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Mapping the evidence of the effects of environmental factors on the prevalence of antibiotic resistance in the non-built environment: Protocol for a systematic evidence map

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ABSTRACT

Background: Human, animal, and environmental health are increasingly threatened by the emergence and spread of antibiotic resistance. Inappropriate use of antibiotic treatments commonly contributes to this threat, but it is also becoming apparent that multiple, interconnected environmental factors can play a significant role. Thus, a One Health approach is required for a comprehensive understanding of the environmental dimensions of antibiotic resistance and inform science-based decisions and actions. The broad and multidisciplinary nature of the problem poses several open questions drawing upon a wide heterogeneous range of studies.

Objective: This study seeks to collect and catalogue the evidence of the potential effects of environmental factors on the abundance or detection of antibiotic resistance determinants in the outdoor environment, *i.e.*, antibiotic resistant bacteria and mobile genetic elements carrying antibiotic resistance genes, and the effect on those caused by local environmental conditions of either natural or anthropogenic origin.

Methods: Here, we describe the protocol for a systematic evidence map to address this, which will be performed in adherence to best practice guidelines. We will search the literature from 1990 to present, using the following electronic databases: MEDLINE, Embase, and the Web of Science Core Collection as well as the grey literature. We shall include full-text, scientific articles published in English. Reviewers will work in pairs to screen title, abstract and keywords first and then full-text documents. Data extraction will adhere to a code book purposely designed. Risk of bias assessment will not be conducted as part of this SEM.

We will combine tables, graphs, and other suitable visualisation techniques to compile a database i) of studies investigating the factors associated with the prevalence of antibiotic resistance in the environment and ii) map the distribution, network, cross-disciplinarity, impact and trends in the literature.

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1. Background

Antimicrobial resistance (AMR) represents a growing threat to human, animal and environmental health. According to some estimations, by 2050, AMR could cause up to 10 million deaths globally annually, a figure compared to the death toll from cancer (see United Nations Environment Programme (2022) and references therein). AMR is commonly attributed to the inappropriate use of antimicrobial compounds, such as antibiotics, for treatment and prophylaxis of infectious diseases (O'Neill, 2014). However, the problem is far more complex and it is becoming increasingly clear that a wide range of biological and non-biological factors are also contributing to this trend. To mention some, effluent discharge from wastewater treatment plants is a known driver of AMR in aquatic and soil environments (Kampouris et al., 2021; Kampouris et al., 2021; Bengtsson-Palme et al., 2018). Local temperature and population density are associated with increasing antibiotic resistance in common pathogens. For instance, antibiotic resistance in *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* is expected to increase by 4.2%, 2.2% and 2.7%, respectively, for an increase in temperature of 10 °C across regions in the United States (MacFadden et al., 2018), implying that current estimations of the burden of antibiotic resistance need to be revisited in consideration of growing population and climate change. Environmental pollutants of anthropogenic origin, such as heavy metals and agrochemicals used in agriculture, have also been recently identified as a potential risk factor for AMR. The presence of pollutants can selectively enrich bacteria carrying antibiotic resistance genes (ARGs), conferring resistance to these compounds and potentially resulting in co-selection (Ramakrishnan et al., 2019; Poole, 2017). Furthermore, the spread of AMR between and among people, animals and other environmental reservoirs is vehicled by different domains like water, soil and air.

This problem has attracted the interest of the scientific community and several systematic reviews have been conducted to assess the drivers impacting antibiotic resistant bacteria (ARB) and 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). According to these studies, prominent drivers of AMR included the dissemination of antibiotics into natural water bodies, which in part has been traced to agricultural effluent discharge and other waste products such as treated industrial wastewater, agricultural practices involving antibiotic use in animals, and agrochemicals such as fertilisers used in food production. Despite the importance of these studies, the scope of their research question was inevitably constrained to target a small subset of the wider evidence base. Unsurprisingly, the authors chose systematic review methodologies as these are well suited to address specific, often dichotomous, research questions where the findings from primary research are aggregated and averaged for robust empirical statements (Gough et al., 2012).

Here, however, we have a broader aspiration and our aim is to identify, collect, organise and publicly share the evidence on the complex nature of the increase, spread and persistence of antibiotic resistance in the environment (Fig. 1). This problem triggers multiple, open research questions since it occurs in different domains (*i.e.*, soil, water, air), involves different causal pathways, depends on a variety of natural/anthropogenic factors and is subjected to multiple potential hazards or stressors (*e.g.*, temperature, soil characteristics, concentrations of trace elements and heavy metals, and drought stress), thereby leading to a heterogeneous body of evidence.

Conceptual frameworks describing the sources of AMR and the pathways of dissemination and transmission have been reported in the literature since 1973 (Singer et al., 2020). In our protocol, we have adapted previous models (Boerlin and White, 2013; Kovalakova et al., 2020) to describe a schematic pathway of antimicrobials from human and animal use to their dissemination in the non-built, non-industrial,

