



## Review article



## Mapping the evidence of the effects of environmental factors on the prevalence of antibiotic resistance in the non-built environment

Iñaki Deza-Cruz <sup>a,b</sup>, Alexandre de Menezes <sup>c</sup> <sup>\*</sup>, Brian Gardner <sup>a,\*</sup>, İlknur Aktan <sup>a</sup>, Sarhad Alnajjar <sup>a</sup>, Martha Betson <sup>a,d</sup>, Adriana Cabal Rosel <sup>e</sup>, Manuela Caniça <sup>f</sup>, Mark A. Chambers <sup>a,g</sup>, Georgina Tarrant <sup>a</sup>, Francesca Contadini <sup>a</sup>, Olukayode Daramola <sup>a,h</sup>, Rani de la Rivière <sup>f</sup>, Bernadette Egan <sup>g</sup>, Abel Ekiri <sup>a</sup>, Catherine Finnegan <sup>a</sup>, Laura C. Gonzalez Villeta <sup>a</sup>, Richard Green <sup>i</sup>, Belinda Hall <sup>g</sup>, Marwa M. Hassan <sup>a</sup>, Martin Hawes <sup>a</sup>, Sara Healy <sup>j</sup>, Lisa Holbrook <sup>a</sup>, Damla Kaya <sup>i</sup>, Prashant Kumar <sup>k,d</sup>, Roberto M. La Ragione <sup>a,g</sup>, Daniel Maupin <sup>i</sup>, Jai W. Mehat <sup>g</sup>, Davide Messina <sup>l</sup>, Kelly Moon <sup>m</sup>, Elizabeth Mumford <sup>a,d</sup>, Gordon Nichols <sup>p,a,n,o</sup>, Daniel V. Olivença <sup>f</sup>, Joaquin M. Prada <sup>a,q,d</sup>, Claire Price <sup>i</sup>, Christopher Proudman <sup>a</sup>, Retha Queenan <sup>a</sup>, Miguel Ramos <sup>f</sup>, Jaime Riccomini Closo <sup>a</sup>, Jennifer M. Ritchie <sup>g,d</sup>, Lorenzo A. Santorelli <sup>g</sup>, Nick Selemetas <sup>g</sup>, Matt Spick <sup>i</sup>, Yashwanth Subbannayya <sup>g</sup>, Shelini Surendran <sup>g</sup>, Pedro Teixeira <sup>f</sup>, Mukunthan Tharmakulasingam <sup>r</sup>, Damian Valle <sup>a</sup>, Arnoud H.M. van Vliet <sup>a</sup>, Marco Videira <sup>f</sup>, Hazel Wallace-Williams <sup>s</sup>, Klara M. Wanelik <sup>g</sup>, Markus Woegerbauer <sup>e</sup>, Sydney Wright <sup>t,b</sup>, Giovanni Lo Iacono <sup>a,q,d</sup> 

<sup>a</sup> School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7AL, Surrey, UK

<sup>b</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, EH25 9RG, Midlothian, UK

<sup>c</sup> School of Biological and Chemical Sciences, Ryan Institute, University of Galway, Ireland

<sup>d</sup> Institute for Sustainability, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>e</sup> Austrian Agency for Health and Food Safety, Austria

<sup>f</sup> National Institute of Health Dr. Ricardo Jorge, Portugal

<sup>g</sup> School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>h</sup> School of Veterinary Medicine, University of Central Lancashire, UK

<sup>i</sup> School of Health Sciences, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>j</sup> The Royal Veterinary College, London, UK

<sup>k</sup> Global Centre for Clean Air Research (GCARE), School of Sustainability, Civil and Environmental Engineering, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>l</sup> School of Veterinary Medicine and Science, Faculty of Medicine and Health Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

<sup>m</sup> Nuffield Centre for International Health and Development, University of Leeds, UK and Priestley Centre for Climate Futures, School of Earth and Environment, University of Leeds, UK

<sup>n</sup> European Centre for Environment and Human Health, University of Exeter Medical School, Truro, Cornwall, UK

<sup>o</sup> University of East Anglia, Norwich, UK

<sup>p</sup> UK Health Security Agency, UK

<sup>q</sup> The Surrey Institute for People-Centred Artificial Intelligence, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>r</sup> Centre for Vision, Speech and Signal Processing, Faculty of Electronics and Physical Sciences, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>s</sup> School of Psychology, University of Surrey, Guildford GU2 7XH, Surrey, UK

<sup>t</sup> University of Missouri, School of Public Health, USA

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## ABSTRACT

\* Corresponding authors.

E-mail addresses: [alexandre.demenezes@universityofgalway.ie](mailto:alexandre.demenezes@universityofgalway.ie) (A. de Menezes), [b.gardner@surrey.ac.uk](mailto:b.gardner@surrey.ac.uk) (B. Gardner).

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**Background:** Antibiotic resistance increasingly threatens the interconnected health of humans, animals, and the environment. While misuse of antibiotics is a known driver, environmental factors also play a critical role. A balanced One Health approach—including the environmental sector—is necessary to understand the emergence and spread of resistance.

**Methods:** We systematically searched English-language literature (1990–2021) in MEDLINE, Embase, and Web of Science, plus grey literature. Titles, abstracts, and keywords were screened, followed by full-text reviews using a structured codebook and dual-reviewer assessments.

**Results:** Of 13,667 records screened, 738 met the inclusion criteria. Most studies focused on freshwater and terrestrial environments, particularly associated with wastewater or manure sources. Evidence of research has predominantly focused on *Escherichia coli* and *Pseudomonas* spp., with a concentration on ARGs conferring resistance to sulphonamides (sul1–3), tetracyclines (tet), and beta-lactams. Additionally, the People's Republic of China has produced a third of the studies—twice that of the next country, the United States—and research was largely domestic, with closely linked author networks.

**Conclusion:** Significant evidence gaps persist in understanding antibiotic resistance in non-built environments, particularly in marine, atmospheric, and non-agricultural settings. Stressors such as climate change and microplastics remain notably under-explored. There is also an urgent need for more research in low-income regions, which face higher risks of antibiotic resistance, to support the development of targeted, evidence-based interventions.

## 1. Background

Antimicrobial resistance (AMR) poses a significant challenge in global health, impacting all societal sectors and posing severe threats to human and animal health, agricultural productivity, and environmental stability. Recent estimates indicate that AMR may have contributed to over 4.71 million human deaths in 2021, a considerable increase from previous years (Naghavi et al., 2024). Forecasts are even more alarming, suggesting that by 2050, AMR could result in up to 8.22 million human deaths annually worldwide, with the most severe effects expected in developing regions such as Sub-Saharan Africa, Southeast Asia, and Oceania (Naghavi et al., 2024). Evidence of contamination of plant-based foods, such as fresh fruit and vegetables, with AMR bacteria and the development of pesticide resistance in plant pathogens exists, yet the impacts of AMR on non-human animals and plants, such as disease burdens in livestock and pets, effects on livestock productivity, and plant production are still under investigation (<https://www.woah.org/en/what-we-do/global-initiatives/antimicrobial-resistance/>). Additionally, our understanding of AMR's impact on ecosystems, including how shifts in soil bacterial populations affect plants and animals, remains limited (Miller et al., 2022).

The misuse and overuse of antimicrobial agents in human and veterinary medicine are well-recognised as primary drivers of AMR (O'Neill, 2014). However, emerging research highlights the role of complex ecological and anthropogenic factors in its emergence and spread. Notably, discharges from wastewater treatment facilities significantly contribute to AMR proliferation in aquatic and terrestrial ecosystems (Kampouris et al., 2021a,b; Bengtsson-Palme et al., 2018). Further studies indicate that a local temperature increase of 10°C could lead to a rise in antibiotic resistance by 4.2%, 2.2%, and 2.7% in *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, respectively, suggesting that current estimates of antibiotic resistance burden must consider growing populations and climate change (MacFadden et al., 2018). Additionally, anthropogenic pollutants, such as heavy metals and various agrochemicals, have been identified as significant factors that enhance bacterial resistance through selective pressure, often resulting in the co-selection of resistant strains (Ramakrishnan et al., 2019; Poole, 2017). The complex interactions among various environmental reservoirs, notably water, soil, and air, further facilitate the propagation of AMR, indicating an intricate web of transmission pathways.

Given the extensive implications of AMR, comprehensive literature reviews have been conducted to assess the primary drivers influencing the emergence and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Coertze and Bezuidenhout, 2019; Duarte et al., 2019; Yang et al., 2018; Chatterjee et al., 2018; Bueno et al., 2017; Huijbers et al., 2015; Kurenbach et al., 2015). These reviews have highlighted critical factors, including antibiotic

dissemination into natural water bodies, agrochemical application, and antibiotic usage in livestock (Coertze and Bezuidenhout, 2019; Duarte et al., 2019; Yang et al., 2018; Chatterjee et al., 2018; Bueno et al., 2017; Huijbers et al., 2015; Kurenbach et al., 2015). Although these reviews offer valuable insights, they often focus on specific, sometimes dichotomous, research questions and aggregate primary research results into generalised findings (Gough et al., 2012).

To address the broader scope of environmental AMR and map out the existing evidence of how antibiotics, ARB, and ARGs spread and persist across terrestrial, aquatic, and atmospheric environments, we followed the protocol for a systematic evidence map (SEM) published by Gardner et al. (2023). The SEM will also conduct a bibliometric analysis to map distribution, network, cross-disciplinarity, impact and trend of the literature. Systematic evidence mapping is a rigorous methodology commonly applied in social sciences and environmental studies, systematically reviewing the existing evidence within a research area, identifying both knowledge gaps and clusters of evidence (Wolffe et al., 2019, 2020). SEMs typically answer research questions with a wider scope, retrieve bibliometric information and other metadata, rather than findings of the research itself, and discourage extraction and statistical analysis of study findings (James et al., 2016). Systematic mapping provides an ideal tool to identify, collect, organise, and publicly share the heterogeneous body of evidence on the complex nature of the increase, spread, and persistence of antibiotic resistance in the environment (Fig. 1 in Gardner et al. (2023)).

The urgency and complexity of AMR necessitate an intersectoral One Health approach to comprehensively consider the factors driving AMR emergence and dissemination as part of the larger system. This approach integrates human, animal, and environmental health to holistically address the issue, in line with recommendations by United Nations Environment Programme (2022). Systematic analyses of human and animal health drivers and impacts are available; our research employs a systematic evidence map (SEM) structure to organise and synthesise the diverse evidence around the environmental impacts on AMR. This format avoids preliminary conclusion drawing from the heterogeneous data, thus paving the way for more targeted future investigations (Wolffe et al., 2019, 2020; Schreier et al., 2022).

### 1.1. Scope, aim and objectives

Our aim is twofold: (i) to systematically map existing evidence on anthropogenic and natural factors that indicate the presence of antibiotic resistance in the outdoor environment, with a particular focus on clinically relevant ARB and ARGs; (ii) to visualise temporal trends, identify emerging research avenues, and reveal disciplinary links using bibliometric analysis. In this context, we refer to samples collected outside built-up areas including locations broadly classified into natural spaces (e.g., grasslands, lakes, and rivers), semi-natural

spaces (e.g., agricultural land), green infrastructures (e.g., gardens and parks) and decommissioned industrial sites. We specifically exclude outdoor areas undergoing intense and continuous industrial activity, such as waste processing plants. This delineation defines our study population, as detailed in the [Methods](#) Section.

