Spatial analysis of antimicrobial resistance in the environment. A systematic review

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Abstract

Antimicrobial resistance (AMR) is a global major health concern. Spatial analysis is considered an invaluable method in health studies. Therefore, we explored the usage of spatial analysis in Geographic Information Systems (GIS) in studies on AMR in the environment. This systematic review is based on database searches, a content analysis, ranking of the included studies according to the preference ranking organization method for enrichment evaluations (PROMETHEE) and estimation of data points per km^2. Initial database searches resulted in 524 records after removal of duplicates. After the last stage of full text screening, 13 greatly heterogeneous articles with diverse study origins, methods and design remained. In the majority of studies, the data density was considerably less than one sampling site per km^2 but exceeded 1,000 sites per km^2 in one study. The results of the content analysis and ranking showed a variation between studies that primarily used spatial analysis and those that used spatial analysis as a secondary method. We identified two distinct groups of GIS methods. The first was focused on sample collection and laboratory testing, with GIS as supporting method. The second group used overlay analysis as the primary method to combine datasets in a map. In one case, both methods were combined. The low number of articles that met our inclusion criteria highlights a research gap. Based on the findings of this study we encourage application of GIS to its full potential in studies of AMR in the environment.

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Introduction

Compared to other recent large-scale problems, such as the COVID-19 pandemic, antimicrobial resistance (AMR) is regarded a long-term issue, with more severe effects (Harring *et al.*, 2021). The modern use of antibiotics was developed during the last decades of the nineteenth century and the first half of the twentieth (Gould, 2016; Nicolaou and Rigol, 2018). From 1937 and onwards the development of natural and synthetic antibiotics exploded. The enormous benefits from the ability to cure bacterial infections resulted in the paramount use of antibiotics, not only for treatment, but also as a prophylactic tool in both medicine and animal husbandry. At the same time, high concentrations of antibiotics released from anthropogenic and agricultural activities can also act as drivers for the development of antibiotic resistant microbial populations (Martinez, 2009; Davies & Davies, 2010; Holmes *et al.*, 2016).

Transmission and dissemination of antibiotic resistance (AR) in the environment can occur over large geographical areas (Singer *et al.*, 2006). A common route for antibiotics and for antibiotic resistant bacteria to enter the environment is through wastewater contaminations from hospitals or intense farming (Baquero *et al.*, 2008). Knowledge of the association between anthropogenic AR in natural ecosystems and possible sources is still scarce (Bueno *et al.*, 2017), and there is a need for multidisciplinary collaboration to find ways to mitigate antibiotic contamination in natural environments (Bueno *et al.*, 2021). Recent AR-research has rather focused on resistant phenotypes than on transmission routes that could explain the influence of anthropogenic activity on natural ecosystems (Miller *et al.*, 2020).

Spatial methods, especially Geographic Information Systems (GIS), have been described as an "invaluable resource for epidemiological research" (Galvin *et al.*, 2013), aiding the understanding of the dynamics of pollution and the development of AR in natural ecosystems (Arya *et al.*, 2013). Spatial statistics, GIS and mathematical modelling can help to determine AR-patterns and high-risk areas (Singer *et al.*, 2006). The burden of AMR is expected to be unevenly distributed geographically. Therefore, there is a need for development of efficient spatial analysis-tools to study its development (Chique *et al.*, 2019; Luz *et al.*, 2022).

The aim of this systematic review is to document how spatial methods have been used to study AMR in the environment, identify commonalities and discuss how the use of GIS methods can be further developed to gain better understanding of AR in natural and semi-natural ecosystems.

Materials and Methods

We conducted a systematic review with focus on the use of spatial methods for studies of AMR in natural ecosystems. Our formulation of the inclusion criteria based on population, exposure, comparator, outcome and study design (PECOS) (Morgan *et al.*, 2018) was the following items: Scientific studies with spatial analysis as an essential research method (S) on the occurrence (E) of AMR (O) in the environment (P) investigating location as a factor (C). An initial database search was performed on 4 January 2021, complemented by updated searches on 21 September 2021, 14 January 2022 and 26 October 2022. All searches followed the same procedure. The databases searched were ProQuest©, PubMed©, Scopus© and Web of Science©. The search terms were the following.