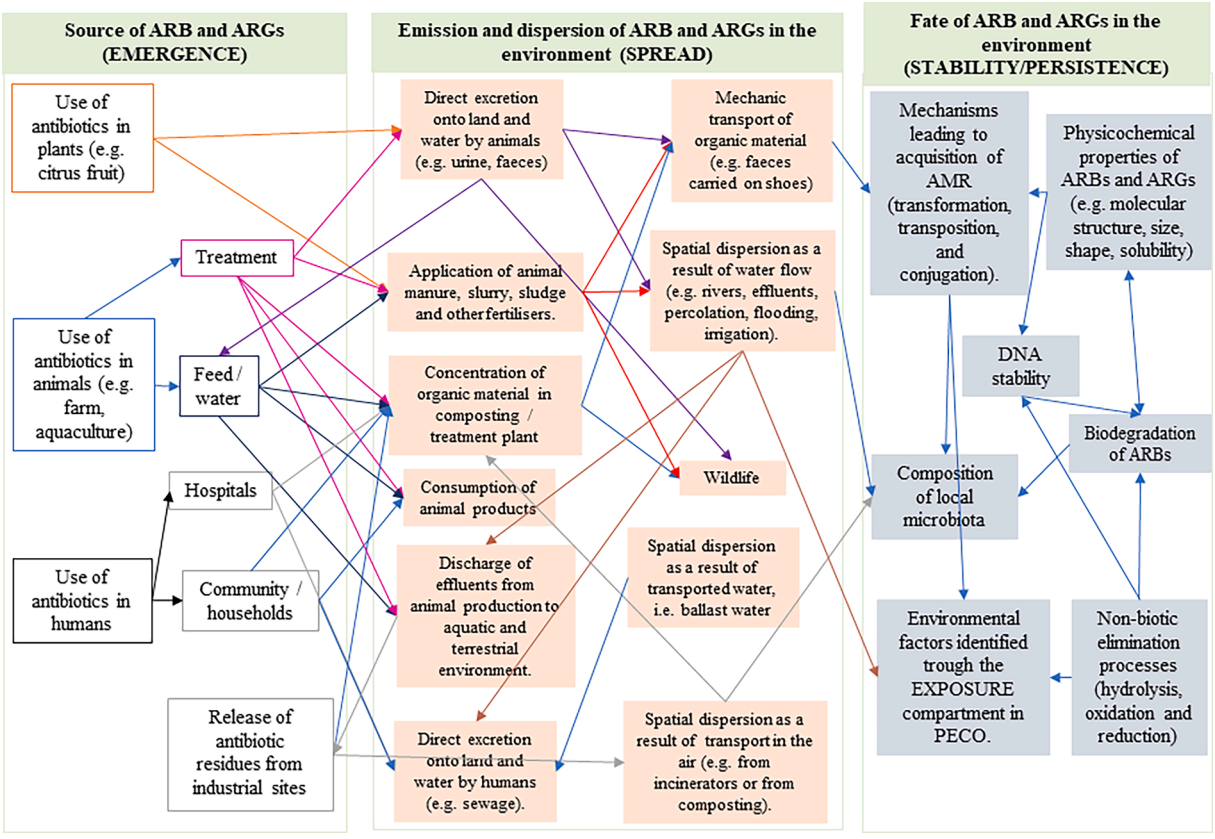

Fig. 1. Conceptual framework for emergence, dispersion and persistence of ARB and ARGs in the environment. The arrows are coloured by topic to assist with visualisation of the pathways. Diagram created by the authors.

environment in line with the PECO statement (Fig. 1). The complexity of the holistic nature of the problem suggests the adoption of a 'configurative' logic where we arrange and interpret the information to identify patterns within heterogeneity, rather than aggregating homogeneous findings (Gough et al., 2012).

As recommended by United Nations Environment Programme (2022), a One Health vision encompassing human, animal and environmental health is required for a comprehensive understanding of the environmental dimensions of antibiotic resistance and to inform science-based decisions and actions. Here, we propose to conduct a systematic evidence map (SEM) to organize and synthesise the (putative) evidence of the effect of local environmental factors on the occurrence of antibiotic resistance with a multi-perspective approach (Wolffe et al., 2020; Wolffe et al., 2019; Schreier et al., 2022). SEMs typically extract metadata (*i.e.*, a set of data that describes and provide information about other data) describing the quantity and the nature of the research in a particular field, rather than findings of the research itself. Extraction and statistical analysis of study findings are discouraged in SEMs (James et al., 2016) to prevent vote counting (*e.g.*, how many studies showed a positive versus negative outcome) of heterogeneous findings and study quality. Accordingly, no statistical analysis of study findings will be conducted in the current SEM. The objectives of evidence maps, however, do not preclude extraction and cataloguing of study results to support future research activities (Schreier et al., 2022). In addition to our systematic mapping of the evidence base we will, to a lesser extent, present a 'narrative synthesis' that might guide future systematic reviews and meta-analyses. Expected outcomes of the SEM process includes the identification of gaps (*i.e.*, under-represented areas of our understanding requiring primary research) and clusters (*i.e.*, established areas that might benefit from secondary research like systematic reviews and modelling) in the current knowledge, nature and level of cross-disciplinary among different fields, trends in the field and potential emerging areas of research; the results will be presented in a visual format and/or database (Miake-Lye et al., 2016).

1.1. Scope, aim and objectives

We aim to systematically map the current body of evidence regarding anthropogenic and natural factors indicative of the prevalence of antibiotic resistance in the outdoor environment, with regards to ARB and ARGs with clinical relevance for humans and/or animals. In this context, we are referring to samples collected outside of a built-up setting: locations which can be broadly classified as natural spaces (*e.g.*, grassland, lakes and rivers), semi-natural spaces (*e.g.*, agricultural land), green infrastructures (*e.g.*, gardens and parks), and former industrial sites no longer used for industrial purposes. We exclude from our consideration outdoor areas subject to intense and constant industrial processes, for example, feedlots and waste processing plants. This description constitutes our population, as shall be elaborated on in the Methods Section, where we follow the population, exposure, comparator, and outcome (PECO) statement to frame our SEM (Table 1). Moreover, we define our exposure as a measure of 'contact' (that can be expressed as the amount of time, frequency, intensity, or their combination) with either a source of antibiotic resistance (hazard) or a medium whose physical, chemical or biological properties can affect the emergence, dispersion, and persistence of antibiotic resistance determinants (stressors). A full definition of our population and exposure can be found in Table 2 and Table S-1, and Figs. S-1–S-4. Accordingly, we aim to address the following over-arching question:

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

Our intention is to gather and collate data to inform future research, policy-relevant systematic review questions, and possibly future funding strategies, concerning risk mitigation for antibiotic resistance emerging in the environment. Considering the breadth and complexity of the topic, the evidence will be organised and presented into homogeneous components by stratifying distinct sub-populations (*e.g.*, soil on farms or surface water), exposures to hazards and stressors (*e.g.*, use of fertilisers or pH of the medium), comparators (*e.g.*, presence/absence of fertiliser or higher/lower temperature) and outcomes (*e.g.*, presence/absence or prevalence of antibiotic resistance determinants).