Here, we adhere to the PECO (Population, Exposure, Comparator, Outcome) framework to structure our Systematic Evidence Map (SEM) as found in [Table 1](#) in [Gardner et al. \(2023\)](#). Furthermore, we characterise our exposure as a measure of ‘contact’—quantifiable in terms of duration, frequency, intensity, or a combination thereof—with sources of antibiotic resistance (hazards) or mediums whose physical, chemical, or biological characteristics may influence the emergence, spread, and persistence of antibiotic resistance determinants (stressors). Comprehensive definitions of our population and exposure are available in [Table 1](#) and [Table S-1](#) in [Gardner et al. \(2023\)](#). Thus, our over-arching research question is:

*What evidence exists on the effect of local environmental conditions on the occurrence of antibiotic resistance in the non-built environment?*

We have compiled and analysed data to guide future research, policy-relevant systematic review questions, and potential funding strategies to mitigate risks associated with the emergence of antibiotic resistance in the environment. Given the topic’s extension and complexity, we organised the evidence into coherent segments by stratifying various sub-populations (e.g., agricultural soil or surface water), exposures to hazards and stressors (e.g., application of manure or proximity to wastewater), comparators (e.g., presence/absence of fertiliser or higher/lower temperatures), and outcomes (e.g., presence/absence or prevalence of ARB or ARGs). Bibliometric analysis was used to map spatiotemporal patterns in the research landscape, including the identification of scientific communities, research productivity, common scientific interests, impact, trends, and diversity.

## 2. Methods

This SEM focuses on antibiotic resistance within non-industrial, non-built environments. The SEM was conducted according to the protocol published by [Gardner et al. \(2023\)](#), which follows established guidelines, and the PRISMA-P statement adapted to SEM methodology ([Elsevier, 2017](#); [Rethlefsen et al., 2021](#)). Minor deviations from the published protocol are described in more detail in [Section 2.5, Deviations from the published protocol](#)

### 2.1. Eligibility criteria

The criteria for study eligibility in this SEM was based on the PECO framework adapted to studies on antibiotic resistance ([Williams-Nguyen et al., 2016](#); [Bueno et al., 2017](#)). The *population* considered comprises samples collected from non-built, non-industrial environmental compartments, encompassing terrestrial and aquatic settings, as well as atmospheric matter. *Exposure* sources included both anthropogenic activities, such as antibiotic use, waste products, chemical pollutants, and natural environmental stressors, such as humidity, soil characteristics, temperature, and wind speed. We also considered exposure sources originating from built-up sites impacting on outdoor locations, e.g., hospitals discharging waste products into the surrounding area. The *comparator* was established by comparing how the outcome measure changes with different levels of exposure, either categorically or numerically. The *outcome* related to the detection or measured abundance of antibiotic resistance determinants (e.g., the abundances of ARGs or presence/absence of ARB) in environmental samples. See details in [Tables 2, S-1, S-3](#) in [Gardner et al. \(2023\)](#).

This SEM excluded studies on samples collected from human, animal or plant hosts, food products, or industrial settings. It also excluded studies on transmission mechanisms from the environment to humans or animals, evolutionary mechanisms of resistance, and the impact of trade and human travel on dissemination.

### 2.2. Information sources and search strategy

Full details on the information sources, search strategy and data management, were discussed in the previously published protocol in [Gardner et al. \(2023\)](#). Accordingly, the literature search was conducted using the following electronic databases: MEDLINE, Embase, and the Web of Science Core Collection as well as the grey literature (full list and details in [S4 File](#) and [Table S-9](#) in [Gardner et al. \(2023\)](#)). The SEM focused on articles published in English from 1990 until September 2021. To assess whether the key evidence gaps identified in our SEM remain relevant, we also conducted a modified update of the search covering the period from 2022 to 2025. This supplementary search was not intended as a full systematic update but rather to provide a broad indication of the number of potentially missing studies and to explore whether recent publications are likely to alter the landscape of evidence in key areas identified as lacking in the original review (see [Section 2.6](#)). The institutional subscription at the University of Surrey and University of Edinburgh ensured access to MEDLINE, Embase (Ovid) and the Web of Science Core Collection.

Additionally, bibliometric information and other details related to the impact of research (e.g., number of citations or Journal Impact Factors) were retrieved from the Web of Science (WoS, <https://www.webofscience.com/wos/>) and from Clarivate’s Journal Citation Reports™ (<https://jcr.clarivate.com/>). This information complemented details that may not be readily available in the manuscript, such as the list of funding organisations that contributed to the research or categories of research areas, among other details. Also, standardised ARG names and antimicrobial information were obtained from the Comprehensive Antibiotic Resistance Database (CARD), <https://card.mcmaster.ca/> (accessed on: 15 September 2024) ([Alcock et al., 2023](#)) and the NCBI National Database of Antibiotic Resistant Organisms (NDARO) (<https://www.ncbi.nlm.nih.gov/pathogens/refgene/>, accessed on: 15 September 2024) as well as further support of authors’ expertise. Other minor deviations from the published protocol are described in more detail in [Section 2.5, Deviations from the published protocol](#).

The search strategy was developed according to the recommendations of the Peer Review of Electronic Search Strategies devised in 2015 (PRESS 2015) for evidence synthesis work ([McGowan et al., 2016](#)). The search strategy we used for MEDLINE (via PubMed) was based on Medical Subject Headings (MeSH), where the terms are conceptually organised into four different groups referring to drug resistance and genetic mechanisms, the environmental location or compartment from which samples are obtained, exposure sources posing as a hazard or stressors for the emergence of antibiotic resistance, and specific exclusion terms. These search concepts are derived from the PECO statement. For the other databases, the search strategies are modelled based on these MeSH terms as closely as possible (see [Table 3](#) in [Gardner et al. \(2023\)](#)).

### 2.3. Data management and study selection

The identified studies were managed using EndNote Version 20.5™ (Clarivate) with duplicates removed automatically. Initial screening based on title, abstract and keywords was conducted by two pairs of independent reviewers (GL/ADM and IDC/FC) with the assistance of ‘SWIFT-Active Screener’ ([Howard et al., 2020](#)), where each pair was randomly allocated 50% of the abstracts. Following initial screening, the retained studies were distributed amongst reviewers for full-text screening. Each full-text study was assessed by a pair of reviewers for further selection using the same criteria as above with the only possible answers to these questions being ‘Yes’ and ‘No’. Criteria for inclusion/exclusion are shown in [Fig. 2](#) in [Gardner et al. \(2023\)](#). Where possible, reviewers were paired so that their scientific background and career stage complemented each other (see [Tables S-6–S-7](#)).

### 2.4. Data extraction and data coding strategies

Each pair of reviewers extracted relevant bibliometric data and

specific information related to the PECO framework from the studies. The extracted data included environmental characteristics, sources of exposure, comparators, and outcomes related to antibiotic resistance. The coding strategy was implemented as described in the protocol, including some minor deviations described in Section 2.5, *Deviations from the published protocol*. The reviewers used a Microsoft Excel® spreadsheet, with the aid of drop-down menus for all closed questions according to the code book (Table S-6, S-7 and Fig. S-6 in Gardner et al. (2023)), and a final column was allocated for reviewer comments (Supplementary Material S2 File), this column enabled reviewers to document extracted data that was otherwise not covered by the available coding variables. A Microsoft Teams page was also set up to allow reviewers to post questions and comments accessible to the entire team. We arranged a webinar to demonstrate how to use the Microsoft Excel® spreadsheet for data extraction, and the recording of this webinar was posted to a dedicated Microsoft Teams page. Each pair of reviewers (Table S-7) was assigned 64 documents, which were equally split: 32 were allocated to the primary reviewer and 32 to the secondary reviewer, with the exception of BG who reviewed 64 manuscripts as the primary reviewer, and MT and DV, who reviewed the grey literature (full list and details of the grey literature are presented in S4 File and Table S-9 in Gardner et al. (2023)). DV and SW also reviewed 10 additional peer-reviewed documents. Data extraction for each pair of reviewers was followed by cross-checking for quality control. This first involved each reviewer double-checking the inclusion/exclusion decisions of the other, with the intent of reaching mutual agreement on their selections (also mediated by a third reviewer, where necessary). Subsequently, fifty percent of the retained documents were selected at random and assigned to the secondary reviewer to double-check the extracted data variables.

Individual manuscripts may investigate more than one geographic site, and within a specific location, they might sample from different environments — for instance, surface water and sediment from the same river, or domestic well water and swine farm well water from the same village. Therefore, here and throughout, we use the term *sampling study* to refer to a study conducted at a *distinct location and environment*, regardless of whether multiple such studies are reported within the same manuscript. This definition is based on the geographic and environmental information provided in the code book at the highest resolution. Relevant coding variables include the *doi* (or an alternative unique document identifier), *research\_country*, *research\_region*, *env\_setting1*, *env\_setting2*, *env\_setting3* (see Table S-6 in Gardner et al. (2023)). We also recorded latitudes and longitudes (*research\_latitude*, *research\_longitude*) and the specific aquatic use (*use\_aquatic*) of the study, but the information was not used in our definition of sampling study. Furthermore, we need to distinguish situations when different exposures are investigated within the same sampling study; for example, sampling from the same agricultural soil that is exposed to both the application of manure and biocides. Thus, we use the term *exposure study* to refer to a sampling study that investigates a *specific stressor*, regardless of whether several stressors are studied within the same manuscript. Therefore, an exposure study represents a unique combination of a sampling study and a specific stressor. This approach uses the same coding variables as used for the sampling study, in addition to variables related to the stressor, such as *stressor\_class* and *stressor\_name* (see Table S-6 in Gardner et al. (2023)). Descriptive statistical analysis was performed using R (Version 4.4.0) (R Core Team, 2018).

### 2.5. Deviations from the published protocol

Whereas the eligibility criteria and screening methods were performed largely as described in the protocol by Gardner et al. (2023), several deviations from the published protocol were necessary in other areas. Namely, the number of co-authors outlined in the protocol was adjusted due to the increasing number of manuscripts being included for full text review and other working commitments. Also, instead

of using an electronic form based on Qualtrics™, a form created in Microsoft Excel was used to gather the required information from reviewers. The code book was also updated to enhance the flow of information. In particular, a new class of stressors, '*stressor\_class*', was included to aggregate specific stressors that had similar characteristics. Reviewers also collected the recorded names of ARBs. In some instances, the available information consisted of the species of bacteria, while in other cases, only the genus or even the class of bacteria was reported. Therefore, the code book was updated to categorise the available information appropriately into genus, family, order, class, and phylum. Finally, the closed options for reporting the occurrence of ARB and/or ARGs, including the antimicrobial classes previously listed in Table S-2 and Fig. S-5 in Gardner et al. (2023), were removed to allow free text entries that better capture the high variability between resistance genes and antibiotics.

