Making Waves: The NORMAN Antibiotic Resistant Bacteria and Resistance Genes Database (NORMAN ARB&ARG) – an invitation for collaboration to tackle antibiotic resistance

Nikiforos Alygizakis[1,2*], Kelsey Ng[1,3], Ľuboš Čirka[4], Thomas Berendonk[5], Francisco Cerqueira[6,7], Eddie Cytryn[8], Geneviève Deviller[9], Gianuario Fortunato[10], Iakovos C. Iakovides[11,12], Ioannis Kampouris[13], Irene Michael-Kordatou[12], Foon Yin Lai[14], Lian Lundy[15], Celia M. Manaia[10], Roberto B.M. Marano[8], Gabriela K. Paulus[16,17,18], Benjamin Piña[7], Elena Radu[19,20,21], Luigi Rizzo[22], Katarzyna Ślipko[21], Norbert Kreuzinger[21], Nikolaos S. Thomaidis[2], Valentina Ugolini[14], Ivone Vaz-Moreira[10], Jaroslav Slobodnik[1], Despo Fatta-Kassinos[11,12]

[1]Environmental Institute, Okružná 784/42, 97241 Koš, Slovak Republic

[2]Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece

[3]RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, Building D29, 62500 Brno, Czech Republic

[4]Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Radlinského 9, Bratislava, Slovak Republic

[5]Technische Universität Dresden, Institute of Hydrobiology, Chair of Limnology, Zellescher Weg 40, 01062 Dresden, Germany

[6]Center for Health and Bioresources, Austrian Institute of Technology, Konrad-Lorenz-Straße 24, 3430 Tulln an der Donau

[7]Institute for Environmental Assessment and Water Research (IDAEA-CSIC). Jordi Girona, 18, 08034 barcelona, Spain

[8]Department of Soil Chemistry, Plant Nutrition and Microbiology, Institute of Soil, Water and Environmental Sciences, The Volcani Center, Agricultural Research Organization, Rishon Lezion, Israel

[9]DERAC Consultancy, 104 Grande Rue, 44240 Sucé-sur-Erdre, France

[10]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

[11]Department of Civil and Environmental Engineering, School of Engineering, University of Cyprus, P.O. Box 20537, Nicosia, 1678, Cyprus

[12]Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, Nicosia, 1678, Cyprus

[13]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

[14]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), SE-75007 Uppsala, Sweden

[15]Lulea Technical University, Lulea, Sweden

[16]KWR Watercycle Research Institute, Groningenhaven 7, 3433PE Nieuwegein, the Netherlands

[17]Delft University of Technology, Faculty of Civil Engineering & Geosciences, Department of Water Management, Stevinweg 1, 2628CN Delft, The Netherlands

[18]Amazon Web Services, Inc. 410 Terry Avenue North, Seattle, WA 98109-5210, U.S.A.

[19]Department of Virology, University of Medicine and Pharmacy Carol Davila, 37 Dionisie Lupu Street, 020021, Bucharest, Romania

[20]Department of Cellular and Molecular Pathology, Stefan S. Nicolau Institute of Virology, 285 Mihai Bravu Avenue, 030304, Bucharest, Romania

[21]Institute for Water Quality and Resource Management, Vienna University of Technology, Karlsplatz 13/226, 1040, Vienna, Austria

[22]Water Science and Technology (WaSTe) Group, Department of Civil Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084, Fisciano, SA, Italy