ProQuest©

TI (“antibiotic” OR “antimicrobial” OR “AMR” OR “resistant bacteria”) AND (TI (“GIS” OR “geographic information system” OR “geographical” OR “spatial analysis” OR “spatio-temporal” OR “point-source”) OR AB (“GIS” OR “geographic information system” OR “geographical” OR “spatial analysis” OR “spatio-temporal” OR “point-source”) OR IF (“GIS” OR “geographic information system” OR “geographical” OR “spatial analysis” OR “spatio-temporal” OR “point-source”)) AND (TI (“environment*” OR “natur*” OR “ecolog*”) OR AB (“environment*” OR “natur*” OR “ecolog*”) OR IF (“environment*” OR “natur*” OR “ecolog*”)).

PubMed©

(“antibiotic” [Title] OR “antimicrobial” [Title] OR “resistant bacteria” [Title]) AND (“GIS” [Title/Abstract] OR “geographic information system” Title/Abstract] OR “geographical” [Title/Abstract] OR “spatial analysis” [Title/Abstract] OR “spatio-temporal” [Title/Abstract] OR “point-source” [Title/Abstract] OR “GIS” [Text Word] OR “geographic information system” [Text Word] OR “geographical” [Text Word] OR “spatial analysis” [Text Word] OR “spatio-temporal” [Text Word] OR “point-source” [Text Word]) AND (“environment*” [Title/Abstract] OR “natur*” [Title/Abstract] OR “ecolog*” [Title/Abstract] OR “environment*” [Text Word] OR “natur*” [Text Word] OR “ecolog*” [Text Word]).

Scopus©

TITLE (“antibiotic” OR “antimicrobial” OR “AMR” OR “resistant bacteria”) AND TITLE-ABS-KEY ((“GIS” OR “geographic information system” OR “geographical” OR “spatial analysis” OR “spatio-temporal” OR “point-source”) AND (“environment*” OR “natur*” OR “ecolog*”)).

Web of Science©

TI=(“antibiotic” OR “antimicrobial” OR “AMR” OR “resistant bacteria”) AND TS=(“GIS” OR “geographic information system” OR “geographical” OR “spatialanalysis” OR” spatio-temporal” OR “point-source”) AND TS=(“environment*” OR “natur*” OR “ecolog*”).

We also manually searched Google Scholar© to find grey literature. The search results were exported in “.ris”-format from each database and imported into Zotero 6.0.15 (Digital Scholar, 2022) to remove duplicates and label according to inclusion and exclusion at each stage.

The first step was title screening, followed by abstract screening and finally full text inspection. All included records were required to be peer-reviewed, primary research articles. Our definition of search terms was based on previous manual searches and references from primary research articles and systematic reviews. Supplementary information (Supplementary Materials, Table S1) provides an overview of all included studies, summarizing the spatial aspects of their studies.

The spatial aspect was the primary focus of this systematic review. Therefore, we did not examine aspects of sample collection or microbiological analysis in the individual studies. The spatial aspect increased the comparability between studies regardless of whether they were based on sample collection or on secondary data. As the sampling effort in relation to the size of the study area is an important feature in spatial research, we estimated the number of samples per km^2 for each study included in the systematic review (Table 1). If not otherwise stated the size of the study area was estimated by drawing polygons in ArcGIS Pro 2.8.2 (ESRI, 2021). For general evaluation, we found approximate values to be sufficient for comparison. To analyse to what degree GIS and spatial analyses had been used we performed a basic content analysis and defined three quality aspects of spatial analyses: spatial design, spatial content and spatial implications. For each aspect we specified two content criteria. The aspect of spatial design is to analyse if individual studies include descriptions of the two criteria: spatial methods and study area. The aspect spatial content is describing if the research output in individual studies includes the two criteria: maps and spatial results, and the aspect spatial implications describes if the study is discussing spatial content in the form of the two criteria: generalisations and recommendations.