### 2.6. Updated search strategies

Additional search strategies were applied following the conclusion of the main SEM analysis, and just prior to publication, to quantify the volume of recent publications potentially relevant to our research topic and assess whether the identified evidence gaps remain. This additional analysis was performed to keep the SEM current with recent publication trends, and furthermore to explore changes in research activity concerning topics of significance that have been identified in this SEM. To this end, the original search strategy was repeated for the target databases (Section 2.2), but with the publication years adjusted to between 1<sup>st</sup> September 2021 and 28<sup>th</sup> April 2025. Additionally, the search strategy was modified to identify publications relevant to specific under-represented topics which were identified as evidence gaps, namely: atmospheric factors, changes in the climate, the presence of microplastics, marine environment, and Africa as an under-represented region of research activity. These additional search strings, customised to the target databases, are summarised in Tables S-1, S-2, and S-3 in the Supplementary Material. For the purposes of identifying trends in the number of publications, and to make consistent comparisons, these modified search strategies were also applied to the original search period between 1990 and September 2021. As described previously, these search results were restricted to those published in English, and duplicates were removed according to DOI and title matches. No further processing steps were applied to these identified studies, thereby giving an *estimation* of the volume of recent publication activity.

## 3. Results

A total of 13,667 manuscripts were screened based on their title, abstract, and keywords against the eligibility criteria to assess their relevance. From these, 1568 manuscripts were selected for full-text screening, of which 738 (47.1% of the reviewed manuscripts) were included for evidence mapping. The full texts of seven manuscripts were not accessible to the reviewers and were therefore excluded. The extracted data are provided in the Supplementary Material as a spreadsheet (S3 File) and as a Microsoft Access Database format (ACCDB) (S4 File). As anticipated in the protocol, a high degree of heterogeneity in the screened studies was found. To mitigate this challenge, we used our code book to stratify the literature according to the PECO categories. We have synthesised the findings by using tables, graphs, and a narrative synopsis to map:

- (A) The evidence concerning factors associated with the prevalence of antibiotic resistance in the environment.
- (B) The distribution, network, cross-disciplinarity, impact and trend of the literature.

**Table 1**

Reported most frequently studied environment. For the cases labelled by \*, the proportion is relative to the total sampling studies, otherwise the proportion is relative to number of sampling studies in the aquatic, terrestrial or atmospheric only.

Population	Subpopulation	Sampling (%)	No manuscripts
<b>Aquatic environment</b>			
All aquatic	All	588 (59%)*	457
Fresh water	All	468 (79.6%)	374
" "	Surface water	401 (68%)	329
" "	Ground water	47 (8%)	40
" "	Pristine water	19 (3%)	18
" "	Recreational uses	27 (4.6%)	16
" "	Domestic use: not-human consumption	20 (3.4%)	17
" "	Domestic use: human consumption	18 (3.1%)	18
" "	Irrigation	5 (0.85%)	5
Brackish water	–	43 (7%)	30
Marine water	–	42 (7%)	37
" "	Recreational uses	10 (1.7%)	10
Coastal areas	–	32 (5%)	31
" "	Recreational uses	2 (0.3%)	2
Mangrove	–	3 (0.5%)	3
<b>Terrestrial environment</b>			
All terrestrial	–	388 (39%)*	317
–	–	224 (58%)	190
Anthropogenic alteration of soil	–	69 (18%)	64
" "	Agriculture soil (livestock)	114 (29%)	98
" "	Agriculture soil (plant)	6 (1.5%)	6
" "	Dismissed Industrial areas	6 (1.5%)	6
" "	Grassland	15 (4%)	9
" "	Green infrastructures	97 (25%)	75
Terrestrial sediment	–	35 (9%)	32
Plant microcosm	–	32 (8%)	29
Natural/minimal anthropogenic alteration of soil	–	1 (0.3%)	29
" "	Alpine Grassland	7 (1.8%)	29
" "	Country walks	1 (0.3%)	29
" "	Desert	7 (1.8%)	29
" "	Forest	1 (0.3%)	29
" "	Savannah	3 (0.8%)	29
" "	Tundra	–	–
<b>Atmospheric environment</b>			
All atmospheric	–	17 1.7%*	16
" "	Airborne Dust and other solid particles	10 (59%)	10
" "	Bioaerosol	7 (41%)	7

### 3.1. (A) Is there evidence of environmental factors being associated with the prevalence of antibiotic resistance in the environment?

In this report, we present an overview of the existing evidence in published literature on the relationship between environmental factors and the prevalence of antibiotic resistance in non-built environments. To this end, we used tables, graphs, and interactive visualisations available in the online repository at [https://public.tableau.com/app/profile/sem.team/viz/SEM\\_antibiotic\\_resistance/Home](https://public.tableau.com/app/profile/sem.team/viz/SEM_antibiotic_resistance/Home). The interactive tool includes heatmaps, geospatial plots, and Sankey diagrams, and allows users to filter and download datasets stratified by populations, exposures, and outcomes. We detail the concentration of evidence, the populations, exposures, and outcomes studied, and the interconnections among these studies, as follows:

**Mapping frequency of occurrence: Population.** Full-text screening of the included manuscripts revealed 993 sampling studies, as defined above. Regarding the population compartments, details of the most studies environments are shown in Table 1 and in Fig. 1.

One manuscript investigated aquatic, terrestrial and atmospheric environments and 50 manuscripts studied both aquatic and terrestrial environments. It is worth noting the limited number of studies focusing on non-agricultural settings (e.g., forests, national parks and other green areas).

**Mapping frequency of occurrence: Exposure.** Fig. 2 shows the number of distinct exposure studies (bubble size) across different combinations of environmental sub-settings (populations) and attribute classes. It is worth noticing that authors explicitly reported *Weather and Climate*

in less than 5% of studies in both aquatic and terrestrial environment, although studies might have implicitly captured some weather-related exposures, like seasonality and temperature. In the Supplementary Material, Fig. S-1 and Fig. S-2 provides an in-depth map of hazards/stressors reported in specific aquatic and terrestrial sub-populations, whereas Fig. S-3 shows the number of exposure studies per stressor in the countries where the research was conducted.

For clarity, the hazards/stressors have been grouped according to a more general stressor class, for example pH and humidity were grouped as 'Physico-chemical Parameters' and specific metals and other chemical elements as 'Chemicals and Heavy Metals' (Supplementary Material, S3 File, coding variable *stressor\_class*). Details of specific hazards/stressors and how these are connected with the population are visualised in the Sankey plot displayed in the online repository [https://public.tableau.com/app/profile/sem.team/viz/SEM\\_antibiotic\\_resistance/Home](https://public.tableau.com/app/profile/sem.team/viz/SEM_antibiotic_resistance/Home)

**Magnitude of association between population and outcome.** Associations between the most frequent ARB families, ARG names and antimicrobial classes vs the most frequent population compartments are visualised in the heatmaps in Figs. 3. Here and throughout, the colours of the heatmap, which use a logarithmic scale due to the large variance in the data, reflect the number of sampling studies which ascertain this association.

The most commonly studied ARB families in both the aquatic and terrestrial compartments are Enterobacteriaceae ( $n = 213$  and  $n = 66$  sampling studies, respectively; mostly *Escherichia* spp.) and Pseudomonadaceae ( $n = 90$  and  $n = 35$ , respectively; mostly *Pseudomonas* spp.). The four most commonly studied ARGs in both the aquatic and terrestrial compartments were genes encoding for sulphonamide resistance (*sul1*, *sul2*) and tetracycline resistance genes (*tet(A)* and *tet(W)*).

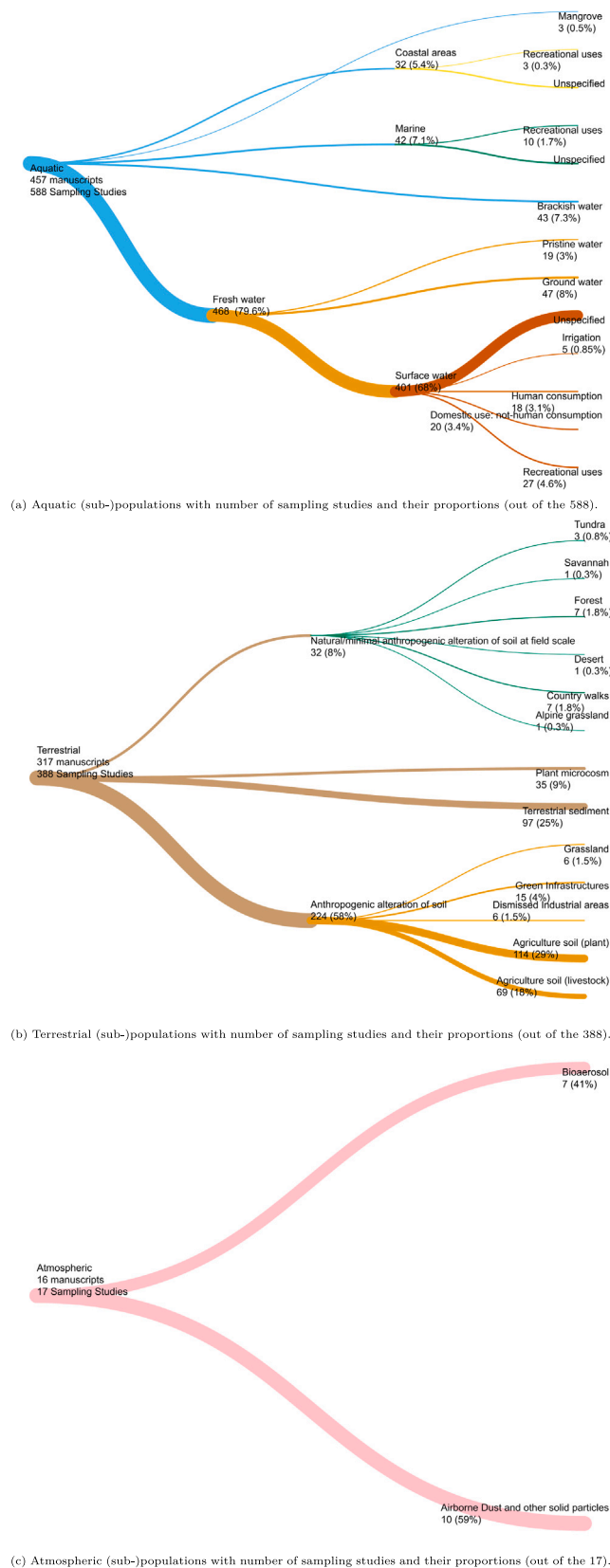


Fig. 1. Tree plots for the aquatic, terrestrial and atmospheric populations and sub-populations.

By combining the studies together, the resulting four most commonly studied antimicrobial classes in the aquatic environment were

Beta-lactams ( $n = 288$  sampling studies), Tetracyclines ( $n = 254$ ), Sulphonamides ( $n = 213$ ) and Quinolones ( $n = 145$ ). In the terrestrial environment, the four most commonly studied antimicrobial classes were Tetracyclines ( $n = 226$ ), Beta-lactams ( $n = 189$ ), Sulphonamides ( $n = 168$ ) and Macrolides ( $n = 156$ ).

Here and throughout, *multidrug resistance* refers to a generic term describing a gene capable of nullifying the effects of several different classes of antibiotics. Additionally, with some flexibility in terminology, metals are also classified as an antibiotic class. Furthermore, Beta-lactams comprise a wide range of antibiotics, including cephalosporins and carbapenems, although they are listed separately here. Lastly, aminocoumarins are technically classified as gyrase inhibitors.

