groundwater of Honghu Lake, China. Environ Sci Pollut Res 27:31995–32005. https://doi.org/10.1007/S11356-020-09487-5/METRICS

Wang F, Xu M, Stedtfeld RD et al (2018) Long-term effect of different fertilization and cropping systems on the soil antibiotic Resistome. Environ Sci Technol 52:13037–13046. https://doi.org/10.1021/ACS.EST.8B04330/SUPPL_FILE/ES8B04330_SI_001.PDF

Wang HT, Chi QQ, Zhu D et al (2019) Arsenic and sulfamethoxazole increase the incidence of antibiotic resistance genes in the gut of earthworm. Environ Sci Technol 53:10445–10453. https://doi.org/10.1021/ACS.EST.9B02277/SUPPL_FILE/ES9B02277_SI_001.PDF

Ward ND, Megonigal JP, Bond-Lamberty B et al (2020) Representing the function and sensitivity of coastal interfaces in earth system models. Nat Commun 11:1–14. https://doi.org/10.1038/s41467-020-16236-2

Waseem H, Williams MR, Stedtfeld RD, Hashsham SA (2017) Antimicrobial resistance in the environment. Water Environ Res 89:921–941. https://doi.org/10.2175/106143017X15023776270179

Wichmann F, Udikovic-Kolic N, Andrew S, Handelsman J (2014) Diverse antibiotic resistance genes in dairy cow manure. MBio 5:1017–1030. https://doi.org/10.1128/MBIO.01017-13/SUPPL_FILE/MBO002141797ST3.DOCX

Wilson DN, Hauryliuk V, Atkinson GC, O'Neill AJ (2020) Target protection as a key antibiotic resistance mechanism. Nat Rev Microbiol 18:637–648. https://doi.org/10.1038/s41579-020-0386-z

Wrighton KH (2018) Discovering antibiotics through soil metagenomics. Nat Rev Drug Discov 17: 241. https://doi.org/10.1038/nrd.2018.36

Yadav S, Kapley A (2021) Antibiotic resistance: global health crisis and metagenomics. Biotechnol Rep 29:e00604. https://doi.org/10.1016/J.BTRE.2021.E00604

Yang Y, Jiang X, Chai B et al (2016) ARGs-OAP: online analysis pipeline for antibiotic resistance genes detection from metagenomic data using an integrated structured ARG-database. Bioinformatics 32:2346–2351. https://doi.org/10.1093/BIOINFORMATICS/BTW136

Yang Y, Song W, Lin H et al (2018a) Antibiotics and antibiotic resistance genes in global lakes: a review and meta-analysis. Environ Int 116:60–73. https://doi.org/10.1016/J.ENVINT.2018.04.011

Yang Y, Zhou R, Chen B et al (2018b) Characterization of airborne antibiotic resistance genes from typical bioaerosol emission sources in the urban environment using metagenomic approach. Chemosphere 213:463–471. https://doi.org/10.1016/J.CHEMOSPHERE.2018.09.066

Ye SH, Siddle KJ, Park DJ, Sabeti PC (2019) Benchmarking metagenomics tools for taxonomic classification. Cell 178:779–794. https://doi.org/10.1016/j.cell.2019.07.010

Yin G, Hou L, Liu M et al (2016) Effects of thiamphenicol on nitrate reduction and N2O release in estuarine and coastal sediments. Environ Pollut 214:265–272. https://doi.org/10.1016/J.ENVPOL.2016.04.041

Yin X, Jiang XT, Chai B et al (2018) ARGs-OAP v2.0 with an expanded SARG database and hidden Markov models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes. Bioinformatics 34:2263–2270. https://doi.org/10.1093/BIOINFORMATICS/BTY053

Yu K, Li P, Chen Y et al (2020) Antibiotic resistome associated with microbial communities in an integrated wastewater reclamation system. Water Res 173:115541. https://doi.org/10.1016/J.WATRES.2020.115541

Zainab SM, Junaid M, Xu N, Malik RN (2020) Antibiotics and antibiotic resistant genes (ARGs) in groundwater: a global review on dissemination, sources, interactions, environmental and human health risks. Water Res 187:116455. https://doi.org/10.1016/J.WATRES.2020.116455

Zhang T, Zhang XX, Ye L (2011) Plasmid metagenome reveals high levels of antibiotic resistance genes and Mobile genetic elements in activated sludge. PloS One 6:e26041. https://doi.org/10.1371/JOURNAL.PONE.0026041

Zhang YJ, Hu HW, Yan H et al (2019) Salinity as a predominant factor modulating the distribution patterns of antibiotic resistance genes in ocean and river beach soils. Sci Total Environ 668:193–203. https://doi.org/10.1016/J.SCITOTENV.2019.02.454

Zhao Y, Tang H, Ye Y (2012) RAPSearch2: a fast and memory-efficient protein similarity search tool for next-generation sequencing data. Bioinformatics 28:125–126. https://doi.org/10.1093/BIOINFORMATICS/BTR595

Zhao H, Yan B, Mo X et al (2019a) Prevalence and proliferation of antibiotic resistance genes in the subtropical mangrove wetland ecosystem of South China Sea. Microbiology 8:e871. https://doi.org/10.1002/MBO3.871

Zhao Y, Liu H, Wang Q, Li B (2019b) The influence of three antibiotics on the growth, intestinal enzyme activities, and immune response of the juvenile sea cucumber Apostichopus japonicus selenka. Fish Shellfish Immunol 84:434–440. https://doi.org/10.1016/J.FSI.2018.10.022

Zheng D, Yin G, Liu M et al (2021) A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. Sci Total Environ 777:146009. https://doi.org/10.1016/J.SCITOTENV.2021.146009