*Corresponding author

Nikiforos Alygizakis

Environmental Institute, Okružná 784/42,

972 41, Koš, Slovak Republic

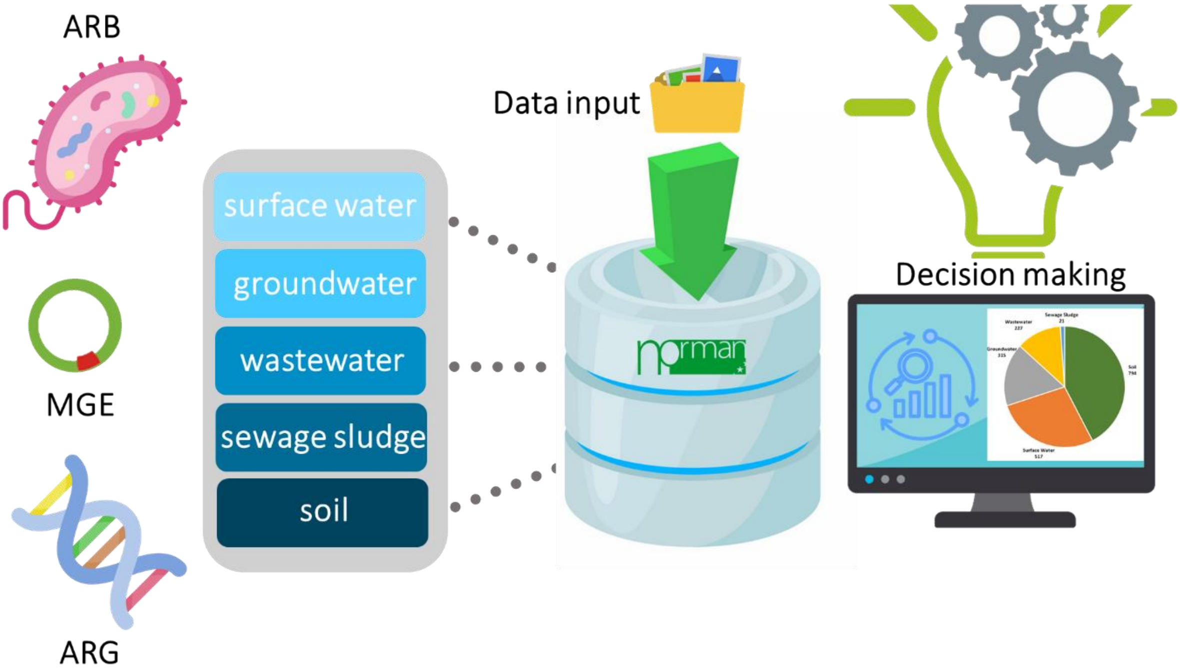
E-mail: alygizakis@ei.sk

Phone: +421 919183401

Highlights

- The NORMAN ARB&ARG enables open and rapid antibiotic resistance (AR) data sharing
- NORMAN ARB&ARG data provides an evidence base for regulators and reveal data gaps
- AR data from three studies were successfully imported to the database as a demonstration

Graphical abstract



Abstract

With the global concerns on antibiotic resistance (AR) as a public health issue, it is pivotal to have data exchange platforms for studies on antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment. For this purpose, the NORMAN Association is hosting the NORMAN ARB&ARG database, which was developed within the European project ANSWER. The present article provides an overview on the database functionalities, the extraction and the contribution of data to the database. In this study, AR data from three studies from China and Nepal were extracted and imported into the NORMAN ARB&ARG in addition to the existing AR data from 11 studies (mainly European studies) on the database. This feasibility study demonstrates how the scientific community can share their data on AR to generate an international evidence base to inform AR mitigation strategies. The open and FAIR data are of high potential relevance for regulatory applications, including the development of emission limit values / environmental quality standards in relation to AR. The growth in sharing of data and analytical methods will foster collaboration on risk management of AR worldwide, and facilitate the harmonization in the effort for identification and surveillance of critical hotspots of AR. The NORMAN ARB&ARG database is publicly available at: https://www.norman-network.com/nds/bacteria/

Keywords: Antibiotic resistant bacteria; antibiotic resistance genes; wastewater treatment plant; wastewater reuse practises

1. Introduction

Antibiotic resistance (AR) has been identified as one of the biggest threats to global health and food security by the World Health Organization (WHO) **(World Health Organization)**. In particular, antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and other mobile genetic elements (MGEs) with the potential to be taken up and expressed by a pathogen are the forms of AR of epidemiological concerns **(Zhang et al. 2021)**. Therefore, the assessment of antimicrobial resistance risk has been proposed as a part of the marketing authorisation for antibiotics in the EU **(European Commission 2021)**. Some agricultural activities, such as application of nonessential antibiotics in livestock farming, contribute to the dissemination of anthropogenic AR in the aquatic ecosystems **(Manyi-Loh et al. 2018)**. Therefore, monitoring of AR has been a requirement for environmental risk assessment plan for reclaimed agriculture wastewater **(The European Parliament and the Council of the European Union 2020)**. In addition, wastewater treatment plant (WWTP) effluents are proven to be a critical point source for the release of AR in the environment as they provide favourable conditions for the development of AR including the close contact between antibiotics and a broad range of bacteria (of environmental, human and animal origins) with stable pH and temperature **(Di Cesare et al. 2016, Kim et al. 2014, Roberto B. M. Marano 2017)**. Aware of this situation, the European Commission recently recommended that by 2025 antibiotic resistance is monitored at least twice a year in WWTPs serving more than 100,000 population equivalents **(European Commission)**. Therefore, it is important to monitor the removal of ARB, ARGs and MGEs at the WWTPs, report their presence in environmental matrices and to share data in open platforms. The scientific community has initiated investigations to assess the status of AR pollution, including studies under the ANSWER H2020-MSCA-ITN-2015/675530 **(European Commission)** and the WATER-JPI StARE **(Parnanen et al. 2019)** projects for example.