Table 1
Population, Exposure, Comparator, Outcome (PECO) statement.

PECO statement	Evidence
Population	Different terrestrial, aquatic and atmospheric non-built-up, non-industrial, environments listed in Table 2 and Fig. S-1.
Exposure	Specific local environmental factors listed in Table S-1 and Figs. S-2–S-4.
Comparator	This refers to one of the following: i) Presence/Absence of the exposure, ii) Increase/Decrease or No Variation in the exposure, iii) No Comparison. 'No Comparison' category is used if the study reports a measure of ARB and/or ARGs even without an explicit comparison, provided that a measure of associated exposure is documented (for example, a study might report a measure of ARB and/or ARGs along with a single measure of the local temperature).
Outcome	Occurrence of antibiotic resistance bacteria and/or antibiotic resistance genes listed in Table S-2 and Fig. S-5 (see also Table S-4).

2. Methods

The protocol for this SEM has been designed following the guidelines described by James et al. (2016), in addition to the PRISMA-P statement as detailed in Moher et al. (2015,) adapted to SEM (Elsevier, 2017). A completed PRISMA-P report (modified) is provided in the supplementary S1 File. In line with these guidelines, the protocol has been registered online on a public repository Gardner (2021). The expected timeline for completion of this SEM is within 18 months from the publication of this protocol.

2.1. PECO statement

To guide the scope of our systematic evidence map, including our eligibility criteria and code book, we structure our SEM according to the PECO framework (Table 1).

2.2. Eligibility criteria

The criteria for study eligibility in this SEM will be based on the PECO framework, as adapted to studies on antibiotic resistance (Bueno et al., 2017; Williams-Nguyen et al., 2016). Each of these items are elaborated on as follows:

- *Population.* The population considered here includes samples obtained from non-built-up, non-industrial environmental compartments, encompassing terrestrial and aquatic locations, and atmospheric matter as listed in Table 2 and Fig. S-1.
- *Exposure.* Sources of exposure to hazards and stressors encompass known or suspected drivers of antibiotic resistance, which could originate from anthropogenic activities which are potentially hazardous (*e.g.*, antibiotics, waste products, chemical pollutants such as herbicides used in agriculture, *etc.*) or otherwise (*e.g.*, trace elements and heavy metals which might occur naturally). Additionally, we also consider the exposure of samples to local environmental conditions, which might be selective for antibiotic resistance traits; examples of potential environmental stressors include humidity, soil characteristics, temperature, wind speed, and other related items. We also consider exposure sources originating from built-up sites impacting on outdoor locations (*e.g.*, hospitals discharging waste products into the surrounding area: Table S-1 and Figs. S-2–S-4). We

Table 2
List of specific populations considered. This encompasses the outdoor locations from which samples are obtained, organised by their environmental compartment.

	Specific Population	Definitions
Terrestrial Environment	Soil in plant microcosm	This includes soils from experimental studies with controlled conditions. It has been incorporated for the reliable and abundant environmental data usually collected in such systems
	Agriculture land (for plant cultivation)	This includes soil from any agricultural area used for plant production
	Agriculture land (for livestock)	This includes soil from any agricultural area used for livestock production
	Former industrial areas	This includes former industrial areas typically left undisturbed, but which represent a hazard
	Green infrastructures	These typically include urban grassland soil with a variety of uses: e.g. Parks and gardens; natural and semi-natural urban green spaces; green corridors; outdoor sports facilities; amenity green space; allotments, community; gardens and city farms; cemeteries and churchyards; accessible countryside in urban fringe areas; civic spaces; tree-lined areas along boulevards
	Country walks	National Parks, hiking areas, walking areas, dog walking areas
	Other terrestrial environment	This includes other non-urban soil: Woodland, forest, and other wooded land; grassland; heathland; scrub and tundra; savanna; desert (see European Environment Agency (2022))
Aquatic Environment	Brackish water; Estuaries	These environments are more saline than freshwater, but not as saline as marine waters. Estuarine environments are where salt and freshwater mix, thus salinity will vary depending on tide.
	Marine	This includes marine waters only.
	Fresh water (ground water)	This includes fresh ground waters only with a variety of uses, *e.g.*, (i) water for domestic use (ii) water for human consumption (iii) water for irrigation (iv) recreational water
	Fresh water (surface water)	This includes fresh waters only with a variety of uses, *e.g.*, (i) water for domestic use (ii) water for human consumption (iii) water for irrigation (iv) recreational water
	Fresh water (pristine water)	This includes fresh waters in pristine environments only.
	Coastal areas; Salt marsh; Tidal marsh	This includes the interface between terrestrial and aquatic environments and may include samples from solid substrate like sand or sediments
	Mangrove	Samples from either solid or aquatic substrate in mangroves.
Atmospheric Environment	Bioaerosol	This includes fungi, bacteria, viruses, and pollen collected from the atmosphere
	Airborne dust and other solid particles	This includes any airborne particles other than bioaerosol.
	Droplets	This includes droplets only

note that the distinction between hazard and stressor can be subtle in some situations, however their classification does not affect the workflow of this SEM. The effect of various forms of exposure is expected to be different according to the stage of antibiotic resistance pathway: from its emergence, spread and through to its persistence. For instance, the proximity of a water body to a waste processing plant could be associated with the emergence of ARB and ARGs; water flow with its dispersion; and UV radiation with its persistence in the environment.

- *Comparator.* This is established by comparing how the outcome measure (defined below) changes with different levels of exposure. These measurements may be expressed categorically or numerically, as summarised in Table S-3.
- *Outcome.* This is the detection or measured abundance of antibiotic resistance determinants (*e.g.*, the abundances of ARGs or presence/absence of ARB) as evaluated from environmental samples. These measurements may be either qualitative or quantitative in description (Fig. S-5).

In this SEM, we shall not investigate: (i) transmission mechanisms from the environment to humans or animals; (ii) antibiotic resistance determinants as evaluated from samples obtained from human or animal hosts, nor food products; (iii) evolutionary mechanisms involved in the emergence of antibiotic resistance; (iv) the impact of trade and human travel on dissemination.

