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Systematic Review Protocol

Title
The role of freshwater environments in antimicrobial resistance spread and implications for public health. A systematic review protocol.

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antimicrobial resistance, antibiotic-resistant bacteria, freshwater environment, transmission, human health

Background
Antimicrobial resistance (AMR) threatens the effectiveness of existing therapies to treat infections, resulting in increasing morbidity and mortality rates worldwide. The role that natural environments play in the spread of AMR to humans is poorly understood. Previous reviews have assessed the dynamics of AMR across humans, environments and animals (1) or assessed control measures for the prevention of AMR dissemination in the environment (2). However, none have evaluated the evidence of human exposure to and transmission of AMR in natural environments. The evidence on this topic was recently described in a systematic map (3) which indicated a pool of studies on AMR in aquatic environments suitable for synthesis in a systematic review. Freshwater environments are subject to environmental release, maintenance and mixing of AMR bacteria and genes associated with mobile genetic elements (4, 5). Human recreational activities in these areas, such as 'wild swimming' (swimming in waters not designated for bathing), are seeing a rise in popularity (6, 7), but increase the risk of exposure to waterborne bacteria, and the risk of AMR transmission. Although current estimates (8) suggest that environment-to-human transmission is minor compared to human-to-human transmission, the risk of AMR transmission through exposure to water bodies contaminated with faecal pollution is typically not accounted for, thereby likely underestimating the role of the environment. Our study aims to evaluate and summarise the available evidence on AMR transmission to the public from freshwater environments within Europe, and to quantify the extent of human exposure to AMR bacteria in freshwater inland water bodies located in the UK. By providing a comprehensive understanding of the risks posed by the presence of AMR bacteria in freshwater environments, our study aims to contribute to the global efforts to address the challenges posed by AMR.

Theory of change or causal model
The Driver-Pressure-State-Exposure-Effect-Action framework considers the environmental dimensions of human health (3, 9-11). While much research has focussed on understanding the State of AMR in the environment and selective Pressures, there is limited understanding regarding their impact on human health (i.e. Exposure and Effect). Recreational use of freshwater involves exposure

to AMR (whether by ingestion, direct contact or inhalation), which could result in colonisation (first stage of infection whereby microbes become established) and/or infection by bacteria that do not respond to antibiotics. This review will assess the evidence of transmission of AMR from this environment to humans.

Stakeholder engagement

This systematic review follows on a recent systematic map published by Stanton et al. (1). The map was developed after consulting with stakeholders across industry, government (including both environmental and NHS departments) and non-government organisations. For the review, we are not planning on consulting these stakeholders again. However, all previously made suggestions are taken into consideration.

Objectives and review question

The objective of this review is to assess the extent to which AMR in natural freshwater environments may pose a threat to public health. The review will adopt the format of the original systematic evidence map by Stanton et al. (1) and will consist of two questions: Q1. What is the extent of human exposure to and transmission of antimicrobial resistance from inland freshwater sources within the geographical area of the European Economic Area (EEA), Switzerland and the United Kingdom (UK)? Q2. What is the prevalence of AMR bacteria and AMR genes in inland freshwater bodies within the UK?

Definitions of the question components

Q1: Population: Humans (including adults and children). Exposure: Recreational exposure to inland freshwater bodies. Outcome: estimated or measured risk of exposure to AMR bacteria, colonisation and/or infection by AMR bacteria, mortality attributed to AMR bacterial infection. Q2: Population: Bacteria. Exposure route: samples of water from inland freshwater bodies. Outcome: Quantified measures of the prevalence of AMR bacteria, AMR genes and other mobile genetic elements (e.g. class 1 integrons) associated with resistance.

Search strategy

We will search for evidence in both published articles and grey literature sources. To ensure a high-quality search, our team is joined by an information specialist (AB), who designed the search strategy for the systematic map by Stanton et al. (1). We will modify and update the database searches, originally developed for the systematic map (1). The search strategy has been developed in Medline via OvidSP and is being adjusted for each relevant database using the Polyglot Translator (an SR-Accelerator tool). We are using a list of key papers identified from the map to optimise the search terms for this systematic review. Our search will be supplemented by reverse and forward citation chasing on included papers to ensure all relevant studies are identified. Geographic limitations will not be included during the search strategy for question one. However, as question two is aimed to inform UK policy-makers, searches will be limited to UK-specific studies. The time range will be limited from 2000 to the present, and language will be restricted to English.