To make full use of emerging datasets and knowledge, it is essential to define ARB, ARGs and MGEs with the highest epidemiological relevance **(Keenum et al. 2022)**, and effectively consolidate the findings from studies on AR. General repositories of ARB and ARG data exist **(Wilkinson et al. 2016)**, but efforts to bring these new contaminants of concern at regulatory level require the application of FAIR principles for sharing of the data and focus on reusability of the data to establish baseline concentration levels in various environmental compartments. Standardized data collection templates (DCTs) have been developed for web-based database for AR data in the ITN MSCA ANSWER project **(European Commission)**. With the knowledge and know-how transfer to the NORMAN Association, the NORMAN ARB&ARG, https://www.norman-network.com/nds/bacteria/ **(NORMAN)** was established to provide open and long living data on AR. The database facilitates the exchange of AR data, enabling the scientific community to generate new knowledge to tackle the AR issue.

The NORMAN ARB&ARG aims to support (1) the automated prioritization of biological risk factors, with potential connection with the NORMAN Working Group 1 (Prioritization of emerging substances) and European Partnership for Chemicals Risk Assessment (PARC) **(Dulio et al. 2020)**; (2) the application of data in models for large-scale projections and policy development, including the establishment of baseline concentration levels; and (3) the derivation of transparent science-based emission limit values (ELVs) for the target ARGs in treated wastewater intended for reuse. The present article covers the infrastructure of the NORMAN ARB&ARG database, and the process of extracting data from and contributing data to the database. It further provides an overview of the current state and the future perspectives on the risk management of AR from chemicals and biological contaminants.

2. Database infrastructures

In order to assure the sustainability of the database, it is hosted by the NORMAN Association, an independent self-funded organisation working on contaminants of emerging concern. The database consists of three sections: the sections “antibiotic resistance bacteria” and “antibiotic resistance genes” contain data on genetic elements including ARB and AR genetic elements that were uploaded to the database respectively (see sections 2.1 and 2.3); the “DCT download” section contains DCTs for the scientific community to contribute their AR data to the database (see section 2.2). The DCTs cover a broad range of matrices including wastewater, surface water, groundwater, sea water, soil, sewage sludge, plant crop and air. All information on the database is publicly available.

2.1 Data sources of the NORMAN ARB&ARG

As of June 2023, 11 studies on the occurrence of AR mainly under the ITN MSCA ANSWER project have been uploaded to the NORMAN ARB&ARG (**Table 1**). In total, 2,557 data points covering a range of environmental matrices were contributed to the database, including soil (n=794), surface water (n=517), groundwater (n=315), wastewater (n=147) and sewage sludge (n=21) from nine countries (Germany, Sweden, Spain, Austria, Israel, Cyprus, Netherlands, Switzerland and Portugal).

Table 1. Summary of the 11 studies with AR data publicly available on the NORMAN ARB&ARG

Countries	**Studied matrices***	**AR data**	**Major findings**	**References**
Austria	Distilled water, WW and SS	ARB & genetic elements	A molecular weight cut off (at filtering membrane for water treatment) smaller than 5000 Da is required to effectively remove AR-encoded free DNA	**(Slipko et al. 2019)**
Austria	SW & Soil	Genetic elements	The first genome-based characterization of an environmental USA300 isolate, providing the ARG profile in surface water and soil samples collected in Austria.	**(Lepuschitz et al. 2018)**
Cyprus	WW	ARB & genetic elements	The inactivation of ARB and reduction of ARG abundance in wastewater by ozonation was found to be governed by the Chick-Watson model-predicted ozone exposure value.	**(Iakovides et al. 2021)**
Germany	GW, WW & Soil	Genetic elements	Irrigation with treated wastewater was found to promote the dissemination of the sulfonamide ARG sul1 and the integrase gene intI1 to groundwater microbiota.	**(Kampouris et al. 2022)**
Germany, Netherlands, Switzerland,	SW	Genetic elements	ARG profiles across the Rhine river were established: 5 Rhine-typical genes (ermB, ermF, Intl1, sul1 and tetM) and the 2 most abundance genes (Intl1 and sul1) were found.	**(Paulus et al. 2020)**