2.3. Information sources

The literature search will be conducted using the following electronic databases: MEDLINE, Embase, and the Web of Science Core Collection (*i.e.*, Science Citation Index Expanded (SCI-EXPANDED) from 1900 to present; Social Sciences Citation Index (SSCI) from 1900 to present, Conference Proceedings Citation Index - Science (CPCI-S) from 1900 to present, Conference Proceedings Citation Index - Social Sciences & Humanities (CPCI-SSH) from 1900 to present, and Emerging Sources Citation Index (ESCI) from 2015 to present). The institutional subscription at the University of Surrey will ensure access to MEDLINE (on the following platforms: ProQuest, EBSCOhost, Web of Science, as well as PubMed without subscription), Embase (Ovid) and Web of Science Core Collection via the Web of Science platform. We shall additionally mine the grey literature by searching relevant databases (see full list and details in Section S4 File). A preliminary search of the grey literature returned a manageable number of records (for most databases the returned records were about 0 −5 and only occasionally the search returned 30 −50 records). We shall include full-text, scientific articles published in English. Based on preliminary searches, we found that most studies about antibiotic resistance in relation to environmental rather than clinical settings were conducted after the year 2000; therefore, to ensure a wide coverage of the literature, we shall include articles published in the last ∼ 30 years (specifically, from the start of 1990 to the present). For each database we record the search strategy and filters applied in adherence to the PRISMA-S statement for reporting literature searches in systematic reviews, which we extend to SEMs (Rethlefsen et al., 2021).

2.4. Search strategy

We have developed and peer reviewed our search strategy, in consultation with an external, senior information specialist, 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). PRESS 2015 is a best practice guideline for performing search strategies and aims to reduce errors by search validation and reporting. The search strategy we use for MEDLINE (via PubMed) will be based on Medical Subject Headings (MeSH); the search string we shall use is shown in Table 3, 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 stressor for the emergence of antibiotic resistance, and specific exclusion terms. These search concepts are derived from the aforementioned PECO statement: the first two groups (*i.e.*, antibiotic resistance and environmental

Table 3
PubMed search strategy.

Concept	Search string
Antibiotic resistance and genetics	("drug resistance, microbial"[MeSH Terms]) AND ("genetic phenomena"[MeSH Terms] OR "genetics"[MeSH Subheading] OR "microbiology"[MeSH Subheading])
	AND
Environmental compartment	("environment"[MeSH Terms] OR "environmental microbiology"[MeSH Terms] OR "geological phenomena"[MeSH Terms] OR "geography"[MeSH Terms] OR "soil"[MeSH Terms] OR "water"[MeSH Terms] OR "animals, wild"[MeSH Terms] OR "environmental pollutants"[MeSH Terms] OR "particulate matter"[MeSH Terms] OR "agriculture"[MeSH Terms])
	AND
Exposure source (hazard or stressor)	("meteorological concepts"[MeSH Terms] OR "inorganic chemicals"[MeSH Terms] OR "animals"[MeSH Terms] OR "animal feed"[MeSH Terms] OR "manure"[MeSH Terms] OR "waste products"[MeSH Terms] OR "agrochemicals"[MeSH Terms])
	NOT
Exclusion terms	("gastrointestinal microbiome"[MeSH Terms] OR "food safety"[MeSH Terms] OR "review"[Publication Type])

compartment) refer to our considered population and target outcome, including antibiotic resistance determinants as sampled from terrestrial, aquatic or atmospheric environments (see Table 2). As expected, the third group described as the 'exposure source' captures the exposure, and by extension comparator, components of our PECO description (see Tables S-1). As stated in our eligibility criteria, included studies will be restricted to those taking samples from non-built-up, non-industrial environments, and so exclude hospital and healthcare settings. Rural areas and agricultural, outdoor sites are included as part of our definition of the sampled environment.

This structuring of the search terms is also inspired by the approach used by two systematic review studies (Bueno et al., 2017; Williams-Nguyen et al., 2016). We enforce a reference to genetic concepts via the MeSH term "genetic phenomena" to narrow the range of returned articles. For the other databases, the search strategies are modelled based on these MeSH terms as closely as possible (see Supplementary Material, S2 File.). Additionally, the search strategies make reference to the somewhat broad concept of antimicrobial resistance for the PubMed and Web of Science databases, which correspond to the controlled search term 'antibiotic resistance' as used by Embase: this was chosen intentionally to support consistency across all the databases. This choice of search term also ensured a comprehensive coverage of the literature, thereby reducing the chances of missing potentially relevant articles. Pilot runs were conducted to validate the selection of these search criteria: on the most recent run, the total number of articles retrieved across all these databases was 17405 (before de-duplication). Hence, the validity of these search results was confirmed by making reference against a core collection of 50 benchmark papers which had been identified from a preliminary scoping exercise. With respect to the grey literature search, the use of Boolean operators was not always possible, which prevented the use of an identical search strategy across the different sources. Thus, to ensure reproducibility of the search, we list the specific search terms for each database (Supplementary Material, Section S4 File).

2.5. Data management

The titles and abstracts of studies identified through the literature search will be imported into EndNote™ (Clarivate) for reference management. Following this, de-duplication of records will be conducted using an EndNote™ built-in tool. Study screening will be assisted using a web-based software application for systematic work, 'SWIFT-Active Screener' (Howard et al., 2020), which accelerates this process by incorporating user feedback with a machine learning algorithm in order to re-prioritise articles as they are screened. Descriptive statistical analysis will be carried out using R (R Core Team, 2018).