All spatial criteria were analysed using an evaluation rubric where we graded all criteria for all the included studies. The grades were defined as 1 = criteria mentioned, 2 = criteria mentioned and explained, and 3 = criteria mentioned, explained and discussed. The sum of all grades from all six criteria resulted in an overall score for each study ranging from 0 to 18 (3 aspects with 2 criteria each equal 6 criteria with a max score of 6×3 = 18). The data from the evaluation rubric was further analysed with the preference ranking organization method for enrichment evaluations (PROMETHEE) as described by Behzadian *et al.*, 2010. PROMETHEE is a decision-making tool establishing a preferential structure between alternatives, where a preference function is used for each criterion. The alternatives are ranked based on the total score in combination with pair-wise comparisons within each category. We used this method to establish a ranking among the included studies based on the individual criterion values for them. The analysis was performed in R v. 4.1.2 (R Core Team, 2021) and R Studio v. 2022.07.2 Build 576 (R Studio Team, 2020) using the package “promethee123” (Angelo Lellis Moreira *et al.*, 2020). To further address possible differences among the included studies the ranking of all studies with the PROMETHEE II method was performed in three different scenarios (S) with different weightings for criteria from the different spatial aspects as follows: (S1) equal weights for all spatial aspects; (S2) spatial design - weight = 0.15

& spatial content - weight = 0.15 & spatial implications - weight = 0.20; and (S3) spatial design - weight = 0.10 & spatial content - weight = 0.15 & spatial implications - weight = 0.25. With this we wanted to see if it was possible to make stronger disseminations among the studies based on the level of progress of their spatial analyses. We regard S1 as a basic GIS analysis with 33.3% of our evaluation based on each of all three aspects. S2 is a more developed GIS analysis with 40% emphasis on the possibility to develop spatial implications, and S3 is most developed with 50% of our evaluation based on spatial implications.

All included articles were peer-reviewed, high quality studies. The overall scores and rankings in our systematic review were used to compare the different studies based on their inclusion and development of the special aspects of problems with AMR in natural environments. They are not intended to be used for a qualitative evaluation of the studies as such, but as an attempt to show to what extent spatial analysis is used at the present research front.

Results

We wanted to find out how spatial methods have been applied in studies on AMR in the environment with a systematic review and content analysis of relevant studies. Although spatial methods have shown useful, these are often not declared as such, or added only as supplements. Only 13 studies fulfilled the review criteria, highlighting a research gap for GIS as a tool for analysing spatial patterns of AMR in the environment. The database searches resulted in 524 records after removal of duplicates (Figure 1). The first two screening stages of titles and abstracts retrieved 45 records for full text appreciation. After full text screening, 13 articles were included in the review. Of the 32 articles that were excluded at the last stage, 22 studies were excluded because they did not include spatial methods as the primary method in their study design. For the remaining 10, a spatial component was included in the study, but was not used as an essential part of the study design (Figure 1). For example Yang *et al.* (2019) included a map for sampled locations, but they did not spatially modify any data. Servais and Passerat's (2009) use of spatial methods was limited to a map on land use. Hu *et al.* (2020) referred to land use as a factor but did not present a map. Yi *et al.* (2019) included maps added as supplements only, indicating lower significance to the study outcome. In one instance antimicrobial resistance served as an indicator for human faecal pollution in natural environments (Kelsey *et al.*, 2003) while Rousham *et al.* (2018) described spatial methods but only as a study protocol and were not included in the final study.