The countries where ARB families, ARGs and antimicrobial classes are most frequently studied are shown in Fig. S-4 in the Supplementary Material.

In line with the overall findings, Enterobacteriaceae is the ARB family most frequently studied across different geographical settings, particularly in the United States ( $n = 26$  and 9 in aquatic and terrestrial settings, respectively), People's Republic of China ( $n = 24$  and 17, respectively) and India ( $n = 24$  and 3, respectively).

*Magnitude of association between exposure and outcome.* Associations between the most frequent ARBs, ARGs and antimicrobial classes vs most frequent exposure factors are visualised in Fig. 4 and in the online repository [https://public.tableau.com/app/profile/sem.team/viz/SEM\\_antibiotic\\_resistance/Home](https://public.tableau.com/app/profile/sem.team/viz/SEM_antibiotic_resistance/Home) (please note that, for visual purposes, in Fig. 4, we filter results with a number of studies higher than a threshold. This may result in a different appearance compared to the plot in the online repository, but the results remain the same).

In the aquatic environment, most studies investigated the association between *Proximity/Contact to Wastewater* with Enterobacteriaceae ( $n = 122$  exposure studies) and Pseudomonadaceae ( $n = 46$ ). Studies investigating exposures to *proximity to wastewater* tend to be conducted over a large spectrum of ARB families.

In the terrestrial environment, most studies investigated the association between *Application of manure* with Enterobacteriaceae ( $n = 16$ ) and Bacillaceae ( $n = 13$ ) in environments closely associated with agricultural settings. Studies investigating *exposures of antimicrobial biocides, exposures to metals farming practices (non-manure application), manure and physico-chemical parameters* tend to be conducted over a large spectrum of ARB families. This, however, could be an effect of the used method. Studies using culture-based antibiotic assays or qPCR tend to focus on a selection of key AMR types/genes and ARBs. In contrast, studies using shotgun metagenomics (for ARGs) and amplicon sequencing (for microbial taxa/ARBs) are less selective and may capture a broader range of ARGs/ARBs.

In the aquatic environment, most studies investigated the association between *proximity/contact with wastewater* and a wide spectrum of ARGs. In the terrestrial environment, multiple studies examined the association between a variety of ARGs and a wide range of hazards/stressors, including *exposure to antimicrobial biocides, exposure to metals, farming practices (non-manure application), application of manure, physical-chemical parameters, and soil chemical composition*. ARGs encoding for sulfonamide resistance (sul1, sul2) were studied over a large variety of hazard/stressors for both aquatic and terrestrial environment.

Studies resulting in the association between antimicrobial classes and exposure were more evenly distributed across various hazards/stressors. However, it is worth noting the limited number of studies in the terrestrial environment, focusing on the associations with pollution, seasonal patterns, and weather and climate.

*Mapping connection concepts.* Fig. 5 displays the network of co-occurring terms used in the titles and abstracts of all included studies. If two terms co-occur in the title or abstract of a publication, they are connected in the network. The strength of each link indicates the number of

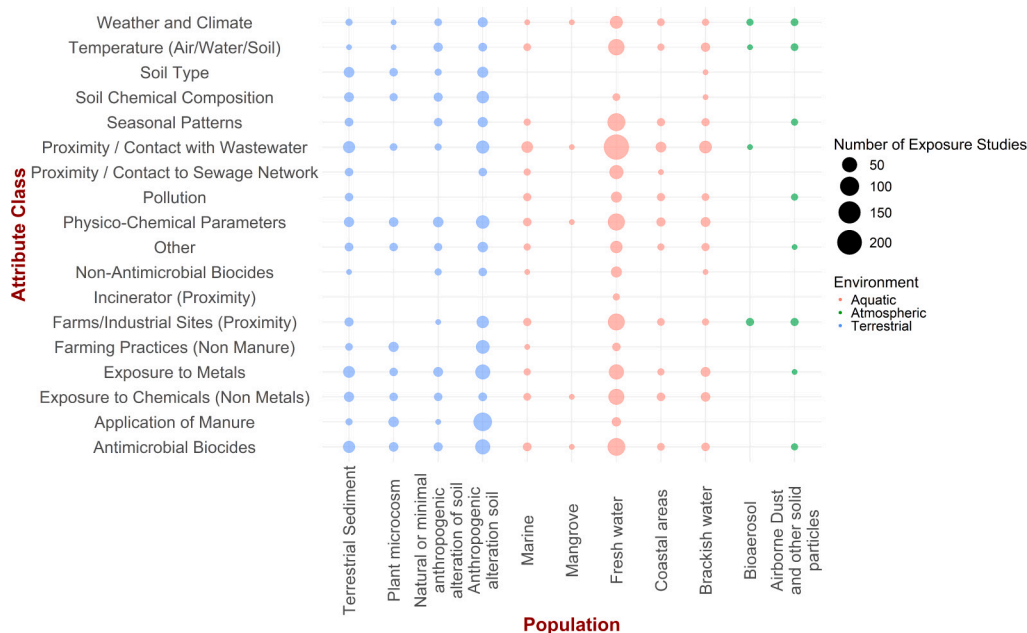


Fig. 2. Bubble plot showing the number of distinct exposure studies (bubble size) across environmental sub-settings and attribute classes.

publications in which the two terms occur together (van Eck and Waltman, 2014, 2010).

The network identifies three clusters of terms that tend to co-occur. The red cluster contains terms typically associated with the aquatic environment, such as ‘surface water’, ‘effluent’, ‘river’, and ‘water sample’. By contrast, ‘seawater’, ‘lake’, and ‘coastal water’ are linked with the green cluster, which is dominated by the term ‘ARGs’ and other microbiological terms. The blue cluster contains terms associated with the terrestrial environment, such as ‘manure’, ‘soil’, and ‘fertiliser’.

*Catalogue of studies reporting statistical distributions of exposure and dose–response relationships.* Given the high heterogeneity of studies and reporting styles, summarising information related to distributions of exposure and dose–response relationships proved challenging. Nevertheless, the three most commonly reported stressors in aquatic environments (i.e., *Proximity/contact with wastewater*, *Exposure to antimicrobial biocides* and *Seasonal patterns*) were primarily measured as categorical variables, e.g., presence of wastewater or season of interest being rainy season, winter or other. Quantitative information was predominantly included for *Exposure to antimicrobial biocides*. In terrestrial environments, the information recorded for the three most commonly stressors, namely *Application of manure*, *Exposure to antimicrobial biocides* and *Exposure to metals* was mostly quantitative (e.g., kg of manure/ha of soil). Quantitative data related to reporting stressors was found in 295 manuscripts (39.9%) investigating 160 aquatic environments, 144 terrestrial environments and 5 atmospheric environments.

In addition, an attempt was made to compile a catalogue of studies reporting on the distribution of exposure and dose–response relationships, which can be found in the Supplementary Material (S5 File). However, it is worth noting the challenges encountered in retrieving this information, as detailed in the Discussion section later.

*Summarise how the outcome is compared in relation to exposure.* The most common effect of the stressors investigated overall was an *Increase of ARG* as described in 483 manuscripts (65.4%). Such positive effect from the stressors on resistant genes was seen consistently in every environment, as reported in 70.2% of aquatic, 67.8% of terrestrial and 78.6% of atmospheric environments. *No effect on ARG* was described in 72 manuscripts (9.8%) and only 10.6% of the manuscripts reported that the stressors studied caused a *Decrease of ARG*. Similarly, the most common effect overall of the stressors investigated on bacteria was an

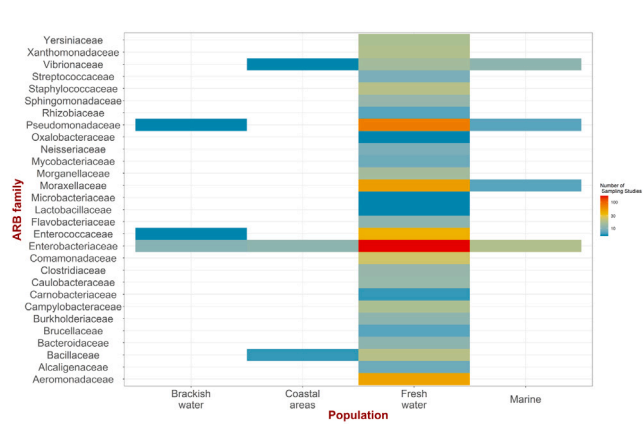
*Increase of ARB* in all the environments as described in 282 manuscripts (38.1%) followed by *No effect on ARB* in 138 papers (18.7%). Only 6.2% of the manuscripts described that the stressors studied caused a *Decrease of ARB*. The substantial proportion of studies reporting a positive effect of stressors in contrast with the proportion of studies reporting a negative or no effect could be due to the repetitive study of known stressors of AMR or could also be indicative of positive bias of reporting in the literature. Furthermore, numerous studies only reported occurrence of ARB or ARG along with a single exposure but did not assess the effect of such stressors on ARB or ARG or an effect could not be extracted during full-text review. As such, 63.1% of the stressors (37.7% in aquatic environments and 25.5% in terrestrial environments) showed *No comparison* for the effect of the stressor on ARB or this could not be extracted. In the case of studies investigating ARG, 36.3% of stressors (20.6% in aquatic and 15.7% in terrestrial environments) showed *No comparison*. This information can help identify gaps in the way differential exposure effects are assessed or reported on the outcome.

### 3.2. (B) Bibliometric Analysis: mapping distribution, network, cross-disciplinarity, impact and trend of the literature

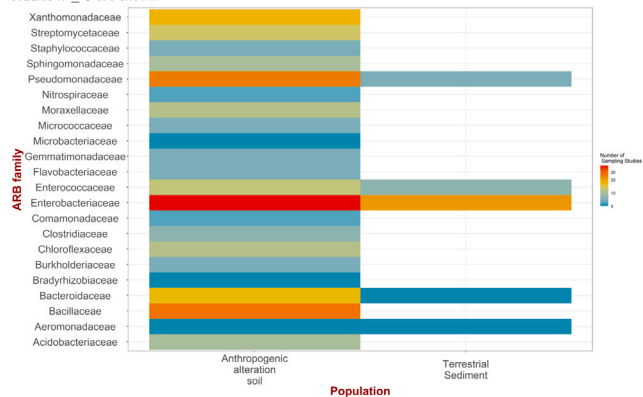
Bibliometric mapping was employed to visualise patterns across the literature. In the following section, the term ‘study’ is used to denote a publication.

*Geo-spatial analysis of publication and funding.* To identify the spatial distribution of research, the countries of origin of the corresponding authors, and funding bodies, we have investigated the following geo-spatial analysis.