Israel	Soil, SW, WW	Genetic elements	The potential dissemination of ARGs via treated wastewater irrigation was investigated: no correlation between gene abundance in irrigation water and those detected in soil	**(Marano et al. 2019)**
Netherlands	WW (from hospital)	Genetic elements	Membrane bioreactor treatment was found to be the most efficient on-site treatment of hospital wastewater in reducing ARGs.	**(Paulus et al. 2019)**
Portugal	Soil	Genetic elements	The commonly used qPCR procedures are not able to quantify ARGs at or below an abundance of one thousand copies per gram of soil dry weight. ARGs at such levels could pose significant risk to environmental and human health.	**(Fortunato et al. 2018)**
Spain	Soil	Genetic elements	Water quality (ARG abundance in the water used for irrigation) was proven to play a significant role in the development of AR in crops	**(Cerqueira et al. 2019a)**
Spain	Soil	Genetic elements	Microbiome composition and ARG levels in crops were found to be influenced by agricultural practices, including the selection of irrigation water.	**(Cerqueira et al. 2019b)**
Sweden	SW	Genetic elements	The first quantitative assessment of ARGs in the aquatic environment of Sweden, which revealed the widespread of 150 ARGs in urban recipient waters	**(Lai et al. 2021)**

* WW: wastewater; SS: sewage sludge; SW: surface water; GW: groundwater

2.2 Contribution to the NORMAN ARB&ARG

Currently, the NORMAN ARB&ARG database contains AR data primarily from surface water and soils from European studies. The development in data coverage of the database in more diverse and globally available geographical locations and environmental matrices would provide a more comprehensive overview of the status of AR distribution across the globe.

The scientific community could contribute to the ARB&ARG database expansion by uploading data from their studies, increasing knowledge on global ARB and ARG occurrence and distribution.

Researchers could include AR data to the database via the DCTs, which are available for download at https://www.norman-network.com/nds/bacteria/downloadDCT.php. The DCTs include columns for detailed descriptions of the studied genes (such as gene description, gene family, associated phenotype and multi-drug resistance phenotype) in order to avoid ambiguity and provide insights on genes of epidemiological relevance. Submitted data would undergo quality check by the NORMAN ARB&ARG team and be publicly available on the database afterwards. The check includes availability of mandatory data fields (see instructions of the DCTs) such as sample matrix, AR abundance, bacterial groups and gene names.

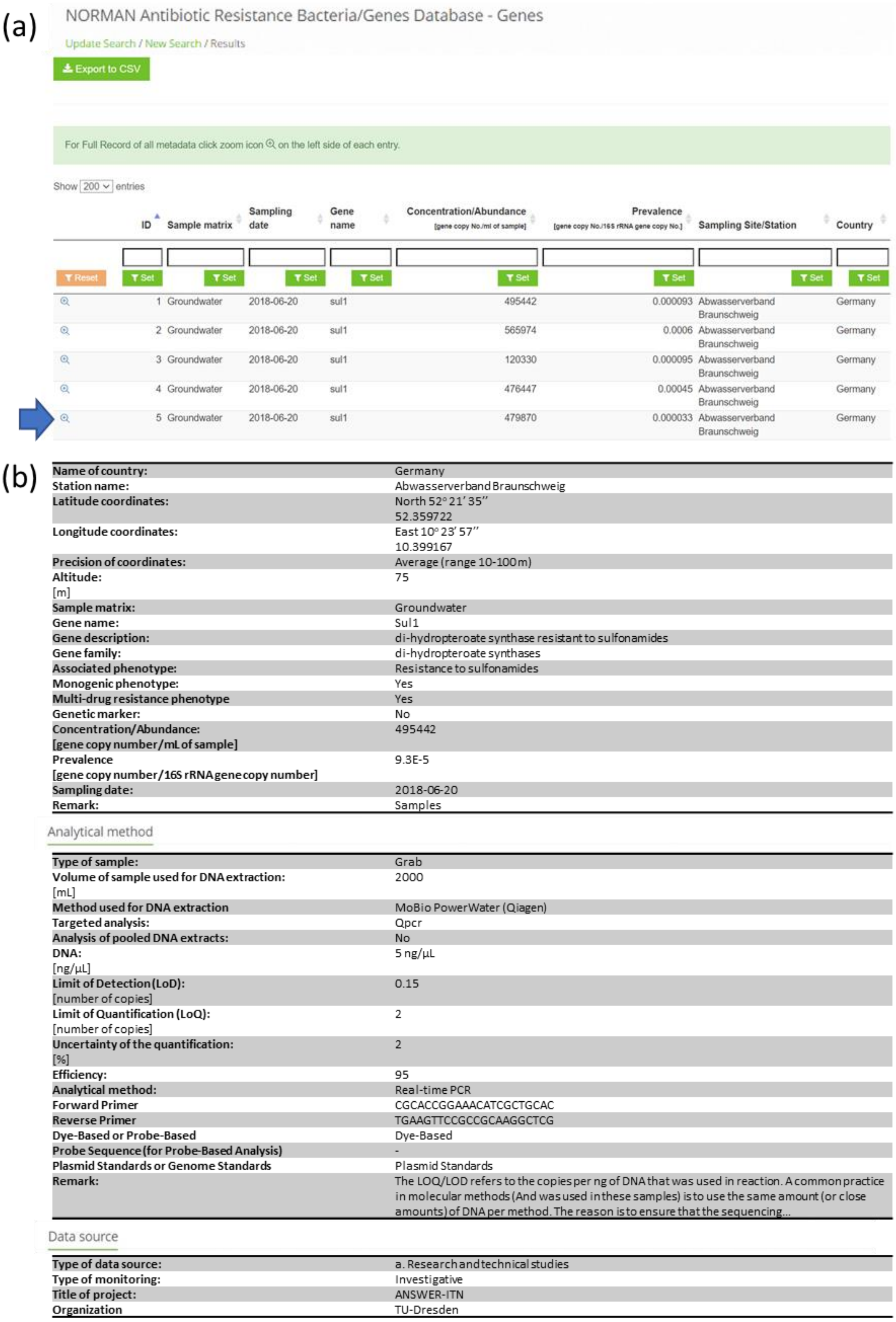
There are separate DCTs for submission of AR data in various matrices (water, soil, air, plant crop and sewage sludge). Each DCT contains 3 modules: data source, analytical method and analysis, which allows the contributors to include relevant information of their studies to the database. The data source sheet contains information about the study such as contact person and relevant literature; the analytical method sheet involves information about the determination method such as limit of detection (LOD) and uncertainty of the analysis; the analysis sheet entails outputs of the analysis such as the individual abundance of ARB or AR genetic elements. AR data of low quality would leave multiple empty spaces in the DCTs including the mandatory fields. Therefore, the DCTs serve as a quality assurance procedure for the AR data submission in term of comprehensiveness of information, such as missing details in sampling process or analytical methods.