Bibliographic databases

The bibliographic databases are all accessed with University of Exeter subscriptions and will include the following: Ovid Medline All (1946-present), CAB Abstracts (2000-present), and Global Health (1973-present) via OvidSP; Web of Science Core Collection (1900-present) and BIOSIS Citation Index (1969-present) via Web of Science; GreenFile; Environment Complete (1888-present) via EBSCOhost; SCOPUS (1788-present), Epistemonikos, ProQuest Dissertations and Theses Global (1637-present) via ProQuest. The searches will include both free text and controlled vocabulary searching when available and relevant. Ovid Medline is used to design the searches for each question (see ‘OVID Medline search strategy – Question 1’ and ‘OVID Medline search strategy –

Question 2’ in additional files) and these will be translated across the other databases. As we are aiming for comprehensiveness, the searches in the smaller databases: GreenFile, Environment Complete, CAB Abstracts and Global Health may be broader, for example, the Boolean term AND may be used instead of the proximity operator, adj.

Web-based search engines
Web-based search engines will not be searched. A Search Summary Table was completed as part of Stanton et al. (1), which showed no additional publications were obtained from web-based searching.

Organisational websites
Organisational website searches will be carried out using the search terms 'antimicrobial resistance', 'antibiotic resistance', and ‘amr’, limited to English only and include the following: Cefas (Centre for Environment, Fisheries and Aquaculture Science) - https://www.cefas.co.uk/ Defra (Department of Environment, Food and Rural Affairs) - https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs EA (Environment Agency) - https://www.gov.uk/government/organisations/environment-agency HPS (Health Protection Scotland) - https://www.hps.scot.nhs.uk/ SEPA (Scottish Environmental Protection Agency) - https://www.sepa.org.uk/ UKCEH (UK Centre for Ecology & Hydrology) - https://www.ceh.ac.uk/ UK HSA (UK Health Security Agency) - https://www.gov.uk/government/organisations/uk-health-security-agency Welsh government - https://www.gov.wales/ NRW (Natural Resources Wales) - https://naturalresources.wales/?lang=en RIVM (Dutch National Institute for Public Health and the Environment) - https://www.rivm.nl/en

Comprehensiveness of the search
We will evaluate the comprehensiveness of the updated search strategy using a list of benchmark papers identified by Stanton et al. (3) as relevant to the questions we aim to answer. The list comprises of four papers relevant to question one and nine papers relevant to question two. The complete list of these is provided as a supplementary file (see 'Benchmark Papers' in additional files). All listed papers were identified by the search strategies for their respective questions.

Search update
The searches will be updated if the study takes longer than 2 years to complete and published after running the searches.

Screening strategy
Search results will be imported into Rayyan AI and duplicate records identified and removed prior to title and abstract screening using both manual and automated deduplication. During this first stage, all members of the team will independently review the titles and abstracts of 100 articles. The next stage of our screening process will involve independent title and abstract screening of the remaining records by the primary reviewer (EP), with at least 10% of these screened by a second reviewer, depending upon the number of records to be screened. The same process will be followed for full-text screening - primarily carried out by EP, with 10% covered by a second reviewer. Any identified papers authored by the reviewing team will be screened by impartial members of the review team at all screening stages.

