A protocol for a systematic review and meta-analysis

Title: Protocol for a Systematic Review on Antimicrobial Resistance and Resistance Genes in Drinking Water on Livestock Farms

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Author contributions
The review (PICO) question and the protocol described in this document were developed with the contribution and final approval of all co-authors. Ronald Vougat Ngom drafted the protocol and all authors provided their input.

Registration
This amendment protocol is archived at Padua Research Archive (handle code: https://hdl.handle.net/11577/3554268) and published online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org/. This protocol is reported using the items (headings) recommended in the PRISMA-P guidelines (Shamseer *et al.*, 2015).

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1. Introduction

1.1. Rationale

The livestock sector plays a crucial role worldwide, contributing significantly to food security and nutrition. Rising incomes, changes in dietary behaviors, and a growing global population have increased the demand for livestock products. However, the intensification of livestock production, where animals are kept in crowded and stressful conditions, has contributed to the emergence, transmission, and amplification of diseases, which are major constraints for the sector (Espinosa et al., 2020; Stevenson, 2023).

Antimicrobial resistance (AMR) is a global concern affecting both human and animal health. Livestock production contributes to the emergence and spread of AMR (de Mesquita Souza, 2020), due to the use of antimicrobials for disease treatment, prevention, and growth promotion. Indeed, humans can contract antimicrobial-resistant bacteria or genes from animals through the consumption of contaminated food of animal origin (Murray *et al.,* 2022).

Water is an essential resource for livestock, supporting hydration, digestion, and overall health. However, the microbial water quality on farms directly influences animal health (Mustedanagic *et al.*, 2023), since water pipelines can act as hotspots for bacterial dissemination (Lethola *et al.*, 2009). Therefore, drinking water can serve as a potential reservoir for the transmission of antimicrobial resistant and/or pathogenic bacteria (e.g., *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli*), especially in situations where sanitation is inadequate and/or cross-contamination from fecal matter occurs (Mantzios et al., 2023).

Mass medication administered through drinking water represents one of the most effective and cost-efficient methods for treating entire herds simultaneously (Hahne *et al.*, 2022). Furthermore, the administration of antimicrobial drugs *via* drinking water can also contribute to the emergence and dissemination of resistant bacteria within the farm environment. Animal waste can further exacerbate this issue, introducing antimicrobials, biocides, metabolites, pathogens, and antimicrobial resistant genes into the environment, including water sources (Collignon *et al.*, 2019; McEwen *et al.*, 2018). The presence of AMR genes in water bodies can also contribute to the spread of resistance (Singh *et al.,* 2022).

AMR in farm drinking water is a critical issue that requires urgent attention and further research to fully understand its implications and develop effective strategies for mitigation. A systematic review of the existing evidence on AMR in drinking water from livestock farms

can provide valuable insights into the extent of the problem and potentially guide future actions.

1.2. Objectives

This protocol aims to review and summarize the available information on antimicrobial resistance, including resistance genes, in drinking water on livestock farms, highlighting the need for sustainable practices and effective water management strategies.

Population: livestock, specifically poultry, pigs, and cattle
Interest: antimicrobial resistance, including resistance gene, in drinking water
Context: Livestock farms

2. Methods

2.1. Eligibility Criteria

- Criteria related to the elements of the PICo question.
- Publication types: Journal articles that provide results of original research and fulfill the study design eligibility criteria.
- Language: Research articles published in English or French.
- Publication date: No limits.
- Geographical location of studies: No limits.
- Study design: All study designs (except for controlled trials) design will be included.

2.2. Information Sources

The search will be carried out using bibliographic databases that provide a high level of article recall across biomedical articles (Bramer *et al.*, 2017). The list of databases to be searched is reported in Table 1. Scopus will be searched *via* the University of Padova (Italy) and Web of Science (WOS), Pubmed, and Agricola *via* Baylor University (Texas, US). All the databases of WOS will be used except those related to conference proceedings, theses, and social sciences.

Table 1. List of databases to be searched.

Database	Interface	URL
MEDLINE	PubMed	https://pubmed.ncbi.nlm.nih.gov/
Web of Science	Web of Science	https://www.webofscience.com/wos/woscc/s

		mart-search
AGRICOLA	EBSCOhost Research Databases	https://web.p.ebscohost.com/
SCOPUS	Elsevier	https://www.scopus.com

2.3. Search Strategy

The search strategy will involve a multi-strand approach that uses a series of searches, with different combinations of concepts to gather all possibly related research and thus achieve high sensitivity (Higgins *et al.*, 2021). Boolean operators (AND and OR) will be used to combine search terms effectively. The search strategy will be tailored to each database to ensure thorough coverage of relevant literature. If less than 10 papers are included, a backward search using Scopus or WOS databases will be performed.

The following search strategy will be used:
([antibiotic resistance] OR [antibiotic resistant genes]) AND [poultry or pig or cattle] AND [drinking water] AND [farm]
#1 (pig* OR swine* OR weaner OR fattener OR sow* OR piglet* OR boar OR boars OR poultry* OR chick* OR broiler* OR layer* OR turkey* OR duck* OR geese OR goose* OR fow * OR avian* OR bird* OR hen OR hens OR flock* OR cattle OR beef OR cow* OR calf OR calves OR heifer* OR bull* OR bovine OR dairy OR "food producing animal*" OR "food-producing animal*" OR "food animal*" OR "animal husbandry*" OR "domestic animal*" OR livestock)
#2 (*water*)
#3 (multidrug* OR "multi-drug*" OR drug* OR antimicrobial* OR antibacterial* OR microbial* or "anti-microbial*" OR "anti-bacterial*")
#4 (resistant OR *resistance* OR susceptible OR susceptibility or sensitivity)
#5 ("antimicrobial resistant gene*" OR "resistance gene*" OR "ARGs" OR "AMR genes" OR "resistant determinant*" OR "mobile genetic elements" OR "MGE")
#6 (*farm*)
#7: #3 AND #4
#8: #7 OR #5
#9: #1 AND #2 AND #6 AND #8

2.4. Study Record

Data Management

All retrieved records will be imported into Zotero Software for deduplication. After duplicate removal, the obtained file will be uploaded in Rayyan software for the screening process.

