A protocol for a scoping review

Title: Antimicrobial resistance and residues in biofilm from livestock farms: a protocol for a scoping review

Authors and their affiliations

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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/11577/3519562) 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-ScR guidelines (Tricco et al., 2018).

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1. Introduction

1.1. Rationale
Livestock sector plays an important role in many countries. In addition to ensuring healthy diets and sustainable food systems, it contributes to poverty reduction in different groups of people (Molina-Flores et al., 2020; FAO, 2023). Factors, such as increasing incomes, changing diets, and population growth, have led to increased demand for livestock products, making the livestock sector one of the fastest growing agricultural sub-sectors. The intensification of livestock production, in which large numbers of animals are kept in crowded and stressful conditions, has led to the emergence,

transmission, and amplification of diseases which are among the main constraints of this sector (Espinosa et al., 2020; Stevenson, 2023).

Livestock diseases are the major drivers of antimicrobial use (AMU) in food-producing animals and AMU has been estimated to increase by 8% by 2030 (Mulchandani et al., 2023). This recurrent and high use of antimicrobials contributes significantly to the emergence of antimicrobial resistance (AMR) (Stevenson, 2023). In animals, AMR can result in treatment failure, and thus represents a threat for the long-term sustainability of the animal industry (Mulchandani et al., 2023). In addition, if not properly used, antimicrobials can result in the presence of antimicrobial residues in food of animal origin (Redwan Haque et al., 2023). Both AMR and antimicrobial residues can be transferred to humans through the food chain, posing a threat to human health (Murray et al., 2022; Redwan Haque et al., 2023). Biofilms present in farms' drinking water distribution systems (DWDSs), which are commonly used for the administration of antimicrobial drugs (AMDs) to large groups of animals, can capture AMDs and thus contribute to both treatment failures and the emergence of resistant bacteria in livestock production.

Biofilms are constituted by complex multi-species bacterial communities embedded in an extracellular matrix of polymeric substances composed of proteins, polysaccharides, nucleic acids, and water (Steenackers et al., 2016). Biofilms commonly develop on abiotic and biotic surfaces; however, they have been also observed moving freely in liquid media (Steenackers et al., 2016). Biofilms represent an important challenge for the livestock sector, since they can act as potential reservoirs for pathogenic and opportunistic bacteria and are known to be a hot-spot for horizontal gene transfer, thus including resistance genes (Ou et al., 2011). Furthermore, the self-produced extracellular polymeric substance can confer increased tolerance to environmental stress and resistance against antimicrobials to the bacteria resident in the biofilm (Costerton, 1999). Biofilms formed by multidrug resistant strains (MDRs) are more problematic, considering AMR a significant public health threat (FAE de Brito, 2022). Of concern, Guéneau et al. (2022a) have shown that the majority of the zoonotic pathogens commonly found in farm facilities are biofilm producers. To support this, the ability of *Campylobacter* spp. to form biofilm was pointed out as one of main sources of *Campylobacter* colonization of poultry in European farms (Newell and Fearnley, 2003; EFSA and ECDC, 2021). As previously suggested (Guéneau et al., 2022b), there is a need to increase the knowledge on biofilms in animal production systems and their impact on antimicrobial treatments and the emergence of AMR.

1.2. Objectives

The objective of this protocol is to review and summarize the available information on antimicrobial resistance (including resistance genes) and antimicrobial residues in biofilms collected in the livestock farm environment. The PICo elements are:

1. **P**opulation: livestock restricted to poultry, pig and cattle

2. **I**nterest: antimicrobial resistance (including resistance genes) or antimicrobial residues in biofilm collected in environment
3. **Co**ntext: Animal farms

2. Methods

2.1 Eligibility criteria

1. Criteria related to the elements of the PICo question.
2. Language: Publications in English and French.
3. Publication types: Journal articles that provide results of original research, fulfills the study design eligibility criteria.
4. Publication date: No limits.
5. Geographical location of studies: No limits.
6. All study designs will be included.

2.2. Information sources

Bibliographic databases that provide a high level of article recall across biomedical articles (Bramer et al., 2017) will be used. Table 1 lists the databases to be searched. Scopus and PubMed will be searched via the University Padova (Italy) and Agricola and Web of Sciences (WOS) will be conducted via Baylor University (Texas, US). All the databases of WOS will be used except for those related to proceeding, 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	http://webofknowledge.com/
AGRICOLA	EBSCOhost Research Databases	https://web.p.ebscohost.com/ehost/
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). If only a few papers (<10) are found to be relevant to the review, in addition to the databases, citations will be extracted from included articles and reviews. In the event of using search reviews, Scopus or Google scholar databases will be used for backward searching.