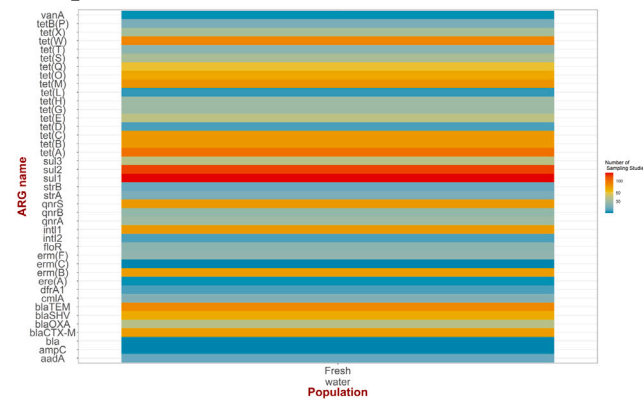
*Geographic distribution of research:* Nearly half (48.5%) of the research evidence identified was collected primarily in two countries: the People’s Republic of China and the United States. While research on AMR in the non-built environment originates from 64 different countries (Fig. S-5 in Supplementary Material), research performed in the People’s Republic of China contributed nearly one-third of the global output in this area ( $n = 250$ , 33.8%), followed by the United States ( $n = 105$ , 14.2%). The third and fourth contributions stemmed from research conducted in India ( $n = 44$ , 5.9%) and in Canada ( $n = 24$ , 3.3%) with fewer publications and each contributing about less than half of the research output compared to the previous country.



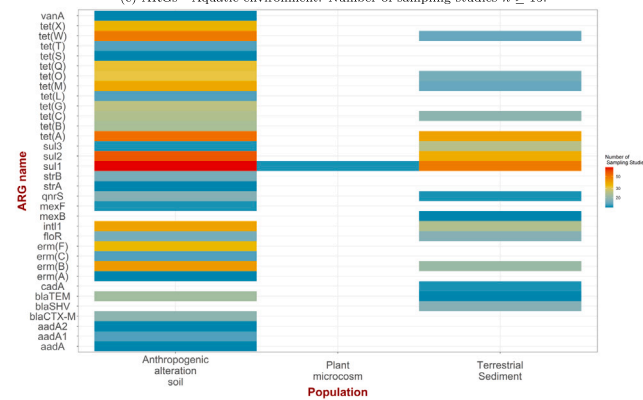
(a) ARBs - Aquatic environment. For visualisation purposes, only cases where the number of sampling studies  $n \geq 5$  are shown.



(b) ARBs - Terrestrial environment. For visualisation purposes, only cases where the number of sampling studies  $n \geq 5$  are shown.

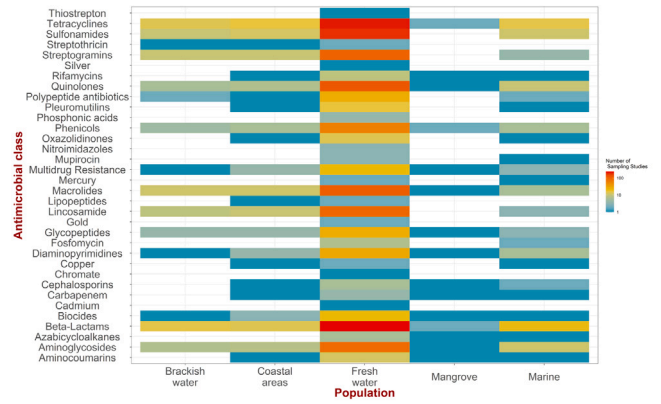


(c) ARGs - Aquatic environment. Number of sampling studies  $n \geq 15$ .



(d) ARGs - Terrestrial environment. Number of sampling studies  $n \geq 13$ .

Fig. 3. Heatmap of hazards/stressors reported in the terrestrial and aquatic environmental settings for different ARB families, ARGs and Antimicrobial class.



(e) Antimicrobial classes - Aquatic environment.



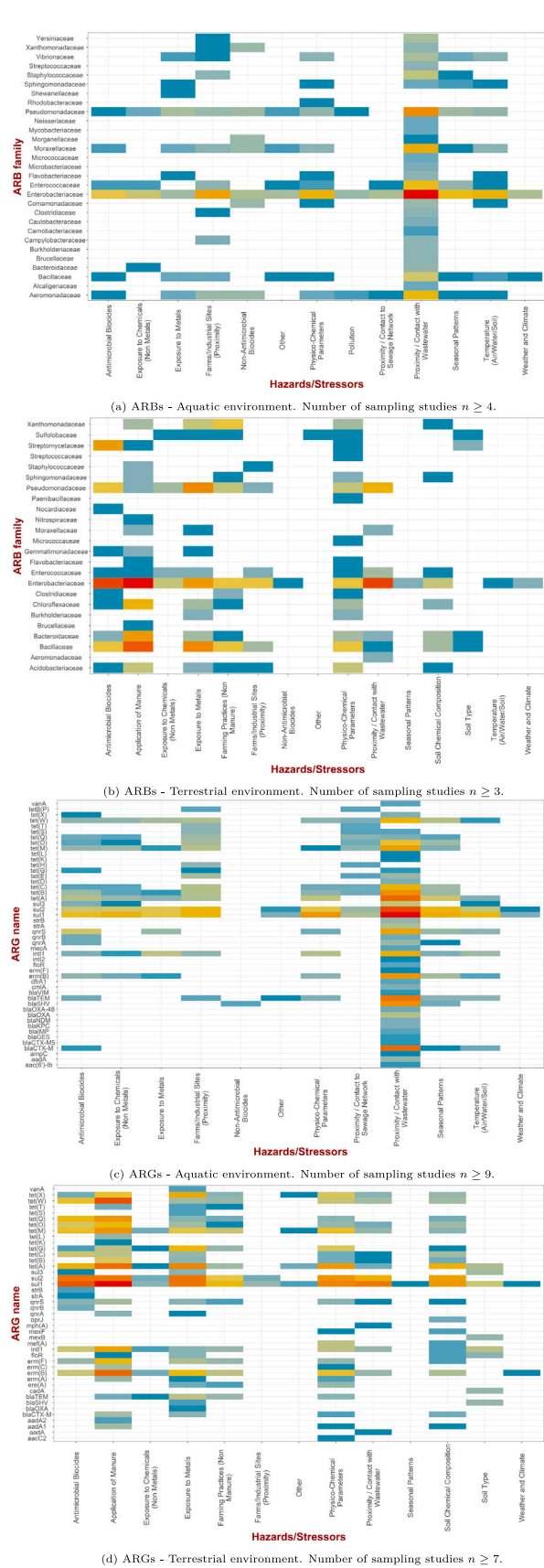
(f) Antimicrobial classes - Terrestrial environment.

Fig. 3. (continued).

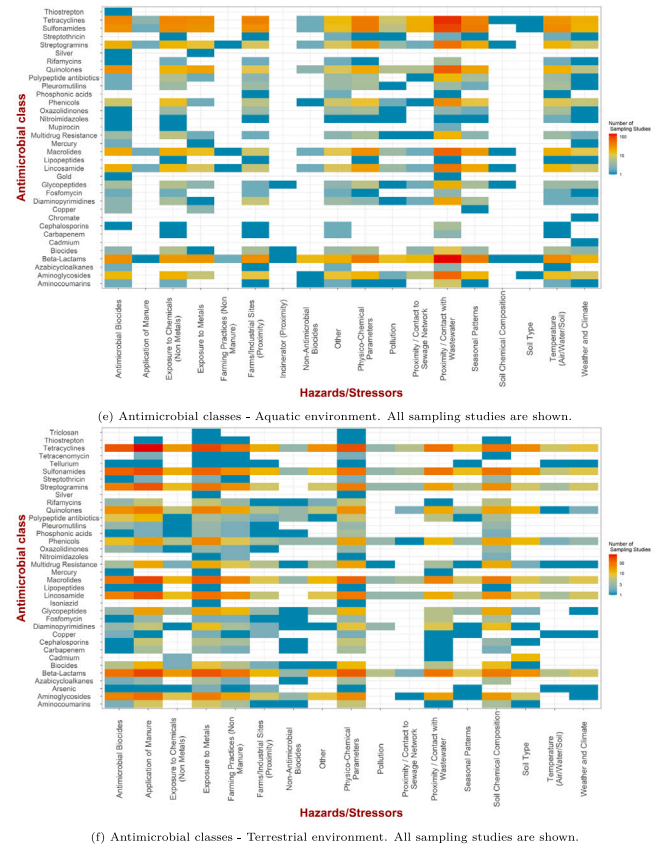
Research was conducted on every continent; however, engagement from African countries was limited, with only 29 studies (3.9%) conducted on the continent of which 19 (2.6%) were led by researchers based at African institutions. This distribution underscores significant geographical disparities in the global research landscape.

**Geographic distribution of corresponding authors:** Most corresponding authors conducted research on occurrences of antibiotic resistance in the environment within their own countries, with only limited international collaborations (Table S-5 in Supplementary Material). For instance, in the two leading nations by total research output, the People's Republic of China and the United States, 95.9% and 91.8% of studies, respectively, were conducted domestically. Also, nearly all of the research activities (91.1%) were performed in 'High Income' or 'Upper-middle Income' countries by researchers based in 'High Income' or 'Upper-middle Income' countries. In contrast, only 6.1% of the manuscripts were led by authors based in 'Lower-middle Income' or 'Low Income' countries, of which merely two manuscripts (0.3%) were led by authors based in 'Low Income' countries out of seven manuscripts (0.9%) conducted in those 'Low Income' countries. The economic status of countries in terms of GDP and the percentage of the GDP dedicated to research and development can explain this variation (Fig. 6).

**Funding:** According to the Web of Science database, a total of 956 different funding agencies contributed to 734 of the studies included in this systematic map. Unsurprisingly, the three funding agencies with the biggest presence originated from the People's Republic of China and included the National Natural Science Foundation of China (NSFC), the National Key Research and Development Program of China and the Chinese Academy of Sciences (Fig. S-6). Funding from these agencies contributed to 272 (36.7%) of the included manuscripts. The National Science Foundation (NSF), a US federal agency, and the European Union (EU) complete the five most common funding organisations named in the included manuscripts and each contributed to 30 and 28 manuscripts, or 4.1% and 3.8% respectively.



**Fig. 4.** Heatmap of hazards/stressors reported in the terrestrial and aquatic environmental settings for different ARB families, ARGs and Antimicrobial class and different stressors.



**Fig. 4. (continued).**

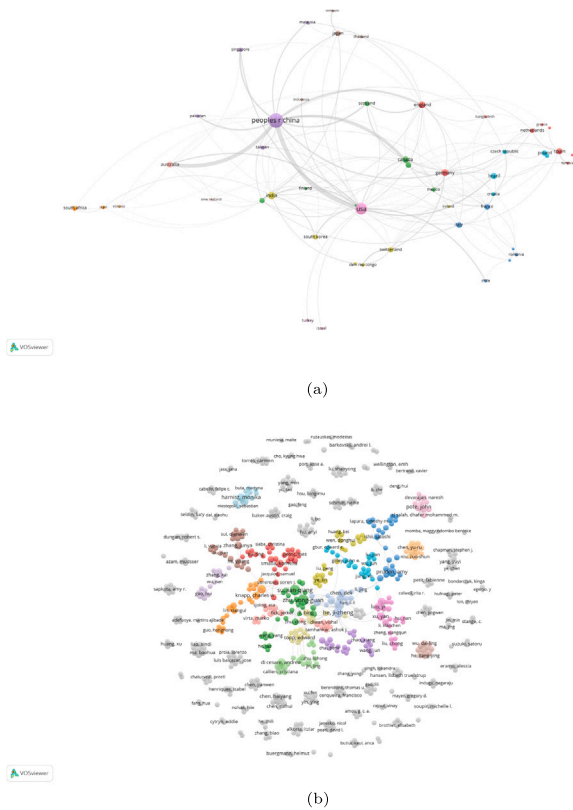
*Identify scientific communities and productivity.* To identify scientific communities and assess their productivity, a co-authorship and citation analysis at the levels of countries, research institutions, and individual researchers was conducted (Fig. 7 and S-7 in the Supplementary Material). In co-authorship maps, two items (e.g., researchers) are connected if they have co-authored a publication together, whereas in citation maps, two items (e.g., researchers) are connected if they have cited each other.

