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Systematic Review: Impact of point sources on antibiotic-resistant bacteria in the natural environment

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Summary

Point sources such as wastewater treatment plants and agricultural facilities may have a role in the dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG). To analyse the evidence for increases in ARB in the natural environment associated with these point sources of ARB and ARG, we conducted a systematic review. We evaluated 5,247 records retrieved through database searches, including both studies that ascertained ARG and ARB outcomes. All studies were subjected to a screening process to assess relevance to the question and methodology to address our review question. A risk of bias assessment was conducted upon the final pool of studies included in the review. This article summarizes the evidence only for those studies with ARB outcomes ($n = 47$). Thirty-five studies were at high ($n = 11$) or at unclear ($n = 24$) risk of bias in the estimation of source effects due to lack of information and/or failure to control for confounders. Statistical analysis was used in ten studies, of which one assessed the effect of multiple sources using modelling approaches; none reported effect measures. Most studies reported higher ARB prevalence or concentration downstream/near the source. However, this evidence was primarily descriptive and it could not be concluded that there is a clear impact of point sources on increases in ARB in the environment. To quantify increases in ARB in the environment due to specific point sources, there is a need for studies that stress study design, control of biases and analytical tools to provide effect measure estimates.

KEYWORDS
antibiotic-resistant bacteria, effect measure, environmental dissemination, point source, systematic review

1 | INTRODUCTION

The widespread use of antimicrobials in human medicine, households, livestock, aquaculture, veterinary medicine and horticulture has enhanced the selection and spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG; Baker-Austin, Wright, Stepanauskas, & McArthur, 2006; Meek, Vyas, & Piddock, 2015; O'Neill, 2015). When present in human pathogens, antibiotic resistance can have a direct negative impact on human health (treatment failure, extended duration of illnesses, death) as well as on the economy (Friedman, Temkin, & Carmeli, 2016).

While research and policy have long focused on the role of the built environment such as healthcare facilities, the potential role of the natural environment in the selection and spread of ARB and ARG has been highlighted in recent research (Hiltunen, Virta, & Laine, 2017; Huijbers et al., 2015; Tripathi & Cytryn, 2017). Environmental systems, most commonly surface waters, often receive treated and untreated waste from human activities such as households, hospitals,

industry and animal agriculture. This waste can contain elevated levels of ARB, ARG and antibiotics (and/or metabolites) which can then be further disseminated via water, land or even through wildlife (Baquero, Martinez, & Canton, 2008; Berendonk et al., 2015; Vittecoq et al., 2016).

The presence of ARB and ARG in the environment likely poses a health risk to humans, animals and the ecosystem, but this is not well understood (Ashbolt et al., 2013). The first step to assess these risks is to quantify the relative contribution of specific point sources on increases in ARB and ARG in the natural environment. Despite an increase in research in this field, the relative impact of specific types of point sources on levels of ARB and ARG in the environment is still unclear and is an area of ongoing scientific investigation (Williams-Nguyen, Bueno, Sargeant, Nault, & Singer, 2016; Williams-Nguyen, Sallach, et al. 2016; Wooldridge, 2012; Woolhouse, Ward, van Bunnik, & Farrar, 2015). The goal of this study was to identify and summarize evidence in the scientific literature pertaining to the association between effluent point sources and the quantity of ARB in adjacent environmental media such as water and soil. We specifically attempted to quantify the strength or magnitude of the effect between a point source (s) and the frequency or concentration of ARB in the surrounding environment. The specific review question was: Is the prevalence or concentration of antibiotic-resistant bacteria in soil, water, air or free-living wildlife higher in close proximity to, downstream from or downwind from, known or suspected sources compared to areas more distant, upstream or upwind from these sources? Epidemiological effect measures (i.e. measures of impact) are often used to understand the magnitude of the relationship between exposures and outcomes. Examples of such measures in the context of our review question could include odds ratios or prevalence ratios (for ARB prevalence outcomes) and mean differences (for ARB concentration outcomes).