The concept of the search strategy will be the following:

[**poultry or cattle or pig**] AND ([**antibiotic resistance**] OR [**antibiotic genes**] OR [**antibiotic residues**]) AND [**biofilm**] AND [**farm**]

Search terms will be amended appropriately to reflect the functionality differences in each database. The general search strategy to identify studies relevant to the PICO of this review will be the following:

#1 (pig* OR swine* OR weaner OR fattener OR sow OR piglet* OR boar OR boars OR chick* OR poultry* OR broiler* OR layer* OR turkey* OR duck* OR geese OR goose OR fowl* 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 "animal farming" OR "domestic animal*" OR livestock)

#2 (multidrug OR MDR OR "multi-drug" OR drug OR antibiotic* OR antimicrobial* OR "anti-microbial*" OR microbial* OR antibacterial* OR "anti-bacterial*" OR bacteria$)

#3 (resistance OR resistant OR sensibility OR susceptibility)

#4 ("resistance gene*" OR ARG OR "AMR gene*" OR "resistance determinant*" OR "mobile genetic element*" OR MGE)

#5: residue*

#6: biofilm*

#7 (farm* or “farm-level”)

#8: #2 AND #3
#9: #2 AND #5
#10: #4 OR #8 OR #9
#11: #1 AND #10 AND #6 AND #7

2.4. Study Records

Data management

All citations retrieved from the databases will be imported into Zotero software for deduplication. After duplicates removal, the file obtained will be uploaded in Rayyan for the screening process.

Selection process

Citations will be screened in two stages, namely i) title and abstract and ii) full text screening. During each stage, six independent reviewers working in two groups will carry out the title and abstract screening. Half of the citations will be assigned to each group. This will guarantee that each reference is screened by three independent reviewers. In each group, conflict will be resolved among the reviewers. At the beginning of each screening phase, a calibration exercise will be conducted among all the six reviewers to enable discussion and solve disagreement before carrying out the full selection process (Sanguinetti *et al.,* 2021). This test will consist of screening at least 10% of the total number of papers available.
For the title and abstract screening, eligibility of studies will be assessed with the following questions:

1. Is the study an original research in English or French? Yes [include], No [exclude], Unclear [include]
2. Does the study concern livestock? Yes [include], No [exclude], Unclear [include]
3. Does the study concern biofilm collected from the farm environment? Yes [include], No [exclude], Unclear [include]
4. Does the study concern antimicrobial resistance, resistance genes or antimicrobial residues? Yes [include], No [exclude], Unclear [include]

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

1. Is a full text available in English or French? Yes [include], No [exclude]
2. Is the population of the study poultry or pig or cattle? Yes [include], No [exclude]
3. Is the interest of the study the antimicrobial resistance or antimicrobial genes or antimicrobial residues in biofilm? Yes [include], No [exclude]
4. Is the study conducted at the farm level? Yes [include], No [exclude]

Data charting process
Two independent reviewers will carry out the data charting by using a Microsoft Excel® spreadsheet. Three other reviewers will check the data extracted. Data to be extracted from eligible studies will include the following items as:

General information
- Duration of the study
- Country where the study was carried out. If not stated, contact study authors or use 'Not Applicable' if the authors do not reply
- Study design (cross-sectional, longitudinal study, etc.)

Population data
- Animal production type: level 1 (species), cattle, poultry, pigs; level 2, dairy cattle, calves, heifers, broilers, layer, turkeys, weaners, finishing pigs, etc.

- Number of farms
- Number of animals
- Type of farm (conventional, commercial, etc.)

Interest and outcomes
- Method of biofilm sampling
- Number of biofilm samples collected per farm
- Total number of biofilm samples collected in the study
- Transportation and storage conditions of biofilm samples before analysis
- Duration between sample collection and analysis
- Bacteria of interest (*Escherichia coli, Salmonella* spp.*, Campylobacter* spp.*, Staphylococcus aureus, Enterococcus* spp.*,* etc.)
- Method used for bacterial isolation
- Method used for sensibility testing
- Method used for DNA/RNA isolation from biofilm samples
- Culture-independent method used for molecular analysis
- Antimicrobials to which bacteria of interest are resistant
- Proportion of resistant bacteria
- AMR genes investigated
- AMR genes identified
- Proportion of AMR genes over samples
- Detection method used for residues determination
- Antimicrobial residues identified

In this review intermediate-resistant samples will be considered as resistant (Ahmed et al., 2019).

2.5. Data synthesis

The scoping review will be reported using the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) statement guidelines (Tricco et al., 2018). The aim of this review is to conduct an overview of results from eligible studies captured with the literature search.
The summary will concern the methodologies used for biofilm sampling, antimicrobial resistance and antimicrobial residues detection in biofilms.

Conclusions

This scoping review will provide an overview of the current evidence regarding antimicrobial resistance and residues in biofilm collected in the environment in livestock farms. Results will be helpful for researchers to improve understanding of the role of biofilms as AMR reservoir and spreader in livestock farms. The results will also be helpful for identifying specific gaps in knowledge related to biofilm sampling methods and antimicrobial resistance and residues detection in this matrix. Moreover, the scoping review will suggest gaps in knowledge that require more research in the future.

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