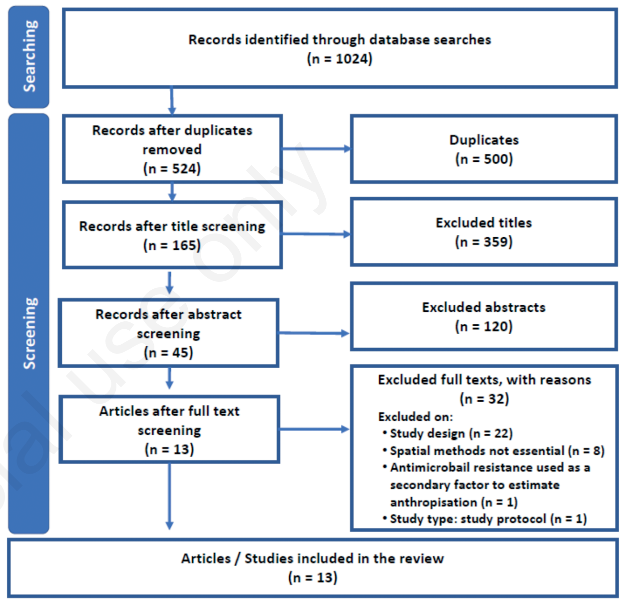

Figure 1. Selection process applied for the systematic review on spatial analysis of antimicrobial resistance in the environment. Searching and screening scheme modified after Haddaway *et al.* (2018).

Table 1. Geographical area and sampling sites for 13 articles included in the systematic review on spatial analysis of antimicrobial resistance in the environment.

Authors (Year)	Geographical location	Study area (km^2)	Sample type	Sampling sites (no.)	Sampling sites (per km^2)
Agga *et al.* (2019)	Agricultural research facility, Kentucky, USA	0.02	Soil	26	1,069.96
Bueno *et al.* (2021)	Minnesota, USA	[i/ii]225 180	Water and sediment	[iii]32	0.00014
Bueno *et al.* (2022)	Minnesota, USA	[i/ii]225 180	Study based on estimates		
Chique *et al.* (2019)	Four counties in Ireland	[i/ii]14 000	Study based on estimates		
Czekalksi *et al.* (2014)	Lake Geneva, Switzerland	[i]15	Sediment	22	1.47
de la Torre *et al.* (2012)	European Union	[i]4 232 040	Study based on estimates		
Ginn *et al.* (2021)	Urban/peri-urban area in La Paz, Bolivia	[i]140	Aerosol	23	0.16
Kucukdogan *et al.* (2015)	Marmara Region, Turkey	67 000	Secondary sample data of manure and soil	10	0.00015
Miller *et al.* (2020)	Various areas in Minnesota, USA	[i]400 000	Faecal, wild owls	78	0.00019
Sacristán *et al.* (2020)	Parts of Chile	[i]600 000	Faecal, wild felids	51	0.00008
Xiang *et al.* (2018)	Peri-urban area outside of Ningbo City, China	85	Soil	32	0.38
Yopasa-Arenas and Fostier (2018)	Brazil	[i]515 767	Study based on estimates		
Zhao *et al.* (2020)	Peri-urban area outside of Ningbo City, China	92	Soil	80	0.87

[i]Study area approximated as it was not stated in the article; [ii]Larger (macro-level) of two scales. Smaller, micro-level not approximated; [iii]Highest number chosen independent of sample type.

Heterogeneous study areas

All included studies were published after 2011. This final set was heterogeneous with diverse study origins where the United States of America (USA) (n = 4) and China (n = 2) were the only countries represented more than once (Table 1). The authors of two of the included articles used the term "macro" to describe the study area: Chique *et al.* (2019) who included four counties in Ireland and Bueno *et al.* (2021), whose study covered the State of Minnesota. Five additional studies fitted the macro-scale definition: Brazil (Yopasa-Arenas and Fostier, 2018), the European Union (de la Torre *et al.*, 2012), the Marmara Region of Turkey (Kucukdogan *et al.*, 2015), different areas in Chile (Sacristán *et al.*, 2020) and (again) the State of Minnesota (Miller *et al.*, 2020). Three author groups also studied smaller areas within a larger "macro" extent: Bueno *et al.*, 2021, 2022 and Chique *et al.*, 2019. The remaining five articles used considerably smaller study areas.

