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Making Waves

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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

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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/.

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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, 2023). 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 and 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 Urban Wastewater, 2024). 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-2015/675,530 (European Commission, 2015) 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/ 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, 2557 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).

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

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.

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.

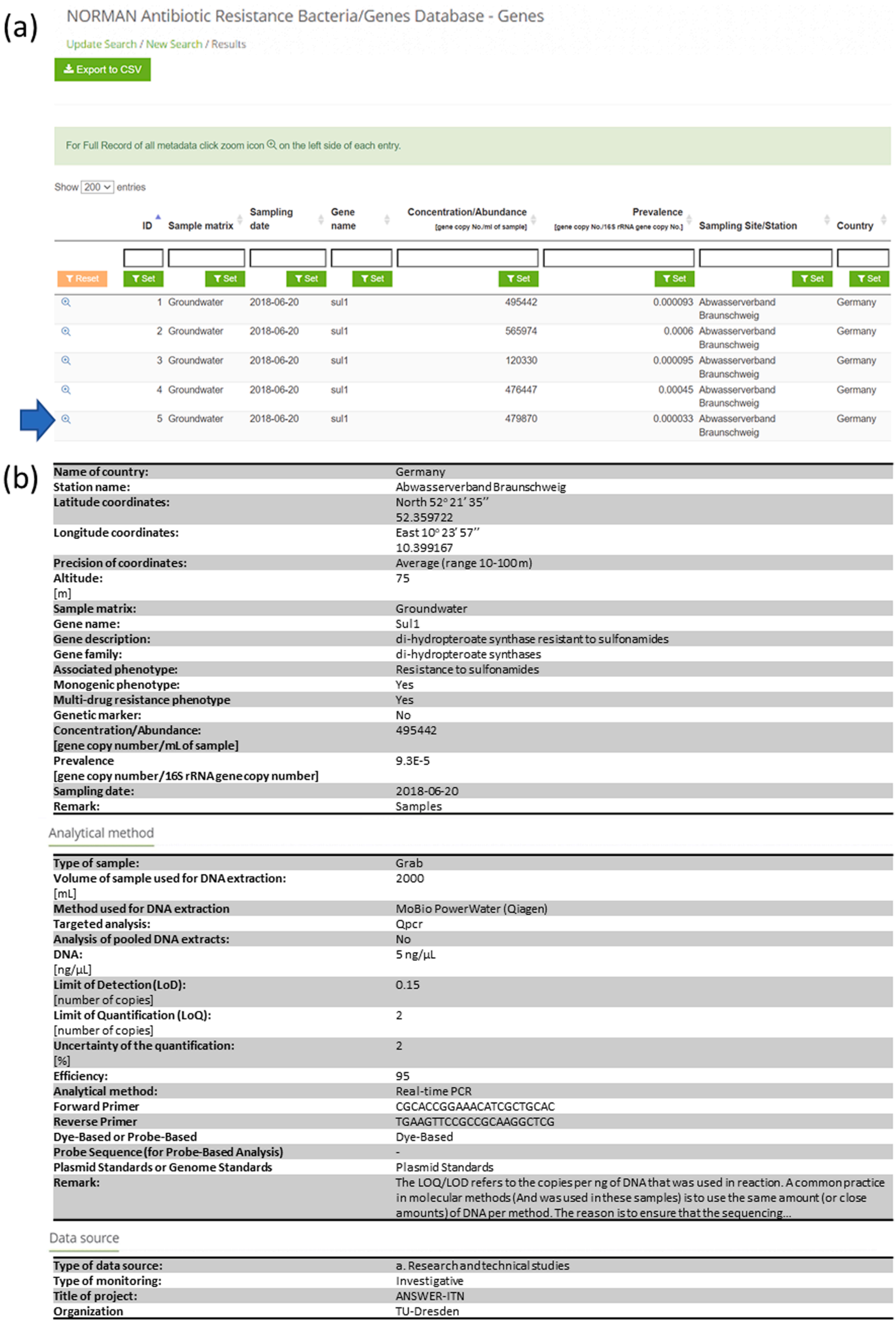
2.3. *Data extraction from the NORMAN ARB&ARG*

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). Fig. 1**a** 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 Fig. 1**a** 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 Fig. 1**b**. The publicly available AR data can be pulled from the database and exported as CSV file for further analysis.

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.



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

Fig. 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).

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.

CRediT authorship contribution statement

Nikiforos Alygizakis: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Kelsey Ng:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Ľuboš Čirka:** Writing – review & editing, Validation, Software, Methodology. **Thomas Berendonk:** Writing – review & editing, Supervision, Resources. **Francisco Cerqueira:** Writing – review & editing, Resources, Data curation. **Eddie Cytryn:** Writing – review & editing, Supervision, Resources. **Geneviève Deviller:** Writing – review & editing, Resources. **Gianuario Fortunato:** Writing – review & editing, Resources. **Iakovos C. Iakovides:** Writing – review & editing, Resources. **Ioannis Kampouris:** Writing – review & editing, Resources. **Irene Michael-Kordatou:** Writing – review & editing, Methodology, Investigation. **Foon Yin Lai:** Writing – review & editing, Resources. **Lian Lundy:** Writing – review & editing, Resources, Project administration. **Celia M. Manaia:** Writing – review & editing, Supervision, Resources. **Roberto B.M. Marano:** Writing – review & editing, Resources. **Gabriela K. Paulus:** Writing – review & editing, Resources. **Benjamin Piña:** Writing – review & editing, Supervision, Resources. **Elena Radu:** Writing – review & editing, Resources. **Luigi Rizzo:** Writing – review & editing, Supervision, Resources. **Katarzyna Ślipko:** Writing – review & editing, Resources. **Norbert Kreuzinger:** Writing – review & editing, Supervision, Resources. **Nikolaos S. Thomaidis:** Writing – review & editing, Supervision, Resources. **Valentina Ugolini:** Writing – review & editing, Software. **Ivone Vaz-Moreira:** Writing – review & editing, Resources. **Jaroslav Slobodnik:** Writing – review & editing, Supervision, Software, Resources. **Despo Fatta-Kassinos:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

All data is available online. The paper presents a database. All sample collections, sample meta-data, and analytical methods are available in the database website.

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