2.6. Study selection

Retrieved studies will be allocated a unique identifier reference and screened against the eligibility criteria to assess their relevance to the research question. Depending on the number of studies retrieved, the full body of abstracts be allocated randomly to two or more subsets (of about 5,000–10,000 records). Each subset will be screened by at least two independent reviewers to select relevant studies based on the title, abstract and keywords. The relevance of the studies requires consensus between the two screening reviewers, with any conflicts being resolved by joint meetings. If a consensus cannot be reached, an additional researcher from the team will be consulted to resolve this. The series of questions used to assess study relevance are framed based on previous, but expanded, protocols regarding antibiotic resistance in the environment (Bueno et al., 2017; Williams-Nguyen et al., 2016), and are as follows:

1. Is the study primary research (*i.e.*, not a literature review) reported in a journal publication, a thesis, or grey literature?
2. Does the study describe any ARB or ARGs in a collected sample?
3. Were the samples collected from a non-industrial environment (as listed in Table 2)?
4. Does the study describe any known or suspected drivers of antibiotic resistance (*i.e.*, hazards and stressors) (as listed in Table S-1)?
5. Does the study report any measurements (either quantitative or qualitative) of the hazards and stressors described?
6. Does the study report the effect (including absence of effect) of those hazards and stressors on ARB or ARGs?

For each of these questions, possible answers include: 'Yes', 'No' and 'Cannot be determined' from the title, the abstract or keywords. If 'No' is stated for any one of these questions, then the study will be excluded from further consideration. Otherwise, the study will be considered potentially relevant to this SEM and retained for full-text reading. SWIFT-Active Screener indicates to the user the estimated percentage of relevant articles already included as screening progresses, which can be used to suggest a cut-off point beyond which screening can safely be stopped. For our purposes, we take 90% as our cut-off for the percentage of relevant articles having been included, in line with a previously established recommendation for this software (Pelch et al., 2019). The retained abstracts will be distributed amongst the reviewers for full-text screening. Each full-text study will then be 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' (Fig. 2). Whenever possible we shall ensure that their scientific background will complement each other. Details of the paired reviewers and their background will be made available in the Supplementary Material. One reviewer (primary) will select the full-text and the second reviewer will confirm the selection. The two reviewers will discuss all disagreements and address potential conflicts. If a consensus cannot be reached, an additional researcher from the team will be consulted to resolve disputes.

2.7. Data extraction and data coding strategies.

The retained full-text studies will be examined by the same pairs of reviewers for data extraction purposes (see Section 2.9). Following the work of Wolffe et al. (2019) and Schreier et al. (2022), some quantitative coding variables, *i.e.*, study findings, will also be extracted and presented as a 'narrative synthesis' to inform future research (see Section 2.10). Key features are summarised in Table S-5. The primary reviewer

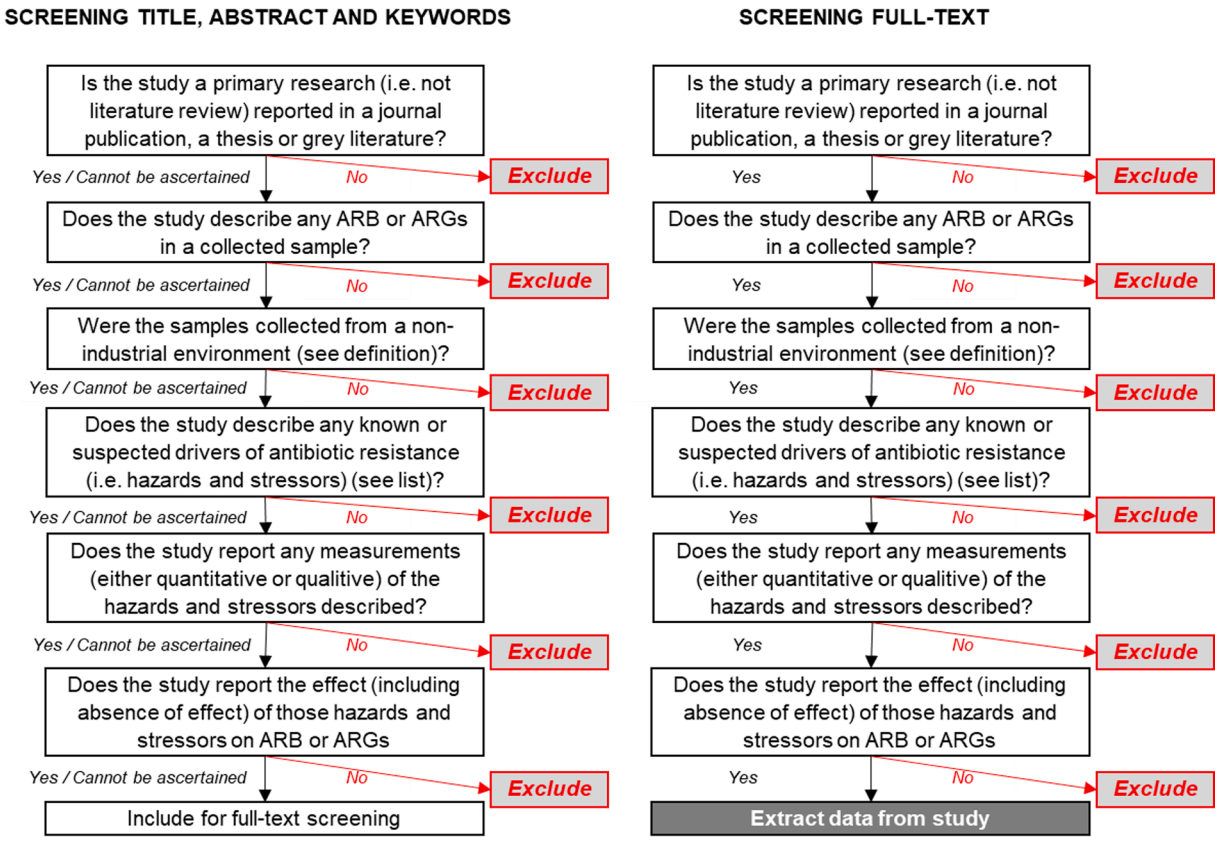

Fig. 2. Decision tree for initial screening of title, abstract and keywords (left) and for screening of full-text studies (right).

will perform data extraction according to the code book (Table S-6). Then, the second reviewer will assess the information extracted from the full-text studies by the first reviewer in a read-only version of the spreadsheets generated by Qualtrics™ electronic forms. The two reviewers will discuss all disagreements and solve potential conflicts. If a consensus cannot be reached, an additional researcher from the team will be consulted to resolve this. Based on our pilot project, we estimated that about 10 −30 documents will be allocated to each primary reviewer. If the allocated number of documents is less than 10, then the second reviewer will double-check extracted information from all documents; otherwise a representative sample (at least 50%) of the allocated documents will be selected at random for cross-checking. The data coding strategy is visualised in Fig. S-6 and implemented by using the web-based electronic form Qualtrics™. The tool will host an electronic form constructed on the inter-related tables (Fig. S-6) that will guide reviewers to extract the required information. The data collected in the Qualtrics™ electronic forms will be exported into a flat table in comma separated value (csv) format. The measure of the outcome and/or exposure can be categorical (presence/absence), ordinal (low, medium, high) or numerical (*e.g.*, prevalence, concentration).