In the present study, a feasibility study was performed by importing AR data from three selected studies from Asia (two from China and one from Nepal) to the NORMAN ARB&ARG **(Amarasiri et al. 2022, Ben et al. 2017, Du et al. 2014)**. It demonstrates how the scientific community could share their AR data to the database, making them publicly available.

2.3 Data extraction from the NORMAN ARB&ARG

The NORMAN ARB&ARG allows search of the uploaded occurrence data of ARB and AR genetic elements by sampling information (for example, sampling site and year) and contaminant information (bacterial group for ARB and gene name for ARGs). **Figure 1a** demonstrates how users could apply filtering when retrieving occurrence data from the database. This includes filtering options for country, sample matrix, sampling site/station, organization, year of analysis, gene name for ARGs and bacterial group for ARB.

The search would provide entries of the available data which match the selected filter, in **Figure 1a** an example: entries that match all 3 search criteria of “Germany” for “Country”; “Groundwater” for “Sample matrix” and “sul1” for “Gene name”. By clicking on the magnifier symbol of the entries (indicated with blue arrow), full record of the metadata from previous studies (abundance, sampling and analytical method, etc.) could be obtained as shown in **Figure 1b**. The publicly available AR data can be pulled from the database and exported as CSV file for further analysis.



NORMAN Antibiotic Resistance Bacteria/Genes Database - Genes

Update Search / New Search / Results

For Full Record of all metadata click zoom icon on the left side of each entry.

Show 200 entries

ID	Sample matrix	Sampling date	Gene name	Concentration/Abundance [gene copy No./ml of sample]	Prevalence [gene copy No./16S rRNA gene copy No.]	Sampling Site/Station	Country
1	Groundwater	2018-06-20	sul1	495442	0.000093	Abwasserverband Braunschweig	Germany
2	Groundwater	2018-06-20	sul1	565974	0.0006	Abwasserverband Braunschweig	Germany
3	Groundwater	2018-06-20	sul1	120330	0.000095	Abwasserverband Braunschweig	Germany
4	Groundwater	2018-06-20	sul1	476447	0.00045	Abwasserverband Braunschweig	Germany
5	Groundwater	2018-06-20	sul1	479870	0.000033	Abwasserverband Braunschweig	Germany

Name of country:	Germany
Station name:	Abwasserverband Braunschweig
Latitude coordinates:	North 52° 21′ 35″ 52.359722
Longitude coordinates:	East 10° 23′ 57″ 10.399167
Precision of coordinates:	Average (range 10-100 m)
Altitude: [m]	75
Sample matrix:	Groundwater
Gene name:	Sul1
Gene description:	di-hydropteroate synthase resistant to sulfonamides
Gene family:	di-hydropteroate synthases
Associated phenotype:	Resistance to sulfonamides
Monogenic phenotype:	Yes
Multi-drug resistance phenotype	Yes
Genetic marker:	No
Concentration/Abundance: [gene copy number/mL of sample]	495442
Prevalence [gene copy number/16S rRNA gene copy number]	9.3E-5
Sampling date:	2018-06-20
Remark:	Samples