2 | MATERIALS AND METHODS

This systematic review was conducted as described previously (Bueno et al., 2017). Briefly, a systematic review of the literature was conducted following a protocol using the population, exposure, comparator, outcome and study design (PECOS) framework (Williams-Nguyen, Bueno, et al. 2016; Williams-Nguyen, Sallach, et al. 2016). The review team comprised six people with expertise in antimicrobial resistance, epidemiology, veterinary medicine and systematic review methodology. Three databases (PubMed©, Commonwealth Agricultural Bureaux (CAB Abstracts©) and Scopus©) were searched on 14 October 2014 from inception date using specific search strategies as shown below and as previously reported (Bueno et al., 2017). The PubMed© controlled-vocabulary search string was as follows:

"drug resistance, microbial"[Mesh] AND ("water pollutants"[Mesh] OR "environment"[MeSH Terms] OR "soil"[MeSH Terms] OR "water"[MeSH Terms] OR "water pollution"[MeSH Terms] OR "air pollution"[MeSH Terms] OR "air pollutants"[MeSH Terms] OR "animals, wild"[MeSH

Terms]) AND ("Animals"[MeSH Terms] OR "humans"[MeSH Terms] OR "animal feed"[MeSH Terms] OR "manure"[MeSH Terms] OR "aquaculture"[MeSH Terms] OR "waste water"[MeSH Terms] OR "sewage"[MeSH Terms] OR "hospitals"[MeSH Terms] OR "hospitals, animal"[MeSH Terms] OR "cities"[MeSH Terms]) NOT "therapeutics"[MeSH Terms] NOT "drug discovery"[MeSH Terms] NOT "aids"[All Fields] NOT "hiv"[All Fields] NOT "influenza"[All Fields].

The search string for CAB Abstracts© was:

("Drug Resistance".mp. and ("environment$" or "soil" or "water" or "water pollution" or "air pollut$" or "wild animals").hw. and ("animals" or "man" or "feeds" or "manures" or "aquaculture" or "wastewater$" or "sewage" or "hospitals" or "animal hospitals" or "urban areas").hw.) not "Therapeutics".af. not "Drug discovery".af. not "aids".af. not "hiv".af. not "influenza".af.

The search string for Scopus© was:

TITLE-ABS-KEY ((antibiotic OR antimicrob*) AND resistan*) AND KEY ("environment*" OR "soil" OR "water" OR "water pollution" OR "air pollut*" OR "wild animals") AND KEY ("animals" OR "man" OR "feeds" OR "manures" OR "aquaculture" OR "wastewater*" OR "sewage" OR "hospitals" OR "animal hospitals" OR "urban areas") AND NOT TITLE-ABS-KEY ("Therapeutics") AND NOT TITLE-ABS-KEY ("Drug discovery") AND NOT TITLE-ABS-KEY ("aids") AND NOT TITLE-ABS-KEY ("hiv") AND NOT TITLE-ABS-KEY ("influenza").

The search was updated on 19 April 2016 using identical search terms. The search did not include any language or geographic limits. All citations were imported into the EndNote reference management software package (Thomson Reuters, Philadelphia, PA), and duplicate records were removed.

The same protocol was followed for culture-independent (ARG) and culture-dependent (ARB) outcomes. Studies with both outcome

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Impacts

- We conducted a systematic review to assess the evidence of increases in antibiotic-resistant bacteria (ARB) in the natural environment from point sources (such as wastewater treatment plants and animal agriculture).

- There is lack of quantitative causal research on the association of point sources and increases in ARB in the environment.

- Improved study design, control of biases and analytical tools to provide effect measure estimates are needed to quantify the effect of point sources on the dissemination of ARB in the environment. This will help with the understanding of how ARB in the environment may affect human and animal health.

types were assessed in a common pool until the data extraction process. At that stage, studies were stratified into ARB and ARG outcomes. More information about studies with ARG outcomes can be found in Bueno et al. (2017).