The type of natural media sampled varied among the included studies. Three studies sampled soil, two wild animal excrement, two sediments, one aerosols and one water (Table 1). One of the studies included data on manure and soil contamination (Kucukdogan *et al.*, 2015). The number of sampling sites per km^2 was mostly below one km^2 (Table 1). The largest number of sampling sites per km^2 was found in the smallest study area, monitoring an agricultural research facility (Agga *et al.*, 2019). The lowest number of sampling sites per km^2 was in a mid-sized study of water bodies in Minnesota (Bueno *et al.*, 2021). Several authors collected multiple samples per sampling site; e.g., Zhao *et al.* (2020) collected 5-8 sub-samples per site. Four publications did not include sample collection (Bueno *et al.*, 2022; Chique *et al.*, 2019; de la Torre *et al.*, 2012; Yopasa-Arenas and Fostier, 2018).

Variation of spatial methods

The methodology of the studies varied considerably (Table S1). Seven studies spatially analysed data from sample collection. Of these, Bueno *et al.* (2021) guided sampling by spatial analysis. Three studies were based on estimates of livestock-related pollution and one publication used various datasets in an exploratory study (Chique *et al*., 2019). Kucukdogan *et al.* (2015) used secondary sample data and estimated variables. The study locations at the smaller scales were peri-urban (n=3), urban (n=1), situated in the vicinity of urban surface waters (n=1), in a setting of wild animal sanctuaries (n=1) and an enclosed agricultural research facility (n=1). Several articles described possible influencing factors with risk for confounders, such as currents and hydrodynamic transport in a lake, which are difficult to predict (Czekalski *et al.*, 2014), water flow dynamics differing between water bodies (Bueno *et al.*, 2021) and the transport of aerosols that fluctuates depending on local variations (Ginn *et al.*, 2021). Kucukdogan *et al.* (2015) argue that subjective decisions on ranking criteria for risk models have an uncertain effect.

Insufficient data were often mentioned as a study limitation (Bueno *et al.*, 2021, 2022; Chique *et al.*, 2019; Czekalski *et al.*, 2014; de la Torre *et al.*, 2012; Ginn *et al.*, 2021; Kucukdogan *et al.*, 2015; Yopasa-Arenas and Fostier, 2018; Zhao *et al.*, 2020). In absence of nation-wide data on antimicrobial use, estimates were commonly based on assumptions, (de la Torre *et al.*, 2012), while in one case there was a lack of accurate data for every parameter used in a calculation model (Kucukdogan *et al.*, 2015). Further, in some papers, there was not sufficient access to livestock data (Yopasa-Arenas and Fostier, 2018) or on the use of fertilizer in each field patch (Zhao *et al.*, 2020). Results at the macro-scale were considered descriptive (Bueno *et al.*, 2021) or limited and in need of further validation with field data (Bueno *et al.*, 2022). de la Torre *et al.* (2012) concluded that their study allowed for qualitative assessment due to limited data for the whole European Union, while Ginn *et al.* (2021) regarded their results observational and Miller *et al.* (2020) saw the low sample size as a hindrance for detecting spatial patterns.

The main reason for applying a spatial GIS-method was often to give a visual impression of the spatial distribution of sample sites and landscape features of a study area. In one case, the overview map was based on satellite imagery (Zhao *et al.*, 2020). When the spatial distribution was analysed, the aim was to identify spatial patterns using heat or cluster maps identifying point-density (Miller *et al.*, 2020). Weighted overlay analyses were often used to combine reclassified parameters in a map (Bueno *et al.*, 2022; de la Torre *et al.*, 2012; Kucukdogan *et al.*, 2015; Yopasa-Arenas and Fostier, 2018). There were also common attempts to use interpolation methods to fill in value gaps between sampling sites with the aim to make a model of the spatial distribution of AMR in an entire study area (Agga *et al.*, 2019; Bueno *et al.*, 2021; Czekalski *et al.*, 2014; Xiang *et al.*, 2018). Buffer zones around sampling sites were used for descriptions of landscape features in the study area (Sacristán *et al.*, 2020) or to generate an input layer for an overlay analysis (Zhao *et al.*, 2020).