Analytical method

Type of sample:	Grab
Volume of sample used for DNA extraction: [mL]	2000
Method used for DNA extraction	MoBio PowerWater (Qiagen)
Targeted analysis:	Qpcr
Analysis of pooled DNA extracts:	No
DNA: [ng/µL]	5 ng/µL
Limit of Detection (LoD): [number of copies]	0.15
Limit of Quantification (LoQ): [number of copies]	2
Uncertainty of the quantification: [%]	2
Efficiency:	95
Analytical method:	Real-time PCR
Forward Primer	CGCACCGGAAACATCGCTGCAC
Reverse Primer	TGAAGTTCCGCCGCAAGGCTCG
Dye-Based or Probe-Based	Dye-Based
Probe Sequence (for Probe-Based Analysis)	-
Plasmid Standards or Genome Standards	Plasmid Standards
Remark:	The LOQ/LOD refers to the copies per ng of DNA that was used in reaction. A common practice in molecular methods (And was used in these samples) is to use the same amount (or close amounts) of DNA per method. The reason is to ensure that the sequencing...

Data source

Type of data source:	a. Research and technical studies
Type of monitoring:	Investigative
Title of project:	ANSWER-ITN
Organization	TU-Dresden

Figure 1. (a) Overview of the search options and criteria for retrieving data from the NORMAN ARB&ARG; **(b)** outputs for the search of ARGs with the selected search criteria on the database and metadata of the first entry that matches the search criteria **(Kampouris et al. 2022)**.

3. Feasibility study and Discussion

The NORMAN ARB&ARG database is a European initiative, therefore, most of the contributed data are from European studies. Currently, limited data from studies of other regions are available on the database. Nonetheless, there is much more data available from published studies from other countries that could be imported to the database. As a feasibility study to demonstrate how the scientific community can share their AR data, study experience and analytical methods to the database, the data from three selected studies of environmental occurrence of AR were imported to the NORMAN ARB&ARG using the DCTs. In the first selected study, the distribution of AR in ten municipal WWTPs in three major cities of China (Beijing, Wuxi and Qingdao) was investigated, which revealed the importance of disinfection during wastewater treatment in removing AR **(Ben et al. 2017)**. In the second study, the occurrence and abundance of 12 resistance genes in five WWTPs in Shanghai and Jiangsu Province of China were also studied, which include ten tetracycline resistance genes and two sulfonamides resistance genes **(Du et al. 2014)**. In the third study, the concentrations of ARGs in the water bodies near Kathmandu Valley, Nepal were evaluated, which include six river water and 30 groundwater samples **(Amarasiri et al. 2022)**. The AR data were taken from the publications of the studies and imported to the database using the DCTs. AR data of the three selected studies are now available on the NORMAN ARB&ARG and can be used to support regulation requests.

With the constant growth of AR data to the NORMAN ARB&ARG, the database could support the establishment of comprehensive profiles of AR and AR monitoring in certain regions/nations. For example, the abundance and diversity of ARB or AR genetic elements in a particular area and matrix could be obtained by applying proper filters for retrieving data from the database. Such findings could be further linked to the concomitant usage of antibiotics in the studied area to characterise the source and status of AR. The availability of data on the database also sheds light on the data gap in AR data in certain countries, which could require further monitoring programs on the occurrence profile of ARB or AR genetic elements, or to extract AR data of the countries from recent publications.

4. Conclusions and future perspectives

- NORMAN ARB&ARG database was designed to facilitate open and FAIR data exchange of AR in environmental matrices
- Data collection is done using excel templates called DCTs, which are publicly available for download on the database
- A feasibility study based on three selected studies (two from China and one from Nepal) successfully demonstrated how the scientific community can share their AR data
- The NORMAN ARB&ARG is expected to grow from the current to more environmental matrices across the world
- The development of NORMAN ARB&ARG can create synergy by fostering collaboration on the risk management of AR worldwide and can facilitate the harmonization in identification and surveillance of critical hotspots of AR.
- The data on the database is crucial and valuable in regulatory applications, including the establishment of baseline concentrations of ARGs and the development of ELVs of ARGs.

Acknowledgements

Authors gratefully acknowledge the International Commission for the Protection of Danube River (ICPDR) for the guidance and support. We are thankful to the researchers who participated in the Joint Danube Survey 4. One of the authors, Kelsey Ng, thanks the RECETOX Research Infrastructure (No LM2023069) financed by the Ministry of Education, Youth and Sports fur supportive background. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 859891 and was supported from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857560. This publication reflects only the authors' view and the European Commission is not responsible for any use that may be made of the information it contains. Foon Yin Lai acknowledges the funding from FORMAS (project number: 2019-01161) and her SLU Career Grant.