Fig. 7(a) illustrates the network of countries based on the number of publications they have co-authored. The People's Republic of China and the United States are the countries with the most joint publications, which is unsurprising given that they have produced the largest number of publications (Fig. S-5). The map also reveals a strong collaboration between Australia and the People's Republic of China. India, despite having a larger number of included publications compared to England, Germany, Australia and Canada (44 vs 34, 33, 27, 29, respectively), has a lower link strength (19 vs 26, 24, 22 and 21, respectively, based on fractional counting; see van Eck and Waltman (2014)), indicating a lower level of international collaboration.

Similarly, Fig. S-7 displays the network of scientific affiliations which have produced at least five publications. The map indicates that the strong link between Australia and the People's Republic of China is largely due to the collaborations between two institutes (the University of Melbourne and the Chinese Academy of Science), while the link between the People's Republic of China and the United States appears to be driven by multiple collaborations between Chinese institutes and a range of US institutions. The map shows nine well-connected clusters, but there are an additional nine clusters (not shown) composed of one or two individual organisations each.

A propensity for isolated working is more evident in Fig. 7(b), which shows the network of 608 authors (out of 3643) who have at least two publications. The map identified 104 clusters, with the largest





**Fig. 7.** Networks of co-authorship. The size of the circle indicates the number of documents produced by researches affiliated in that country (a) or researchers (b). The thickness of the lines indicate the strength of the link based on fractional counting i.e., when a country has co-authored a document with  $n$  other countries, this yields a strength of  $1/n$  for each of the  $n$  co-authorship links.

Transfer Protocol Secure) moved from being ranked 248<sup>th</sup> position in the  $\leq 2010$  study period to 11<sup>th</sup> position in the  $\geq 2020$  study period.

For the terrestrial environment, the terms ‘soil’, ‘resistance’, ‘manure’, ‘antibiotic(s)’, ‘gene(s)’, ‘bacterial’, and ‘sample(s)’, were among the top 15 most frequently used words across the three study periods (Supplementary Material S1 File). Similarly, the terms ‘ARGs’ and ‘ARG’ were practically absent in documents prior to 2010, with weighted percentages less than 0.01%, but which respectively increased to 0.63% and 0.18% (ranked 5<sup>th</sup> and 28<sup>th</sup>) during the 2011–2019 study period, and to 0.83% and 0.24% (ranked 3<sup>th</sup> and 21<sup>st</sup>) for the  $\geq 2020$  study period. The increasing frequency of the term ‘ARG(s)’ presumably reflects the rise in metagenomics, along with mass sampling and the reconstruction of metagenome-assembled genome (Zhang et al., 2022b).

Similarly to the aquatic environment, the usage of the word ‘HTTPS’ also increased during these study periods, moving from 242<sup>th</sup> ranked in the  $\leq 2010$  study period to 12<sup>th</sup> ranked in the  $\geq 2020$  study period. An exploratory text search of ten specific random documents anecdotally shows that the increasing use of the word ‘HTTPS’ is due to references to the journal online Supplementary Material, online repositories (e.g., bioinformatics databases like <https://blast.ncbi.nlm.nih.gov/Blast.cgi> or platforms for hosting source codes like <https://github.com/>), perhaps following a trend in the implementation of Open Science principles (Anonymous, 2021), but also due to list of references explicitly using hyperlinks.

**Identify common scientific interests.** Two objects (e.g., journals, institutions, or researchers) are co-cited if there is a third object (again, journals, institutions, or researchers) citing both; thus, co-citation is interpreted as a measure of similarity or relatedness between these

objects (Boyack and Klavans, 2010). For example, two journals are co-cited when a third research article cites papers from both journals. The co-citation analysis of journals (Fig. S-9a) of scientific publications reveals three major clusters. These can be broadly grouped based on the most common Web of Science Categories which classify the journal, based on its subject area or discipline.

The most common categorisations in the blue cluster were ‘Environment’ and ‘environmental Science’ with 35 out of 78 categories (the cluster contains 45 journals, but journals can have more than one category); whereas the most common categories in the red cluster were typically associated with biological disciplines, namely: ‘Microbiology’/‘Applied Microbiology’ (27 out of 103 categories across 85 journals), ‘Infectious Diseases’ (11), ‘Pharmacology’/‘Pharmacy’ (10) and ‘Biotechnology’ (6); finally, the third cluster (green) appears to be more equally distributed across disciplines, with journals categorised as: ‘Microbiology’/‘Applied Microbiology’ (7 out of 39 categories over 58 journals), ‘Environment’ and ‘environmental Science’ (7) and ‘Multidisciplinary Sciences’ (4). Maps of co-cited publications (receiving at least 20 citations, fractional counting as in Fig. S-9a) also reveal that the landscape of publications can be grouped into four clusters; one cluster of publications is dominated by the ‘Environment’ and ‘Environmental Science’ categories, while the difference among the other three clusters is less obvious (not shown).

**Assessing the diversity of research according to disciplines.** All of the included studies were published in the scientific literature as a *Journal Publication* in 165 journals (Table 2). Thirteen manuscripts had been also disseminated in conferences prior to their peer reviewed publication and were also classified as a *Proceedings Paper*. None of the included evidence in our map was published in a book or in the grey literature. The journal with the highest number of relevant articles published was ‘*Science of the Total Environment*’, with 108 manuscripts (14.6%), followed by *Environmental Pollution*, with 54 manuscripts (7.3%).

The 734 manuscripts indexed in the Web of Science, were categorised in 31 different research areas. The most common research area was *Environmental Sciences & Ecology* and included more than half of the manuscripts ( $n = 467$ , 63.6%), followed by *Microbiology* ( $n = 169$ , 23.0%), and *Engineering* ( $n = 105$ , 14.2%). The ten most common research areas can be seen in Supplementary Material Fig. S-10. Numerous manuscripts were included in multiple categories.

**Assessing impact and trend.** We also investigated the temporal distribution of the bibliographic records, trends in citations over time, and the impact of the journals at the time the manuscripts were published.

**Publication year:** Full-text screening of manuscripts published between 1991 and 2021 revealed that 2020 was the most prolific year, with 130 manuscripts found in the literature (17.6% of the studies reviewed), followed by 2019 with 102 manuscripts (13.8%) and 2021 with 101 manuscripts (13.7%). Nearly half of the studies ( $n = 333$ , 45.1%) were published in the three most recent and most productive years between 2019 and 2021, and nearly the other half of the studies ( $n = 360$ , 48.8%) were found in the preceding 10 years (2009–2018), indicating the recent rise in concern for the role of environmental factors in the spread and persistence of antibiotic resistance (Fig. S-11a).

**Impact:** To assess the impact of research, we investigated the number of citations in the literature for each of the included manuscripts, and the impact factor of each journal for the year the respective manuscripts were published. We found that the 734 manuscripts indexed in the Web of Science had received a total of 47,660 citations up to 27 September 2024, with an average of 64.8 citations per manuscript. Thirteen manuscripts were classified as ‘*Highly cited papers*’, as they have received enough citations to place them in the top 1% of their academic field, which included Environment/Ecology ( $n = 6$ ), Engineering ( $n = 3$ ), Microbiology ( $n = 2$ ), Agricultural Sciences ( $n = 1$ ) and Biology & Biochemistry ( $n = 1$ ). It was observed that the number of citations



**Table 2**

Ten most common journals and their research areas as indexed by Web of Science. N: number of manuscripts, %: percentage of manuscripts.

Journal name	Research area	N	%
Science of the Total Environment	Environmental Sciences & Ecology	108	14.6
Environmental Pollution	Environmental Sciences & Ecology	54	7.3
Frontiers in Microbiology	Microbiology	40	5.4
Environment International	Environmental Sciences & Ecology	31	4.2
Environmental Science & Technology	Engineering; Environmental Sciences & Ecology	31	4.2
Journal of Hazardous Materials	Engineering; Environmental Sciences & Ecology	27	3.6
Water Research	Engineering; Environmental Sciences & Ecology; Water resources	27	3.6
Applied and Environmental Microbiology	Biotechnology & Applied Microbiology; Microbiology	26	3.5
PLoS One	Science & Technology - Other Topics	23	3.1
Ecotoxicology and Environmental Science & Technology	Environmental Sciences & Ecology; Toxicology	22	3.0

**Table 3**

The volume of publications before and after the original Sept. 2021 search date, using targeted search strategies.  $N_{pre}$  and  $N_{post}$  are the unscreened counts before and after Sept. 2021, respectively;  $N_{est}$  is the estimated number of studies retained from  $N_{post}$  if screening was applied.

Search strategy	$N_{pre}$	$N_{post}$	$N_{est}$	Comments
Original	13,667	9,060	489	
Atmospheric effects	211	226	12	$N_{post} \approx 2.4\%$ (226/9,060) of papers retrieved after Sept. 2021 vs 1.5% (211/13,667) for $N_{pre}$ .
Climate change	60	101	5	$N_{post} \approx 1\%$ of papers retrieved after Sept. 2021 vs 0.4% for $N_{pre}$ .
Microplastics	75	285	15	$N_{post} \approx 3\%$ of papers retrieved after Sept. 2021 vs 0.5% for $N_{pre}$ .
Africa	591	290	16	$N_{post} \approx 3\%$ of papers retrieved after Sept. 2021 vs 0.5% for $N_{pre}$ .
Marine	524	334	18	$N_{post} \approx 4\%$ of papers retrieved after Sept. 2021 similar to 4% for $N_{pre}$ .

regarding antibiotic resistance in the non-built environment have primarily focused on terrestrial and aquatic settings. Such interest can be explained by the direct and more traceable impact these environments have on the dissemination of antibiotics (Chatterjee et al., 2018; Coertze and Bezuidenhout, 2019; Duarte et al., 2019). Furthermore, the greater focus on freshwater over marine environments in studies concerning antibiotic resistance reveals an important gap in current research. This uneven emphasis can partly be explained by several factors. Freshwater systems are often more accessible and directly connected to human populations and agricultural activities, where antibiotics are most commonly used and can leach into surrounding environments (Chen et al., 2017; Wang et al., 2020a). As such, these systems are both easier to sample and more visibly impacted by human activity either from agricultural practices (Brooks et al., 2020), freshwater aquaculture systems (Nakayama et al., 2017; Lastauskiene et al., 2021; Bai et al., 2022), municipal waters (Bird et al., 2019; McInnes et al., 2021), including hospital wastewater (Girijan et al., 2020; Kalasseril et al., 2020), or others (Tong et al., 2020; Lu et al., 2020), thus gaining more immediate research interest compared to

marine environments. In contrast, marine environments can be significantly harder to access and study, especially in more remote or deeper locations. Additionally, the dilution effect in ocean waters (Lu et al., 2021) may lead researchers to hypothesise a lesser degree of antibiotic concentration and visibility of resistance compared to smaller, more contained freshwater systems. Nevertheless, the potential for antibiotic resistance to develop and spread in marine environments (Heuer et al., 2002; Chen et al., 2015), affecting a broad range of marine life and ecosystems (Taviani et al., 2008; Matyar et al., 2008; Miller et al., 2009; Shah et al., 2014; McCann et al., 2019), and the potential for dispersion to new areas (Ng et al., 2015), underscores the need for a better-balanced research approach that includes more significant attention to these under-represented areas. This could help ensure a comprehensive understanding of the pathways and impacts of antibiotic resistance across different aquatic systems.