Antimicrobial pollution and the environment

The general result emerging from the included studies was that there existed significant positive associations between anthropogenic activities, antimicrobial pollution and the presences of antimicrobial genes in natural ecosystems (Table S1). This was true both for studies relying on empirical data on antimicrobial pollution from soil, faecal or water samples as well as for studies estimating concentrations of antimicrobials based on register data (Table S1). Three studies focused on continuous pollution gradients based on large-scale land use patterns. Ten studies assessed the effects from antimicrobial pollution from point sources. Those that not referred to watershed pollution connected to human activities (Xiang *et al.*, 2018; Zhao *et al.*, 2020) and non-point manure pollution from livestock (de la Torre *et al.*, 2012). Selective exposure in nature due to antibiotic pollution was mentioned in all articles, among these were increasing stress on ecosystem safety (de la Torre *et al.*, 2012), toxic effects and degradation of soil and water quality (Kucukdogan *et al.*, 2015) and risk for worsened soil security (Zhao *et al.*, 2020).

The result of the content analysis was analogous with above findings for each study. Of all spatial categories "Study Area" and "Maps" were the only ones where no study reached the highest grade. The totals by category confirmed that "Generalisation" and "Recommendations" were the least mentioned while "Methods" and "Results" scored the highest. The categories "Generalisation" and "Recommendations" were also the only ones that registered zero points. Five studies with the highest score in the content analysis reached above 13 out of the18 possible points (Table 2).

Discussion

"GIS" or "spatial analysis" in the title or abstract were strong indicators for inclusion in this systematic review, but they were rarely found. The selection process resulted in a large proportion of exclusions in the last step of full text screening. However, four articles allowed for certain inclusion already at the "title and abstract"-stage (Chique *et al.*, 2019; de la Torre *et al.*, 2012; Kucukdogan *et al.*, 2015; Yopasa-Arenas and Fostier, 2018). Our first finding was that only a few studies, compared to the overall number of environmental studies on AMR, applied GIS as the main research method. As only 13 studies met our inclusion criteria, we considered it difficult to assess the publication bias. Nevertheless, we have confidence in the outcome based on a thorough manual search for grey literature in Google Scholar with the same search terms, which did not show any unpublished studies on preprint servers.

Application of GIS

Results from our content analysis revealed a large gap between the highest and lowest ranked studies in the PROMETHEE-analysis. Our attempt was to determine to what degree GIS was applied in each study. The ranking order remained similar independent of how we weighted the three categories: Design, Content and Implications. We found it fair to expect formulation of explanation and discussion of the spatial design, for example in the form of considerations related to the size of the study area, but the included studies neither discussed these aspects nor the spatial content and the sub-group "maps" in detail. Implications as spatial category had the lowest summed score suggesting that spatial methods are rarely the main research method with none or only brief attempts at formulating any generalisations or recommendations. However, the spatial implications category was also the one with the largest variation among the included studies indicating large differences in competence in spatial analysis among different researchers.