Reviewers will first gather bibliometric data including details from the corresponding author such as the affiliation institution, country of affiliation and key words from the publication among other details (Table S-7). Also, information related to the type of research, the location of the study and funding received will be investigated (Table S-6). Next, reviewers will scan the selected publications to obtain data related to the SEM. Questions connected to the 'population' section of the PECO statement are included in Table S-6 and consist of environmental information such as the type of environment (*i.e.*, Terrestrial, Aquatic or Atmospheric) and deeper levels of habitat information (*e.g.*, marine water, fresh water, brackish water or coastal areas in the case of aquatic environments), the use of such environment and anthropogenic alteration of the environments. Sources of 'exposure' and 'comparators' will be studied in the 'hazards' information table (Table S-6). Hazards to be studied will include physio-chemical variables of the sampling site (*e.g.*, Temperature; Altitude; Soil composition; Soil type; pH, *etc.*), metals and other chemicals (*e.g.*, Cd; Cu; Ag; Zn; As; Cr; Hg; Pb; Microplastics; other), exposure to biocides, proximity to farms or industrial sites and weather patterns among others. The complete list of hazards can be seen in Table S-1. The 'outcome' section will be extracted with the questions detailed in Table S-6 related to antimicrobial information. These will comprise the name of ARBs and ARGs, the class of antibiotic to which are resistant and clinical relevance. Where possible, we shall extract data concerning: i) the taxonomic classification of the resistant bacteria (*e.g.*, *Salmonella*) and of the antibiotic class; ii) the resistance mechanism (intrinsic or acquired); iii) if the antibiotic resistance was related to veterinary or human medicinal products; (iv) if the hazard is likely to be associated with: emergence, dispersion, persistence of antibiotic resistance determinants, (v) insights on the causal pathway if discussed in the full-text, and finally, vi) the mechanisms of HGT involved in the transmission of antibiotic resistance, and possibly the environmental factors associated with these mechanisms.

2.8. Risk of bias assessment

Risk of bias assessment will not be conducted in this SEM.

2.9. Evidence mapping and presentation

The qualitative coding variables will be synthesised by using tables, graphs, and other suitable visualisation techniques(see *e.g.*, Vendl et al. (2021, 2016)) to map:

A) the evidence of the factors associated with the prevalence of antibiotic resistance in the environment (Fig. 3).

B) the distribution, network, cross-disciplinarity, impact and trend of the literature (Fig. 4).

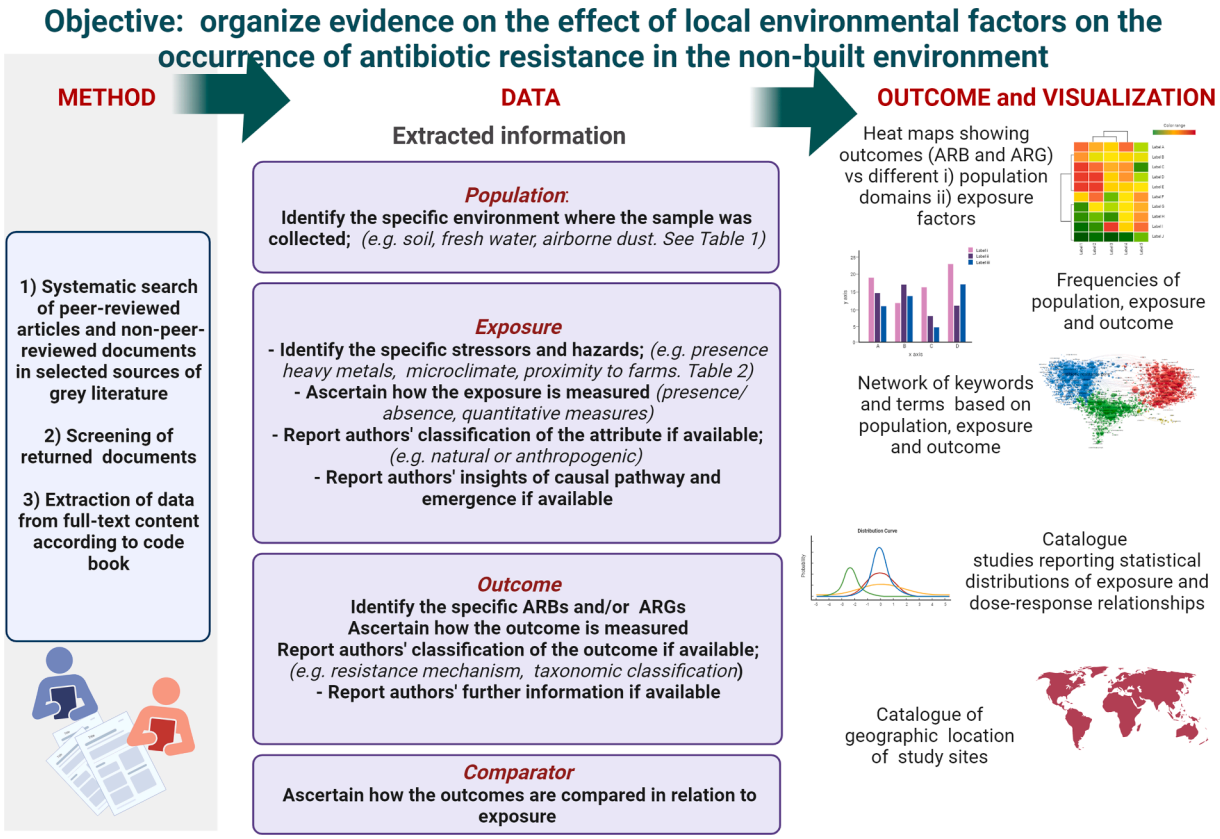

Fig. 3. Simplified and exemplary schematic of the SEM process with expected outcomes and type of visualization. Created with BioRender.com (2022).