Selection Process

The screening of the retrieved citations will take place in two steps: i) title and abstracts screening, and ii) full text screening. Six independent reviewers working in two groups will perform the screening. Each group will be assigned half of the citations to ensure each reference is screened by at least three independent reviewers. Discrepancies will be resolved through discussion or by consulting a fourth reviewer (Affengruber *et al.,* 2022). A calibration exercise will be conducted at the start of each step using at least 10% of the records to align reviewer judgments and minimize conflict (Shamseer *et al.,* 2015).

For title and abstract screening, eligibility of the studies will be assessed using the following questions:

- Is the study original research in English or French? Yes [include], No [exclude], unclear [include]
- Does the study focus on drinking water or water on livestock farms? Yes [include], No [exclude], unclear [include]
- Does the study focus only on animals (e.g., swabs or feces) or environmental samples (e.g., biofilm, manure, wastewater)? Yes [exclude], No [include], unclear [Include]
- Does the study focus on livestock? Yes[include], No[exclude], unclear [include]
- Does the study concern bacterial antibiotic resistance and/or resistant genes? Yes [include], No [exclude], unclear [include]

The records meeting the inclusion criteria will pass to the next stage. During the full text screening, eligibility of the studies will be assessed using the following questions:

- Is the full text available in English or French? Yes [include], No [exclude]
- Is the study original research? Yes [include], No [exclude]
- Is the population of study poultry (i.e., broilers, layers, turkeys, and ducks), cattle or swine? Yes [include], No [exclude], unclear [exclude]
- Is the interest of the study antibiotic resistance and/or resistance genes from drinking water? Yes [include], No [exclude], unclear [exclude]
- Is the study observational research (excluding cohort and case studies) where drinking water, collected at farm level, is analyzed? Yes [include], No [exclude], unclear [exclude]

Data extraction

Four independents reviewers (in pairs) will extract data using a Microsoft Excel spreadsheet. A calibration exercise using at least 10% of randomly selected papers will be conducted prior to extraction.

Data to be extracted from eligible studies will include the following items as:

General Information

- Name of the first author
- Publication year

- Study duration
- Country (location of sample collection); if not stated, contact authors or mark as 'Not Applicable'
- Study design (e.g., cross-sectional, longitudinal, etc.)

Population data

- Animal species (poultry, pig, or cattle)
- Productive category (e.g., dairy cattle, calves, heifers, broilers, layers, turkeys, weaners, finishing pigs)
- Number of farms
- Number of animals per farm
- Type of farms (e.g., conventional, commercial)

Interest and Outcomes

- Source of drinking water
- Sampling method
- Sample size
- Sample transport and storage conditions
- Time elapsed from collection to analysis
- Bacteria of interest (*Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Enterococcus* spp., etc.) of the study
- Method for bacterial isolation
- Method for susceptibility testing
- Method for DNA/RNA isolation from isolates
- Molecular techniques used on bacterial DNA/RNA
- DNA/RNA isolation from drinking water
- Culture-independent molecular techniques
- Bacteria identified in drinking water samples
- Antimicrobials to which isolated bacteria were resistant
- Prevalence of resistant bacteria in drinking water
- AMR genes investigated
- AMR genes identified
- Prevalence of AMR genes in isolates or water

2.5. Risk of bias assessment

Study quality will be assessed using a standardized tool for systematic reviews of prevalence (Munn et al., 2014). Criteria include study design, sample representativeness, and outcome measurement. If needed, alternative tools will be adopted based on study design.

2.6. Data Synthesis

The review will follow the PRISMA guidelines (Page *et al.*, 2018). A quantitative synthesis (meta-analysis) will be conducted if data from a consistent number of studies are available. For each study, the proportion of resistant isolates will be calculated. Meta-analysis will be performed using Review Manager (RevMan 5.4.1). A random-effects model will be used to estimate pooled AMR prevalence, with corresponding 95% confidence intervals (CI). The random-effects model is preferred due to its ability to account for heterogeneity by incorporating both within-study and between-study variances (Nyaga *et al.*, 2014). Pooled estimates will be generated if ≥4 studies report on the same bacterium-antibiotic pair. Subgroup analyses will consider variables such as region, year, income level, farm type, and species. Intermediate-resistant samples will be classified as resistant (Vougat Ngom *et al.*, 2024). If meta-analysis is not feasible, median resistance (MR) and interquartile range (IQR) will be reported. For all analyses, statistical significance will be set at $p < 0.05$.

2.7. Sensitivity assessment

Heterogeneity will be assessed using Cochran's Q test and I^2. If heterogeneity is high ($I^2 > 50\%$, $P > 0.05$), potential sources will be explored via meta-regression (Bohning *et al.,* 2021).

2.8. Reporting bias across studies

If at least ten studies are included in the meta-analysis, publication bias will be evaluated using a funnel plot and Egger's regression (Marvridis *et al.*, 2014).

3. Conclusions

This review aims to provide valuable insights into the current status of antimicrobial resistance in drinking water from livestock farms and contribute to the advancement of knowledge in this field**.** The findings will highlight the significant impact of antimicrobial-resistant bacteria in livestock drinking water sources on human and animal welfare. The results will also be helpful in identifying specific gaps in knowledge related to drinking water sampling methods, bacterial isolation, and antimicrobial resistance identification within this matrix.

4. References

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