In general, with only 13 included articles in this systematic review, the diverse application of spatial analyses indicates a great potential for GIS in studies of AR in natural and semi-natural ecosystems. We found two distinctive groups among the included studies, i) laboratory detection of antibiotics and AR complemented with GIS-methods (Agga *et al.*, 2019; Bueno *et al.*, 2021; Czekalski *et al.*, 2014; Ginn *et al.,* 2021; Miller *et al.*, 2020; Sacristán *et al.*, 2020; Xiang *et al.*, 2018; Zhao *et al.*, 2020), and ii) GIS as the main method by means of areal overlay analysis (Bueno *et al.*, 2022; Chique *et al.*, 2019; de la Torre *et al.*, 2012; Kucukdogan *et al.*, 2015; Yopasa-Arenas and Fostier, 2018). In the first group, GIS was used to visualize laboratory results, in maps and point distributions used for interpolation (Agga *et al.*, 2019; Bueno *et al.*, 2021; Czekalski *et al.*, 2014; Xiang *et al.*, 2018) and in point-density methods (Bueno *et al.*, 2021; Zhao *et al.*, 2020). In general, data cannot cover each point in a geographic area, especially not in large areas. While values at the exact sample location were confirmed, interpolation values returned estimates based on known data. When interpolation was applied in the studies above, it was not described in any detail. Interpolation maps were often found in supplementary material, again suggesting that spatial methods were not considered as the primary method.

The second group used overlay analyses combining several data layers to generate risk-estimates or probability maps for accumulation of antibiotic resistance bacteria. Input data came from secondary sources, such as cattle density, soil types or water flow direction. Overlay analysis in these studies was conducted to generalise findings for medium to large geographical areas. Bueno *et al.* (2021) combined the approaches of both groups by designing sampling at the micro-level based on information from the larger macro-level. In their discussion, Bueno *et al.* (2021) described results from the "macro"-level as descriptive, owing to the uncertainty of sample data in comparison to the extent of the study area. In practice, probability maps could be created based on estimates to be verified or falsified by control-sampling in the field. This procedure would allow for calibration of the input data for overlay analysis and lead to improved probability maps, but this approach is also time-consuming.

Table 2. Content analysis of the 13 included articles in the systematic review based on six different criteria categorized under one of three spatial aspects.

Article	Methods	Study area	Maps	Results	Gen.	Rec.	Total score	Design (S1)	Content (S2)	Implication (S3)
Bueno *et al.*, 2021	3	2	2	3	3	2	15	0,9445	0,9585	0,9585
Kucukdogan *et al.*, 2015	3	2	2	3	2	3	15	0,9445	0,9585	0,9583
Zhao *et al.*, 2020	2	2	2	3	3	3	15	0,8332	0,9168	1,0665
Bueno *et al.*, 2022	2	2	2	3	3	2	14	0,6388	0,6835	0,7750
Yopasa-Arenas and Fostier, 2018	3	1	2	3	3	2	14	0,5831	0,6335	0,7416
Chique *et al.*, 2019	3	2	2	1	2	2	12	0,2776	0,3002	0,2417
Miller *et al.*, 2020	2	2	2	2	1	2	11	-0,0555	-0,0583	-0,0832
Czekalski *et al.*, 2014	2	2	2	2	2	0	10	-0,2222	-0,2585	-0,3332
de la Torre *et al.*, 2012	2	2	2	1	1	1	9	-0,4167	-0,4419	-0,5249
Ginn *et al.*, 2021	2	2	2	2	0	1	9	-0,4167	0,4919	-0,6250
Agga *et al.*, 2019	1	1	2	3	0	0	7	-0,8886	-0,9668	-0,9669
Xiang *et al.*, 2018	1	1	1	3	1	0	7	-1,0832	-1,0918	-1,0418
Sacristán *et al.*, 2020	2	1	1	2	1	0	7	-1,1388	-1,1418	-1,1666
Sum of grades	28	22	24	31	22	18				

Gen=generalization; Rec=recommendations. Grading of criteria are based on three levels (from basic to comprehensive).The Total Score is equal to the sum of all grades for each study. Ranking is based on the PROMETHEE method using three different scenarios. S1 equal weights for all spatial aspects including methods and study area; S2 = spatial design including. maps and results - weight = 0.15, spatial content - weight = 0.15; spatial implications - weight = 0.20; S3 spatial design - weight = 0.10, spatial content - weight = 0.15; spatial implications - weight = 0.25. The combined grades of all studies are summed up for each category. The highest possible sum is 39 for every category.