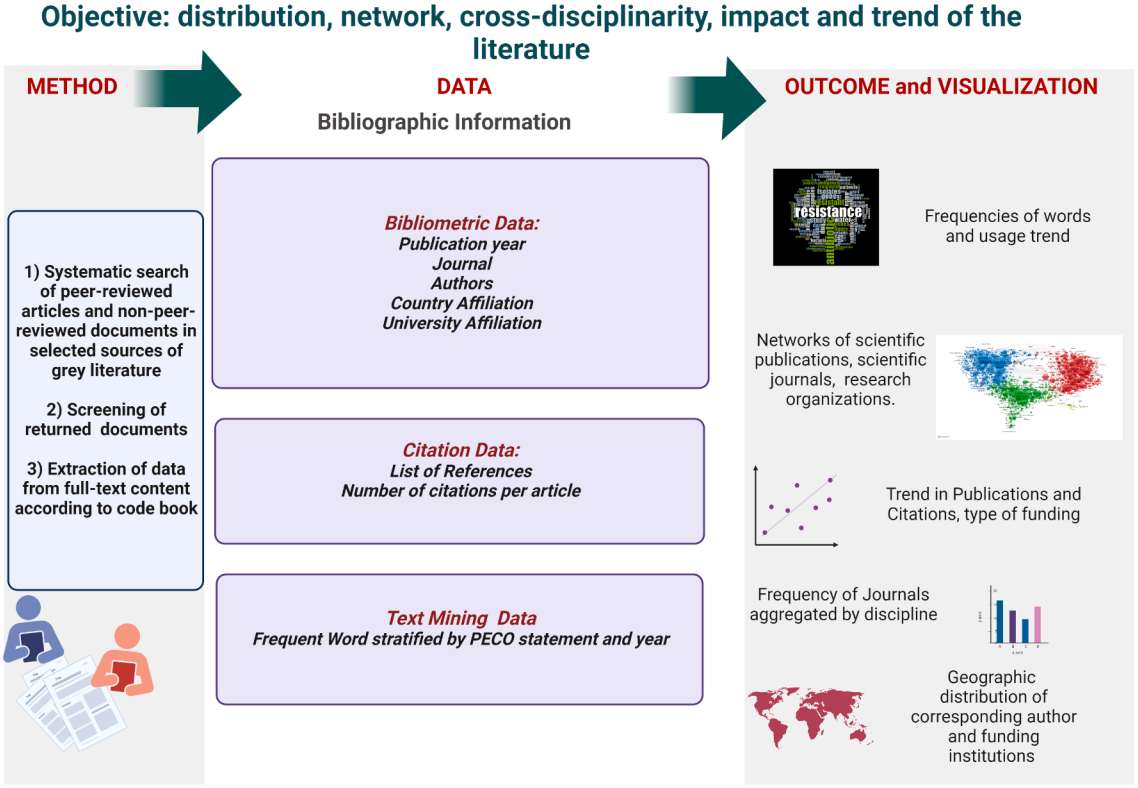

Fig. 4. Simplified and exemplary schematic of the bibliometric analysis process with expected outcomes and type of visualization. Created with BioRender.com (2022).

2.9.1. Is there evidence of environmental factors being associated with the prevalence of antibiotic resistance in the environment?

Due to the broad scope of our research question, it is expected that most of the studies will have a high degree of heterogeneity. To mitigate the problem, we shall use our code book to stratify the entire literature according to PECO categories (for instance, synthesise the evidence according to aquatic, terrestrial and atmospheric population). This exercise will assess how much evidence there is, where the evidence is, which population, exposure and outcome have been studied, and how the studies are connected. Specifically:

Mapping Frequency of Occurrence. Ascertain the frequency (tables and bar-charts) of specific ARBs, ARGs, population domains and hazard/stressors (identified in Tables 2 and S-1) among the different studies.

Magnitude of association: population. Visualise the association between most frequent ARBs and ARGs and most frequent population domains in two-dimensional heatmaps. The colors of the map reflect the number of documents which ascertain the association. Discuss bias and limitation in the use of the map.

Magnitude of association: exposure. Visualise the association between most frequent ARBs and ARGs and most frequent exposure factors in two-dimensional heat maps. The colours of the map reflect the number of documents which ascertain the association. Discuss bias and limitation in the use of the map.

Mapping connection concepts. Produce a network of co-occurring words used in the titles, keywords and abstracts (van Eck and Waltman, 2014; van Eck and Waltman, 2010) (see example in Supplementary Material Fig. S-7). The network will identify clusters of papers with higher *similarity*, providing insights of how scientific concepts are connected (the strength of a link indicates the number of publications in which two words occur together). The exercise will be done for 3–5 particular subsets of the literature based on the PECO stratification (*e.g.*, network of terms for documents focusing on ARB in the terrestrial environment exposed to heavy metal). The criteria for the stratification will be based on the frequency of ARBs/ARGs, population domains and hazard/stressors identified above.

Catalogue studies reporting statistical distributions of exposure and dose–response relationships. Systematically catalogue how many studies, and which ones, report i) statistical distributions of exposure and ii) dose–response relationships according to the populations of interest. The database of these distributions and relationships can be used to inform secondary synthesis other than systematic review, such as future modelling work to identify potential hot-spots of antibiotic resistance conditioned to the environment.