Conflict of interest

The authors declare no conflict of interest.

References

Amarasiri, M., Takezawa, T., Malla, B., Furukawa, T., Sherchand, J.B., Haramoto, E. and Sei, K. (2022) Prevalence of antibiotic resistance genes in drinking and environmental water sources of the Kathmandu Valley, Nepal. Front Microbiol 13, 894014.

Angelakis, A.N. and Gikas, P. (2014) Water reuse: Overview of current practices and trends in the world with emphasis on EU states. Water Utility Journal 8, 67-78.

Ben, W., Wang, J., Cao, R., Yang, M., Zhang, Y. and Qiang, Z. (2017) Distribution of antibiotic resistance in the effluents of ten municipal wastewater treatment plants in China and the effect of treatment processes. Chemosphere 172, 392-398.

Cerqueira, F., Matamoros, V., Bayona, J., Elsinga, G., Hornstra, L.M. and Pina, B. (2019a) Distribution of antibiotic resistance genes in soils and crops. A field study in legume plants (Vicia faba L.) grown under different watering regimes. Environ Res 170, 16-25.

Cerqueira, F., Matamoros, V., Bayona, J. and Pina, B. (2019b) Antibiotic resistance genes distribution in microbiomes from the soil-plant-fruit continuum in commercial Lycopersicon esculentum fields under different agricultural practices. Sci Total Environ 652, 660-670.

Di Cesare, A., Fontaneto, D., Doppelbauer, J. and Corno, G. (2016) Fitness and Recovery of Bacterial Communities and Antibiotic Resistance Genes in Urban Wastewaters Exposed to Classical Disinfection Treatments. Environ Sci Technol 50(18), 10153-10161.

Du, J., Ren, H., Geng, J., Zhang, Y., Xu, K. and Ding, L. (2014) Occurrence and abundance of tetracycline, sulfonamide resistance genes, and class 1 integron in five wastewater treatment plants. Environ Sci Pollut Res Int 21(12), 7276-7284.

Dulio, V., Koschorreck, J., van Bavel, B., van den Brink, P., Hollender, J., Munthe, J., Schlabach, M., Aalizadeh, R., Agerstrand, M., Ahrens, L., Allan, I., Alygizakis, N., Barcelo', D., Bohlin-Nizzetto, P., Boutroup, S., Brack, W., Bressy, A., Christensen, J.H., Cirka, L., Covaci, A., Derksen, A., Deviller, G., Dingemans, M.M.L., Engwall, M., Fatta-Kassinos, D., Gago-Ferrero, P., Hernández, F., Herzke, D., Hilscherová, K., Hollert, H., Junghans, M., Kasprzyk-Hordern, B., Keiter, S., Kools, S.A.E., Kruve, A., Lambropoulou, D., Lamoree, M., Leonards, P., Lopez, B., López de Alda, M., Lundy, L., Makovinská, J., Marigómez, I., Martin, J.W., McHugh, B., Miège, C., O'Toole, S., Perkola, N., Polesello, S., Posthuma, L., Rodriguez-Mozaz, S., Roessink, I., Rostkowski, P., Ruedel, H., Samanipour, S., Schulze, T., Schymanski, E.L., Sengl, M., Tarábek, P., Ten Hulscher, D., Thomaidis, N., Togola, A., Valsecchi, S., van Leeuwen, S., von der Ohe, P., Vorkamp, K., Vrana, B. and Slobodnik, J. (2020) The NORMAN Association and the European Partnership for Chemicals Risk Assessment (PARC): let's cooperate! Environmental Sciences Europe 32(1).

European Commission ANtibioticS and mobile resistance elements in WastEwater Reuse applications: risks and innovative solutions.

European Commission Urban wastewater - Ensuring that urban wastewater is properly dealt with to protect the environment and human health.

European Commission (2021) Evaluation and revision of the general pharmaceutical legislation.

Fortunato, G., Vaz-Moreira, I., Becerra-Castro, C., Nunes, O.C. and Manaia, C.M. (2018) A rationale for the high limits of quantification of antibiotic resistance genes in soil. Environmental Pollution 243, 1696-1703.

Iakovides, I.C., Manoli, K., Karaolia, P., Michael-Kordatou, I., Manaia, C.M. and Fatta-Kassinos, D. (2021) Reduction of antibiotic resistance determinants in urban wastewater by ozone: Emphasis on the impact of wastewater matrix towards the inactivation kinetics, toxicity and bacterial regrowth. Journal of Hazardous Materials 420.