Eligibility criteria
Question 1: Relevant studies will include 1) human populations; 2) estimated or measured recreational exposure to natural surface water in the environment, including rivers, streams, lakes, ponds, reservoirs, canals, quarries, estuaries; 3) outcomes associated with exposure to or transmission of AMR bacteria in water, including quantitative estimates of exposure to AMR bacteria in water, and/or human colonisation by, infection by or death as a result of AMR bacteria; 4) studies

written in English; 5) studies published after 2000; 6) studies on freshwater sites from the EEA, Switzerland and the UK; 7) experimental, observational, descriptive, modelling study designs, or systematic reviews. Question 2: Relevant studies will include 1) bacteria. Studies measuring other microorganisms of public health concern will be excluded; 2) studies of water (and/or sediments) samples collected from natural surface water environments, including rivers, streams, lakes, ponds, reservoirs, canals, quarries, estuaries; 3) studies reporting quantitative data on the prevalence (percentage, or relative abundance) and type of AMR bacteria, genes and mobile genetic elements (eg. class 1 integrons) within eligible environmental samples. Studies reporting absolute abundance of resistance); 4) studies written in English; 5) studies published after 2000; 6) studies of sites in the United Kingdom (England, Scotland, Wales, and Northern Ireland) only, as this is consistent with Stanton et al (1), size of evidence base expected and is relevant to UK stakeholders; 7) longitudinal, transects, cross-sectional study designs. Full details for inclusion and exclusion criteria are provided in the additional file 'Inclusion and Exclusion Selection Criteria'.

Consistency checking

After screening and discussing the titles and abstracts of 100 articles, the level of agreement between all reviewers will be assessed using the Kappa Coefficient. Scores that are below or equal to 0.6 will initiate further discussions, refinement of eligibility criteria where necessary, and then an additional evaluation of 100 articles. Next, the primary reviewer (EP) will proceed to screen all titles and abstracts against the finalised inclusion and exclusion criteria, with an eligible second reviewer (i.e., a reviewer who has not authored any of the materials being assessed) screening a minimum of 10% of the results. Any further discrepancies will be discussed by the two screening reviewers and, if necessary, the wider review team. For the next stage, full texts identified during the first stage of screening will be assessed for eligibility. The primary reviewer (EP) will screen all articles, with again a minimum of 10% being screened by an eligible member of the review team.

Reporting screening outcomes

The systematic review will adhere to the ROSES flow diagram for reporting screening outcomes. The number of identified records from each database will be documented along with the count of duplicates removed. The number of articles or datasets identified through searching grey literature will be reported as well. The number of full texts for inclusion will be reported, along with the number of excluded full texts and the reason for their exclusion. The ROSES diagram will be included in the final systematic review to provide a clear and transparent summary of the process and the number of articles included at each stage. Studies excluded at full-text screening will be listed with the reason for exclusion.

Study validity assessment

Each study included after full-text screening will be assessed for bias based on an adapted version of the Cochrane Collaboration tool, originally published by a systematic review on a similar topic (12) (see 'Risk of Bias Assessment Tool' in additional files). Several types of potential bias were identified, including selection bias, attrition bias, and reporting bias. Selection bias may arise from inconsistencies in sampling methods across different locations, such as collecting different volumes of water, varying frequency of sampling, or selecting different numbers of colonies for characterisation. Performance bias may arise during processing or detection of AMR if methods differ in their outcome assessment. Attrition bias may result from missing data for any of the samples collected or analysed, for instance, due to unfavourable weather conditions. Reporting bias may arise from a preference for certain outcomes over others, leading to a disproportionate representation of these results in the reported study. Studies will be categorised as of ‘high’, 'low', or ‘unclear’ risk of bias, depending on whether the study design and methods are described at a sufficient level to justify the reported results as reliable. EP will conduct the validity assessments, which will be double-checked by another member of the review team.

Consistency checking
Critical appraisal will be carried out by the primary reviewer (EP) and double-checked by another member of the review team. Where disagreements arise, these will be discussed between the two reviewing members, and where a decision cannot be reached, disagreements will be discussed as a team.

Data extraction strategy
Data extraction forms for each of the two questions will be drafted based on the review question and data reported in the benchmarking papers. Lists of benchmark papers and the meta-data extraction strategy can be found in additional files. Where data is missing or unclear in an article, this will be reported in our final analyses, along with a record of all collected data. We will attempt to reach study authors where further information might be needed and report the outcome of these.

Meta-data extraction and coding strategy
As above.

Consistency checking
Data will be extracted by the first author (EP) and double-checked for consistency by a minimum of one other member of the team. If disagreements arise, data extraction criteria will be revised and checked again until consistency is agreed upon between all members of the team.