Summarise how the outcome is compared in relation to exposure. We will summarise how many studies compare their outcome for different levels of exposure, how the levels are measured (*e.g.*, presence/absence alone, ordinal scale, range of continuous values), how this depends on the most studied (3 −5) populations/exposures according to the PECO stratification. Furthermore, from our pilot project, a comparator component in these studies is not always present or evident. For example, some studies might report occurrence of ARB/ARG along with a single exposure, like proximity to a waste processing plant, but not assess if/how the occurrence of ARB/ARG changes for changes in the exposure. Situations like this example will be reported as 'No comparison'. This will help in identifying gaps in the way the effects of differential exposure on the outcome is assessed.

2.9.2. Bibliometric Analysis: mapping distribution, network, cross-disciplinarity, impact and trend of the literature

Bibliometric mapping will be used to visualise dynamic patterns in the literature landscape during a specified temporal domain. The aim is to monitor how the field is progressing and possibly to identify emerging avenues of research. This will provide insights into how disciplines are connected and the prevalent mode of investigation. Specifically, the tool will assess spatiotemporal patterns of the research landscape, research productivity and impact, patterns in the disciplines, and clusters in research collaborations. As above, the exercise will be done for 3–5 particular subsets of the literature based on the PECO stratification.

Detect potential emerging trends. We shall plot word clouding based on the full document stratified according to the PECO statement. This will be repeated for different periods (*i.e.*, 2000–2005, 2005–2010, 2010–2015, 2015–2020) to detect variations in word usage. By comparing potential shifts in the use of keywords, this qualitative tool will provide insight into emerging trends.

Identify common scientific interests. Two objects (*e.g.*, journals, institutions or researchers) are co-cited if there is a third object citing both; thus, co-citation is interpreted as a measure of similarity or relatedness between objects (Boyack and Klavans, 2010). We shall perform a co-citation analysis of scientific publications to identify common scientific interests.

Identify scientific communities and productivity. We shall perform a co-authorship analysis of researchers, research institutions, and countries based on the number of publications they have authored jointly.

Assessing the diversity of research according to disciplines. We shall identify prevailing research areas of the literature by assessing the distribution of journal disciplinary scope according to the Web of Science classification (see example in Fig. S-8).

Assessing impact and trend. We shall plot the temporal distribution of the bibliographic records, temporal trends of citations and the impact of the journals where the paper was published. This extends to the frequency and temporal trends of major types of funding (government, publicly funded but independent research councils, industry, NGOs, charity, and other).

Geo-spatial analysis of publication and funding. We shall plot geographic maps of the country of the corresponding author and country of the funding bodies. We shall compare them with geographic maps of the locations where studies were conducted. The files generated for the network analyses (as in Fig. S-7) will be made available in the Supplementary Material to be used interactively in the freely available VOSviewer software (van Eck and Waltman, 2010) to exploit its full functionality (*e.g.*, Zoom in or redo the analysis with different parameters). Word clouding will be generated using NVivo™, a qualitative data analysis software (QSR International Pty Ltd., 2020).

2.10. Narrative synthesis.

To support future research, as well as inform future funding strategies and provide an additional resource for policy-makers, the following quantitative coding variables will be extracted and catalogued (Schreier et al., 2022).

Geo-spatial reporting of study sites. Plotting geographic maps of the locations where the study was conducted. This point can assist funding bodies and future research to identify those settings and environments where research activity is scarce. Comparisons of the geographic maps of the study sites with respect to the country of the corresponding author and/or funding bodies will provide insight into how resources are allocated.

The additional points will be further considered in the narrative synthesis:

- Ascertain if the work clearly categorises the hazard/stressor being of anthropogenic or environmental origin.
- Describe and discuss the frequency of specific outcomes in terms of the taxonomic classification of resistant bacteria (*e.g.*, *Salmonella*) and/or antibiotic class (*e.g.*, β-lactam antibiotics). Ascertain if this is clinically relevant to humans or animals.
- Ascertain if the study clearly focused on specific stages of the antibiotic resistance pathway: emergence, dispersion and persistence. Explore i) potential associations of these stages with specific

populations and/or exposures and ii) if some stages are underrepresented in the literature as compared with others.
- Summarise potential insights of the causal pathway discussed in the studies. Identify potential gaps in the PECO statement and the conceptual model (Fig. 1). Propose their potential revisions for future research in the light of the findings.
- Identify specific, closed questions for future confirmatory systematic reviews; discuss their feasibility based on the number of studies returned.

Authors' contributions: CRediT Author Statement

Brian Gardner: Software, Methodology, Writing - Original Draft, Writing - Review and Editing, Visualization; **Martha Betson:** Writing - Review and Editing; **Adriana Cabal Rosel:** Writing - Review and Editing; **Manuela Caniça:** Writing - Review and Editing; **Mark A. Chambers:** Writing - Review and Editing; **Francesca M. Contadini:** Writing - Review and Editing; **Laura C. Gonzalez Villeta:** Writing - Review and Editing; **Marwa M. Hassan:** Writing - Review and Editing; **Roberto M. La Ragione:** Writing - Review and Editing; **Alexandre de Menezes:** Writing - Review and Editing; **Davide Messina:** Writing - Review and Editing; **Gordon Nichols:** Writing - Review and Editing; **Daniel V. Olivença:** Writing - Review and Editing; **Revati Phalkey:** Writing - Review and Editing; **Joaquin M. Prada:** Writing - Review and Editing; **Werner Ruppitsch:** Writing - Review and Editing; **Lorenzo A. Santorelli:** Writing - Review and Editing; **Nick Selemetas:** Writing - Review and Editing; **Mukunthan Tharmakulasingam:** Writing - Review and Editing; **Arnoud H. M. van Vliet:** Writing - Review and Editing; **Markus Woegerbauer:** Writing - Review and Editing; **Iñaki Deza-Cruz:** Methodology, Validation, Writing - Original Draft, Writing - Review and Editing, Visualization, Project administration; **Giovanni Lo Iacono:** Conceptualization, Methodology, Validation, Writing - Original Draft, Writing - Review and Editing, Visualization, Project administration. All authors approved the final manuscript. Brian Gardner is the guarantor of the review.

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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 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.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envint.2022.107707.

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