Kampouris, I.D., Alygizakis, N., Klumper, U., Agrawal, S., Lackner, S., Cacace, D., Kunze, S., Thomaidis, N.S., Slobdonik, J. and Berendonk, T.U. (2022) Elevated levels of antibiotic resistance in groundwater during treated wastewater irrigation associated with infiltration and accumulation of antibiotic residues. J Hazard Mater 423(Pt B), 127155.

Keenum, I., Liguori, K., Calarco, J., Davis, B.C., Milligan, E., Harwood, V.J. and Pruden, A. (2022) A framework for standardized qPCR-targets and protocols for quantifying antibiotic resistance in surface water, recycled water and wastewater. Critical Reviews in Environmental Science and Technology 52(24), 4395-4419.

Kim, S., Yun, Z., Ha, U.H., Lee, S., Park, H., Kwon, E.E., Cho, Y., Choung, S., Oh, J., Medriano, C.A. and Chandran, K. (2014) Transfer of antibiotic resistance plasmids in pure and activated sludge cultures in the presence of environmentally representative micro-contaminant concentrations. Sci Total Environ 468-469, 813-820.

Lai, F.Y., Muziasari, W., Virta, M., Wiberg, K. and Ahrens, L. (2021) Profiles of environmental antibiotic resistomes in the urban aquatic recipients of Sweden using high-throughput quantitative PCR analysis. Environ Pollut 287, 117651.

Lepuschitz, S., Huhulescu, S., Hyden, P., Springer, B., Rattei, T., Allerberger, F., Mach, R.L. and Ruppitsch, W. (2018) Characterization of a community-acquired-MRSA USA300 isolate from a river sample in Austria and whole genome sequence based comparison to a diverse collection of USA300 isolates. Sci Rep 8(1), 9467.

Manyi-Loh, C., Mamphweli, S., Meyer, E. and Okoh, A. (2018) Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. Molecules 23(4).

Marano, R.B.M., Zolti, A., Jurkevitch, E. and Cytryn, E. (2019) Antibiotic resistance and class 1 integron gene dynamics along effluent, reclaimed wastewater irrigated soil, crop continua: elucidating potential risks and ecological constraints. Water Res 164, 114906.

NORMAN NORMAN Antibiotic Resistance Bacteria/Genes Database.

Parnanen, K.M.M., Narciso-da-Rocha, C., Kneis, D., Berendonk, T.U., Cacace, D., Do, T.T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J.L., Michael, S.G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H., Sorum, H., Stedtfeld, R.D., Tiedje, J.M., Giustina, S.V.D., Walsh, F., Vaz-Moreira, I., Virta, M. and Manaia, C.M. (2019) Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. Sci Adv 5(3), eaau9124.

Paulus, G.K., Hornstra, L.M., Alygizakis, N., Slobodnik, J., Thomaidis, N. and Medema, G. (2019) The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. Int J Hyg Environ Health 222(4), 635-644.

Paulus, G.K., Hornstra, L.M. and Medema, G. (2020) International tempo-spatial study of antibiotic resistance genes across the Rhine river using newly developed multiplex qPCR assays. Sci Total Environ 706, 135733.

Roberto B. M. Marano, E.C. (2017) Antimicrobial Resistance in Wastewater Treatment Processes (ISBN: 9781119192435). Patricia L. Keen, R.F. (ed).

Schymanski, E.L. and Bolton, E.E. (2022) FAIRifying the exposome journal: Templates for chemical structures and transformations. Exposome 2(1).

Slipko, K., Reif, D., Wogerbauer, M., Hufnagl, P., Krampe, J. and Kreuzinger, N. (2019) Removal of extracellular free DNA and antibiotic resistance genes from water and wastewater by membranes ranging from microfiltration to reverse osmosis. Water Res 164, 114916.

The European Parliament and the Council of the European Union (2020) Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse (Text with EEA relevance).

Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes, A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C.T., Finkers, R., Gonzalez-Beltran, A., Gray, A.J., Groth, P., Goble, C., Grethe, J.S., Heringa, J., t Hoen, P.A., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E., Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone, S.A., Schultes, E., Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M., van der Lei, J., van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P., Wolstencroft, K., Zhao, J. and Mons, B. (2016) The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018.

World Health Organization Antibiotic resistance.

Zhang, A.N., Gaston, J.M., Dai, C.L., Zhao, S., Poyet, M., Groussin, M., Yin, X., Li, L.G., van Loosdrecht, M.C.M., Topp, E., Gillings, M.R., Hanage, W.P., Tiedje, J.M., Moniz, K., Alm, E.J. and Zhang, T. (2021) An omics-based framework for assessing the health risk of antimicrobial resistance genes. Nat Commun 12(1), 4765.