Potential effect modifiers/reasons for heterogeneity
Reasons for heterogeneity are likely to arise due to differences in: - Study designs - Study populations and comparator groups, if applicable - Microbiological targets and assays - Time of sampling (difference in seasons and weather conditions prior to and during sampling) - Type of environment sampled - Proximity of sampling sites to known sources of pollution (e.g., Waste water treatment sites, combined sewer overflows, farms) - Methods for estimating exposure (question 1 specifically) - Methods for processing and characterising and analysing AMR bacteria and genes This list is not exhaustive and may be expanded during the review.

Type of synthesis
A narrative approach will be taken to synthesise the current state of knowledge on AMR prevalence in freshwater bodies and the associated risks to human health. All studies going through data extraction and validity assessment will be summarised in a narrative synthesis. Where sufficient quantitative data exists for comparable studies, a meta-analysis will be performed (e.g., AMR prevalence).

Narrative synthesis methods
Our narrative synthesis will use tabulation and textual descriptions of the evidence base, to report a summary of the data extracted from the studies. We will explore both the similarities and differences within and between the final set of studies, including the quality of the study designs, and investigate any patterns in the available data (13). An overall assessment of the strength of the evidence and our ability to draw conclusions about the health effects resulting from exposure to environmental AMR in freshwaters will be given. The summary will focus on the following main elements: - Describe the distribution and type of evidence available to understand human exposure to AMR in natural freshwaters and of transmission to humans. Summarise the findings from included research studies. Consider the types, quality, and amount of evidence in terms of causality, using the Bradford Hill criteria (14) as a framework. Identify knowledge gaps. - List study methods and identify their benefits and limitations. - Organise studies to describe human health risk estimates from exposure to AMR in freshwater (Question 1). - Organise studies to describe patterns, types and prevalence of AMR (Question 2). - Describe the nature and extent of the evidence base quantifying

AMR prevalence in natural freshwaters in the UK (Question 2). Summarise the findings from included studies in terms of sources of pollution. Identify knowledge gaps. - Where possible, explore for associations. - Provide a summary information table on the descriptive data and risk of bias for each study.

Quantitative synthesis methods
Based on the data available in benchmark publications, differences in study design, AMR targets measured, and the type of environment sampled, all present issues to sensibly combining quantitative data by random effects meta-analysis. If sufficient data is available, and it is appropriate to do so, studies identified in question 1 may be combined using random effects meta-regression to summarise relative risks of AMR-related outcomes in exposed populations compared to unexposed populations. Where sufficient information is available, we will estimate recreational user exposure to AMR in freshwater, using methods reported by Leonard et al. (12), for example, if AMR is expressed as (or sufficient information is available to calculate) AMR per millilitre of water. Data on AMR prevalence in the UK in the second part of the review may be meta-analysed using the R package 'metaphor' to summarise the prevalence of AMR in inland water bodies in the UK. The details of the quantitative analyses will be determined once articles have been screened and data extracted to understand the amount, distribution and types of data available. If meta-analyses are performed, heterogeneity will be assessed by calculating the I2 statistic.

Qualitative synthesis methods
N/A

Other synthesis methods
N/A

Assessment of risk of publication bias
If there is sufficient quantitative data for a meta-analysis, we will create funnel plots to assess publication bias.

Knowledge gap identification strategy
An important aim of the review will be to identify knowledge gaps, in terms of human health outcomes associated with recreational exposure to waterborne AMR in natural freshwaters, and types and abundances of AMR in these environments. We will produce heat maps cross-tabulating key categories of data.

Demonstrating procedural independence
Reviewers who have authored papers that are identified during the search will not be involved in the assessment of these papers at any stage of the review process. Instead, an impartial reviewer will be responsible for screening, assessing and extracting data from these publications, ensuring an objective and unbiased process. EP and NE have not previously worked with members of the group and have no previous publications with the other authors.

Competing interests
The authors of this systematic review have no competing interests to declare.

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Author's contributions
All authors contributed to the conceptualisation and design of the review. EP wrote the first draft and all authors contributed to the revision of the draft. All authors approve the submitted protocol.

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