We have also observed a substantial number of studies investigating exposure to wastewater and to manure (Sengelov et al., 2003; Ji et al., 2012a; Zhu et al., 2013; Czekalski et al., 2014; Udikovic-Kolic et al., 2014; Chen et al., 2016; Zhang et al., 2019). Such considerable clusters of research evidence, particularly in agricultural settings, reflect a clear recognition of these factors as significant vectors for the spread of ARB and ARGs. However, this focus may overshadow other equally critical pathways and environments that also require attention. Only a few studies have investigated the dissemination of AMR in urban green spaces (Han et al., 2016; Yan et al., 2019) and there is a need to explore the extent of dissemination of AMR in rural green spaces or forestry areas not primarily associated with farming. The focus of most terrestrial studies on agriculture results in a knowledge gap about how human, animal, and environmental interactions affect AMR in these non-agricultural settings. For example, AMR could spread to wild animal and soil microbiota in such non-agricultural environments, representing an unknown threat to public health.

Likewise, the impact of weather, climate and especially climate change on antibiotic resistance is relatively understudied (van Bavel et al., 2024), although some studies investigated exposure to factors such as seasonality and temperature (Hoa et al., 2011; Zheng et al., 2018; Muurinen et al., 2017) and the number of studies are increasing, especially during the past 5–6 years (van Bavel et al., 2024). This knowledge gap is important in a One Health context, as climate change may make humans and animals more susceptible to AMR infections due to changes in behaviour, impacts on mental health, and other negative socio-ecological determinants health. In particular, the potential roles of microplastics in aquatic (Yang et al., 2019; Wang et al., 2020b) and terrestrial (Sun et al., 2018) environments as vectors of antibiotic resistance, as well as various forms of environmental pollution (Ji et al., 2012b; Hu et al., 2017; Zhao et al., 2019; Knapp et al., 2011), including contaminants (Wu et al., 2016), which remain understudied, signal significant gaps in the current research landscape, although there is an indication that interest is increasing rapidly in the last years.

A critical gap was the relative lack of research on the atmospheric environment. This finding agrees with other reviews on airborne antibiotic resistomes across various contexts (Gwenzi et al., 2022). The under-representation of atmospheric studies could be attributed to several factors. Firstly, the complexity and technical challenges associated with analysing microbial communities from bioaerosols may deter researchers and funding partners from undertaking and supporting such research (Gollakota et al., 2021). Airborne microbial communities are less concentrated and more transient than those found in water or soil. These microbes, attached to solid particles and liquid droplets, remain airborne for prolonged periods and travel extensive distances, complicating both their detection and consistent monitoring (Becsei et al., 2021). Secondly, until recently, there might have been an underestimation of the role that the atmospheric environment plays in the transmission and propagation of antibiotic resistance genes (Jin et al., 2022) and how wind and aerosols may link AMR in humans and animals across ecosystem barriers. Furthermore, policy, public health

and funders priorities may skew research focus primarily towards water and soil concerns, attributed to their clearer and more direct connections to human health outcomes (Brevik et al., 2020; Samreen Ahmad et al., 2021). Nevertheless, existing evidence indicates air's role as a vector in certain urban areas (Messi et al., 2015; Hu et al., 2018; Sun et al., 2020), near concentrated animal operations (Gibbs et al., 2006; Blaak et al., 2015; Wooten et al., 2019; de Rooij et al., 2019; Ruiz-Ripa et al., 2020), and other environments known for persistent AMR (Korzeniewska and Harnisz, 2013). This identified gap underscores a need for more research to comprehensively understand and mitigate antibiotic resistance across all environmental vectors, including atmospheric environments.

As illustrated in the online repository [https://public.tableau.com/app/profile/sem.team/viz/SEM\\_antibiotic\\_resistance/Home](https://public.tableau.com/app/profile/sem.team/viz/SEM_antibiotic_resistance/Home), the predominant focus on bacteria such as *Escherichia coli* and *Pseudomonas* spp. in studies regarding antibiotic resistance can be explained by multiple factors. First, both *E. coli* and *Pseudomonas* are well-characterised in the scientific community, making them model organisms for studying microbial behaviour, genetics, and resistance mechanisms. *E. coli*, in particular, is a common indicator organism for faecal contamination in water and has been extensively studied in both clinical and environmental settings. Its presence is often associated with the risk of other pathogens in the same environment, making it a critical focus for public health and their inclusion in routine water quality monitoring programmes (Blount, 2015). *Pseudomonas* spp., especially *P. aeruginosa*, is notorious for its resistance to antibiotics and its role in hospital-acquired infections. This makes it a key organism for studying in the context of antibiotic resistance, not just in clinical settings but also in environmental reservoirs that may act as sources of resistance genes. Secondly, both organisms are relatively easy to culture and manipulate in laboratory settings, allowing for detailed genetic and biochemical analyses. This practical ease extends to the ability to monitor their resistance patterns over time and across various environmental conditions. However, focusing primarily on these bacteria might lead to a skewed understanding of the broader spectrum of microbial resistance in the environment. This gap underscores the need to broaden research to include a wider variety of bacteria that may play significant roles in antibiotic resistance ecology. This would help provide a more comprehensive map of resistance dynamics and inform more effective environmental and public health strategies to combat antibiotic resistance on a broader scale.

Some ARG types or mechanisms were reported more frequently, such as ARGs conferring resistance to Sulphonamides (sul1, 2 and 3 genes), Tetracyclines (tet genes) and Beta-lactams (e.g. blaCTX-M, blaKPC, blaOXA genes). Sulphonamide antibiotics were among the first synthetic antibiotics produced and have been widely used in both human and veterinary medicine (Gillings et al., 2015; Berendonk et al., 2015). Therefore, there has been long-term selective pressure for sulphonamide resistance in contaminated environments, and some sulphonamide resistance genes, such as sul1, have become widespread in areas that are exposed to human and domestic animal waste, including aquatic ecosystems contaminated by wastewater and livestock farms (Gillings et al., 2015). Sulphonamide resistance genes, such as sul1, are routinely included as primary targets in ARG detection panels due to their longstanding and widespread use. Sul1 is strongly associated with class 1 integrons (Partridge et al., 2018), which are involved in the horizontal gene transfer of antibiotic resistance genes, resulting in widespread distribution in contaminated environments (Domingues et al., 2015). Since tetracycline has been used heavily in livestock, and the genes that confer the resistance are included in veterinary antimicrobial susceptibility panels (O'Dwyer et al., 2017), it is frequently detected in farm soils exposed to animal faeces, as well as in soils that are exposed to manure and slurry (Zhang et al., 2022a). Penicillins such as amoxicillin are frequently used in veterinary medicine, and their inclusion in tests for antimicrobial susceptibility may account for the numerous reports of ARGs conferring resistance to beta-lactam

antibiotics (Martin et al., 2020). Furthermore, extended spectrum beta-lactamase genes (e.g. blaCTX-M, blaKPC, blaOXA) are frequently investigated in environmental studies due to their clinical relevance (Mills and Lee, 2019; Bleichenbacher et al., 2020; Yuan et al., 2024).

It was observed that a majority of the studies indicated stressors were associated with an increase in ARG or ARB. In contrast, only a few studies found stressors to have no impact on either ARG or ARB. The prevalent reporting of positive effects, as opposed to minimal or negative effects, might be attributed to the frequent re-examination of well-known AMR stressors. Moreover, this trend could also be indicative of a positive publication bias, extensively documented in the existing literature (Knobloch et al., 2011; Fanelli, 2012; Simonsohn et al., 2014).

Research on antibiotic resistance predominantly occurred within the research area of 'Environmental Sciences & Ecology', representing over half of the current studies, with significant contributions from the 'Microbiology' and 'Engineering' areas. A pattern in the clustering of citations among journal was also observed with publications within 'Environmental Sciences & Ecology' were cited more often than across other areas. This distribution is perhaps not surprising, since this SEM focused on the environment. Additionally, we have observed numerous clusters of authors who worked mostly in isolation with co-citations mirroring co-authorship within the cluster. Our network mapping shows clusters in the co-authorship maps between countries and institutions, indicating a possibly higher tendency for scientists to reference work from their collaborative networks. These distinct clusters can lead to citation biases (Mattiazzi and Vila-Petroff, 2024) and siloed research efforts, where scientists may lack engagement with other aspects of resistance or environmental influences on microbial behaviour investigated by authors outside their cluster of collaborations. This bias can, at least in part, be detected using appropriate metrics, such as 'author bias', which takes into account the frequency of contributions from specific authors, providing a measure of the potential effect of overrepresented individuals (Le Clercq, 2024). This siloed research, though typical in mainstream science, hampers comprehensive approaches to managing antibiotic resistance effectively. To address this, fostering interdisciplinary collaboration in research and analysis is essential. For instance, integrating environmental science with microbiology could link detailed molecular insights with broad ecosystem processes. Such an integrated approach would not only expand the scientific foundation but also strengthen practical outcomes for risk management, creating a powerful alliance against the global challenge of antibiotic resistance. Furthermore, studying AMR through the One Health prism necessitates that funding agencies recognise the benefits of funding research across sectors and disciplines, and that journals accept multidisciplinary studies encompassing humans, animals and environmental factors. For example, AMR can spread across ecosystem barriers (e.g. from the human gut to freshwater and marine environments). Studies that include such a wide variety of ecological compartments can be challenging for funding agencies and journals that focus on a particular scientific domain or type of ecosystem.