Table 3. Recommendations to advance spatial analysis as a method of environmental studies of antimicrobial resistance based on the results of the systematic review

-	Document spatial methods adequately and include spatial results in the main publication.
-	Discuss spatial methods
-	Gather appropriate and sufficient data to answer your research question.
-	Explore Geographic Information Systems to test hypotheses or to guide sample taking.
-	Consider running statistical power tests on your datasets, especially in relation to the size of study area.
-	Take confounders into consideration when designing the study, namely secondary point-sources, and variations in the environmental setting.

Complexity and confounding factors

Environmental studies of AMR have been described as complex, with a risk of flawed study design by not taking into account possible confounders (Singer *et al.*, 2006) especially at moderate to larger scales (Bueno *et al.*, 2017). The examined environmental media also has spatial implications (Bueno *et al.*, 2017, 2018; Luz *et al.*, 2022) adding further complexity. Concentration of bacteria is influenced by the environmental media in which they reside, where water and soil have different characteristics. Dilution in combination with predation and antibiotic degradation in aquatic environments possess a natural resilience capacity (Goulas *et al.*, 2020). The density of bacteria, and therefore the detection by measurement, is higher in soils and sediments bacterial movement is lower. Density is also higher in sewage water compared to other types of water (Kümmerer, 2009). The study of Bueno *et al.* (2021) sampled both soil and water to document long-term effects in soil samples and the expected short-term contamination in water samples. Beyond the scope of this study, factors that influence spatial analyses, *e.g.*, buffer size and distances of sample location to the point source of contamination need to be examined with regard to possible confounding.

Suitable data density and recommendations

Even if data density varied greatly among the included studies, estimates of data points per km^2 (Table 1) should bring about reflections on data density. However, data density must not have a direct connection to study quality as methods and study settings differed in the papers. Two out of four articles with the highest ranking for content (Table 2) were also among the articles with lowest data density. If generalisation is the preferred outcome, representative sample data that merit this scope are crucial. It is always important to reflect on whether or not data are suitable in relation to the study area, *e.g.*, obtainable data should be tested statistically in relation to the hypothesised outcome. An article by Young *et al.* (2018) can pose as such an example from studies on blue carbon ecosystem by analysing the statistical power at different sample sizes associated with landscape variables. The power of association of sample values to landscape use should preferably be tested at different geographical scales. Of the included studies in this systematic review, only one used a statistical test to compare associations between variables based on data estimated from buffers of different sizes (Zhao *et al.*, 2020).

A set of recommendations based on this review are summarised in Table 3. We feel that maps and considerations regarding the size of the study area are central components of any spatial analysis that need to be satisfyingly represented both in the methods section and as spatial results. Maps can present data in many ways (although at times misleading). Decision on study area size is a conscious geographical delimitation that ought to be based on the data intended to be used. We think that a study design that *a priori* relates the data available to the study area, may guide decisions towards appropriate spatial methods and tools.

Conclusions

We identified two distinctive groups of spatial analysis. One using areal overlay analysis of relevant data layers to generate risk maps and one focusing on detection of AMR and antimicrobial pollution in environmental samples. In the latter group we identified GIS as a secondary method to analyse patterns of point values estimated from environmental samples. A combination of both approaches, demonstrated by one of the included studies, allows for a synthesis of distributional knowledge of AMR. Initial GIS analysis based on distributions of risk factors can guide sample collection or AMR-surveillance, which in turn can lead to further calibration of risk maps. Spatial methods are important, but still underexplored in the studies of AMR. With our set of recommendations based on a synthesis of studies in this field (Table 3) and our content analysis, we encourage application of GIS in studies of AMR in the environment.

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Online supplementary material:

Results of PROMETHEE-ranking. Generated in RStudio v. 2022.07.2 Build 576.

Table S1. Summary of methods and results related to spatial aspects of 13 articles included in the systematic review on spatial analysis of AMR in the environment, published between 2012-2022.