The geographic imbalance in antibiotic resistance research, predominantly concentrated in high-income countries such as those in Europe, the United States, Australia, but also the People's Republic of China, significantly under-represents low-income regions, particularly Africa, although the trend appears to have changed during the recent years. This disparity highlights a critical global health challenge. Several reasons may explain this situation: Higher income countries often have more developed scientific infrastructures and greater access to research and development funding (World Bank, 2024). These resources enable higher income countries to undertake elaborate, long-term research projects, equipped with advanced technologies and skilled personnel. The concentration of antibiotic resistance research in the non-built environment in the People's Republic of China, where the output of manuscripts significantly surpasses that of any other countries, also highlights various systemic and strategic factors influencing research

tendencies. Such dominant position in antibiotic resistance research of the People's Republic of China may be attributed to its governmental prioritisation and substantial funding directed towards tackling AMR. As one of the most populous countries with a massive agricultural sector and widespread use of antibiotics in healthcare and farming (Lin et al., 2020; Shao et al., 2021), the country recognises AMR as a significant threat to its public health and food safety (Thomas and Lo, 2019). Notwithstanding, the cash-per-publication reward policy has also been attributed to the increasing number of publications emerging from this country (Quan et al., 2017). In contrast, the limited presence of research from Africa, despite its potentially higher risk factors for antibiotic resistance, particularly clinical misuse of antibiotics (Chokshi et al., 2019), underscores deep global disparities in scientific output and focus. Many African and other low-income countries, where health budgets are strained, struggle to allocate resources for surveillance and research into antibiotic resistance (Chokshi et al., 2019; Pokharel et al., 2019; Simpkin et al., 2019; Iskandar et al., 2021; World Bank, 2024). Moreover, the research agenda in high-income countries might not align with the pressing needs or the unique cultural and sociopolitical conditions found in low-income countries (Dimitris et al., 2021; Eichbaum et al., 2021). It is worth noting that we reviewed only documents written in English and, therefore, has not captured the full extent of research reported in other languages. Additionally, challenges to publication of research conducted in low-income countries in international literature are well known. High-impact journals often favour studies from more established research economies due to factors such as networking, access to higher quality data, and the perceived rigour of methodological approaches (Harris et al., 2017a,b). This can perpetuate a cycle where research in low-income countries remains under-cited and less visible (Mattiuzzi and Vila-Petroff, 2024). Due to the global threat of AMR, strengthening global health responses to antibiotic resistance requires an intentional shift towards more inclusive, collaborative international research frameworks that prioritise resources for research and capacity building to low-resource settings. Programs that foster partnerships between high-income and low-income countries, provide training, share technologies, and financially support research initiatives in lower income countries in Africa and other areas, such as the Fleming Fund Fellowship (<https://www.flemingfund.org/>), are vital. Encouraging the local ownership and context-specific adaptation of research projects can also ensure that findings are relevant and can effectively inform public health strategies tailored for relevant communities.

In constructing our systematic evidence map, we encountered several challenges. Due to the extremely large volume of outputs, the review was concluded in September 2021, as outlined in the protocol. Nonetheless, the findings are considered robust given the exceptionally high number of included studies. To assess the potential impact of newer research, we conducted a modified search update from September 2021 to April 2025. Our estimate suggests that fewer than 18 additional studies (4% of articles retrieved post-September 2021) addressed the identified research gaps, indicating these areas remain under-represented. Notably, studies focusing on microplastics and Africa have increased from 0.5% to 3%, suggesting a rapidly increase interest.

The diversity of publications in terms of aims, methodology and presentation of findings increased the difficulty in mapping available scientific evidence. In some cases, authors presented several dozens of ARGs in the form of images, which had to be manually inputted by our reviewers. Additionally, in other instances, the specific species of ARB were omitted and only the bacterial family name or phylum was reported instead. Therefore, to streamline the process of evidence mapping and facilitate the synthesis of knowledge, it is crucial to implement universally accepted minimum information reporting standards for antimicrobial resistance data, similar to those used in other fields such as gene expression (Brazma et al., 2001) or autophagy (Klionsky

et al., 2021). While initiatives like the EPI-Net One Health reporting guidelines (Babu Rajendran et al., 2023) offer frameworks, wider adoption and harmonisation across journals of such guidelines would vastly improve data extraction, data comparability and analysis. By implementing such standards, researchers could improve annotation of diverse datasets on antimicrobial resistance.

Also, a significant caveat with bibliometric maps is their sensitivity to the initial parameters such as the choice of databases, keyword sets, and the inclusion of grey literature. Moreover, bibliometric analysis can be influenced by publication bias, where studies with significant results are more likely to be published and hence more likely to be included in the map. Finally, the use of Clarivate Analytics' Journal Impact Factor (JIF) as a measure of impact has several limitations. Since the JIF is a metric calculated from citations and data indexed in the Web of Science Core Collection, it is subject to many factors that influence citation rates, such as the volume of publication and citation characteristics of the subject area and type of journal. Also, it is not openly accessible, and some authors consider similar measures an inadequate metrics to capture the complexity and multiple dimensions of research impact (Haustein and Larivière, 2015).

During the screening of our systematic mapping, we have used the assistance of predictive models such as 'SWIFT-Active Screener' (Howard et al., 2020). Future uses of artificial intelligence (AI), particularly natural language processing and machine learning, can assist in systematic evidence maps and other reviews to interrogate the literature (Gardner et al., 2024). For instance, AI technologies can automate repetitive tasks and help reduce human error due to tiredness or distraction from tedious tasks (Wu et al., 2019). They can also increase consistency, as it eliminates the possibility of human variability in interpreting research (Burger et al., 2023) and can analyse images and identify patterns, such as text from figures or tables, extracting valuable data that might otherwise require manual interpretation (Davenport and Kalakota, 2019; Sidorov et al., 2020). Further future applications of AI could also help create continuous literature reviews that are always up to date and summarising the current state of the research, especially for resource-intensive and large-scale reviews like the current one, (living systematic reviews, see <https://community.cochrane.org/review-development/resources/living-systematic-reviews#what>) (Burger et al., 2023).

Future research on antibiotic resistance should diversify to include systematic reviews examining multifactorial contributions to resistance, regional disparities, and intervention strategies, particularly in under-researched locations as described in this evidence map. Funding bodies are advised to prioritise interdisciplinary projects that take a One Health approach, support long-term studies and access to datasets (see e.g., (The Center for Disease Dynamics, Economics & Policy, 2024)), invest in emerging economies, and facilitate global collaborations to ensure a comprehensive and uniform global response. Additionally, identifying resistance hotspots via spatial and temporal mapping, predictive modelling, and field studies could be vital (Zhang et al., 2022b). AMR researchers and funding partners should increase focus on the interconnections between human, animal and environmental drivers, impacts and risks, and scientific journals must be accommodating of the multidisciplinary aspect of One Health research. These strategies would enable more accurate predictions and targeted interventions, enhancing our understanding and management of antibiotic resistance globally.

## 5. Conclusions

This systematic evidence map has revealed a substantial concentration of AMR research in the non-built environment originating mainly from two countries and high-income areas. Available reports predominantly explore fresh water environments, reflecting a geographic and thematic focus that may not provide a comprehensive view of AMR distribution globally. The observed biases in AMR research have implications for understanding AMR emergence, dissemination, and risk in a

One Health context. The majority of identified studies have investigated key environmental stressors such as wastewater exposure and manure application; while these are well-recognised vectors for AMR dissemination, our map indicates a scarcity of research in non-agricultural or less common environments. The primary organisms under study have been *E. coli* and *Pseudomonas* spp., with a focus on ARGs that confer resistance to sulphonamides, tetracyclines and beta-lactams. These findings underscore the need for an expanded scope of research to include diverse environments and geographic regions, particularly low-income countries, to develop a more global understanding of AMR dynamics. The evident emphasis on certain bacteria and resistance mechanisms suggests a potential to diversify research efforts towards lesser-studied microbes and ARGs, possibly presenting new insights into mitigating AMR spread.

#### CRediT authorship contribution statement

**Iñaki Deza-Cruz:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Formal analysis, Conceptualization, Writing – original draft, Validation, Software, Project administration, Investigation, Data curation. **Alexandre de Menezes:** Investigation, Writing – review & editing, Funding acquisition. **Brian Gardner:** Writing – review & editing, Resources, Investigation, Software, Methodology. **Ílknur Aktan:** Writing – review & editing, Investigation. **Sarhad Alnajjar:** Writing – review & editing, Investigation. **Martha Betson:** Writing – review & editing, Investigation. **Adriana Cabal Rosel:** Investigation, Writing – review & editing, Funding acquisition. **Manuela Caniça:** Writing – review & editing, Funding acquisition, Investigation. **Mark A. Chambers:** Investigation, Writing – review & editing, Funding acquisition. **Georgina Tarrant:** Investigation, Writing – review & editing. **Francesca Contadini:** Investigation, Writing – review & editing. **Olukayode Daramola:** Investigation, Writing – review & editing. **Rani de la Rivière:** Investigation, Writing – review & editing. **Bernadette Egan:** Investigation, Writing – review & editing. **Abel Ekiri:** Investigation, Writing – review & editing. **Catherine Finnegan:** Investigation, Writing – review & editing. **Laura C. Gonzalez Villeta:** Investigation, Writing – review & editing. **Richard Green:** Investigation, Writing – review & editing. **Belinda Hall:** Investigation, Writing – review & editing. **Marwa M. Hassan:** Investigation, Writing – review & editing. **Martin Hawes:** Investigation, Writing – review & editing. **Sara Healy:** Investigation, Writing – review & editing. **Lisa Holbrook:** Investigation, Writing – review & editing. **Damla Kaya:** Investigation, Writing – review & editing. **Prashant Kumar:** Investigation, Writing – review & editing. **Roberto M. La Ragione:** Writing – review & editing, Funding acquisition, Investigation. **Daniel Maupin:** Writing – review & editing, Software, Visualization, Investigation. **Jai W. Mehat:** Writing – review & editing, Investigation. **Davide Messina:** Writing – review & editing, Investigation. **Kelly Moon:** Writing – review & editing, Investigation. **Elizabeth Mumford:** Writing – review & editing, Investigation. **Gordon Nichols:** Writing – review & editing, Investigation. **Daniel V. Olivença:** Writing – review & editing, Investigation. **Joaquin M. Prada:** Writing – review & editing, Investigation. **Claire Price:** Writing – review & editing, Investigation. **Christopher Proudman:** Writing – review & editing, Investigation. **Retha Queenan:** Writing – review & editing, Investigation. **Miguel Ramos:** Writing – review & editing, Investigation. **Jaime Riccomini Closa:** Writing – review & editing, Investigation. **Jennifer M. Ritchie:** Writing – review & editing, Investigation. **Lorenzo A. Santorelli:** Writing – review & editing, Investigation. **Nick Selemetas:** Writing – review & editing, Investigation. **Matt Spick:** Writing – review & editing, Investigation. **Yashwanth Subbannayya:** Writing – review & editing, Investigation. **Shelini Surendran:** Writing – review & editing, Investigation. **Pedro Teixeira:** Writing – review & editing, Investigation. **Mukunthan Tharmakulasingam:** Writing – review & editing, Investigation. **Damian Valle:** Writing – review & editing, Investigation. **Arnoud H.M. van**

**Vliet:** Writing – review & editing, Investigation. **Marco Videira:** Writing – review & editing, Investigation. **Hazel Wallace-Williams:** Writing – review & editing, Investigation. **Klara M. Wanelik:** Writing – review & editing, Investigation. **Markus Woegerbauer:** Investigation, Writing – review & editing, Funding acquisition. **Sydney Wright:** Investigation, Writing – review & editing. **Giovanni Lo Iacono:** Writing – original draft, Validation, Software, Project administration, Investigation, Formal analysis, Conceptualization, Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Data curation.

#### Registration

This protocol was registered on the Open Science Framework (OSF), with project DOI: 10.17605/OSF.IO/A8GV6 (<https://osf.io/a8gv6>).

#### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ELM - (the Edinburgh (access to) Language Models) (<https://information-services.ed.ac.uk/computing/comms-and-collab/elm>). ELM is the University of Edinburgh's AI innovation platform to safer access of Generative Artificial Intelligence (GAI) via Large Language Models (LLMs). This tool was used to assist in enhancing grammar and spelling in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: RLR is a scientific advisor for Vidiia (<https://www.vidiia.com>). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.envint.2025.109634>.

## Data availability

Data are available in the Supplementary Material and external repository provided.

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