The first screening stage (relevance screening) consisted of an evaluation of titles and abstracts of all records retrieved to retain only those relevant to the review question. Inclusion criteria required that papers (i) were primary research; (ii) collected environmental samples (soil, water, sediment, air, biological samples from wildlife); and (iii) reported prevalence or concentration of ARB. An exclusion criterion that was not reported in the original protocol (Williams-Nguyen, Bueno, et al. 2016; Williams-Nguyen, Sallach, et al. 2016) was added and asked: "Does the study use microbial source tracking techniques?". If yes, then the study was excluded. Microbial source tracking techniques compare characteristics of faecal bacteria isolated from environmental sources with characteristics of faecal bacteria from known sources in an effort to identify the source of environmental isolates. These types of studies often do not explicitly compare sites based on physical distance or direction from the source (e.g. Dickerson, Hagedorn, & Hassall, 2007; Edge & Hill, 2005; Mthembu, Biyela, Djarova, & Basson, 2010; Murugan, Prabhakaran, Al-Sohaibani, & Sekar, 2012). As a result, such studies do not provide evidence for this systematic review question.

Any study that did not meet all these criteria was excluded. Those studies for which it was unclear whether criteria were met on the basis of title and abstract passed through to the following screening phase for further review.

Remaining articles were subjected to a second screening stage (design screening) which consisted of an evaluation of the methods section of the full-text to determine whether the methodology used for each study was adequate to answer the systematic review question. Inclusion criteria therefore required that studies a) reported proximity to, or direction from a potential point source and b) had a comparison group (i.e. samples taken a fixed distance from or/upstream from the source) or compared across a range of distances (i.e. samples taken at different distances from the source). Studies that did not meet these criteria were excluded. An additional question not reported a priori in the protocol (Williams-Nguyen, Bueno, et al. 2016; Williams-Nguyen, Sallach, et al. 2016) was added at this screening stage and asked: "Does the study implicitly or explicitly define a point source to which a comparison is made?". Also, during this screening phase, articles not written in English were identified, and if needed, an effort was made to translate the full text as the language fluency of the review team allowed.

The systematic review process was pre-tested by reviewing a sample of articles among all the citations from the complete database. Four articles that featured comparison groups based on information in the title and/or abstract were chosen. Studies of this kind were specifically selected to ensure testing of the second screening level (design screening). Two independent reviewers evaluated this phase, and improvements to the screening process and data entry were made based upon their feedback.

Records which passed both screening processes were entered into a spreadsheet designed for this systematic review (Microsoft Office Excel 2013® Microsoft Corporation, Redmond, WA, USA). For both screening phases (relevance and design), two reviewers independently assessed each study. Consensus was required between the reviewers, and conflicts were resolved through phone conferences and e-mail.

After the screening stages, the full text of each included study was evaluated for potential threats to internal validity (risk of bias assessment) by two independent reviewers. Data for the risk of bias assessment were entered into a customized relational database (Microsoft Access 2013®). First, a qualitative rubric (explained below) was pre-tested by reviewing a sample from the included full-text articles after the two screening stages by three independent reviewers. A total of three articles were evaluated for this purpose. Pre-testing improved the interpretation of the risk of bias assessment across reviewers, as well as the consistency of data entry.

Articles were divided equally between each participating reviewer. A qualitative rubric of low, high and unclear was assigned to each study for the potential risk of bias in the reported effect measure or other outcome variable due to selection bias, information bias and confounding (Williams-Nguyen, Bueno, et al. 2016; Williams-Nguyen, Sallach, et al. 2016). The risk of bias assessment was conducted at the study level and not at the outcome level due to the large number of possible outcomes per study. Selection bias was defined as systematic differences between the comparison groups with respect to how samples were collected in the study (methods used across sites). Information bias was defined as systematic differences in the methods for ascertaining ARB between comparison groups (i.e. use of different laboratory methods for the samples in the comparison groups). Confounding was evaluated with respect to the presence of other point or non-point sources that were likely to release ARB, ARG and/or antibiotics to the environment that could have affected the study outcome(s). It was assumed that a study that assessed the impact of a point source using sampling locations within a large spatial scale (e.g. 100 km distance between sampling locations) was at higher risk of confounding than a study where the spatial scale was smaller (e.g. a 10 km scale) due to the possible influence on the outcome of a larger number of alternative point and non-point sources (and thus release of ARB, ARG and/or antibiotic metabolites) unless adequate confounding control measures were described. For all three types of biases, strategies to control or minimize the impact of these biases on the internal validity of the study were factored into the decision to classify them as low, unclear or high. Generally, a study was classified as "high" risk of bias if there were differences across the comparison groups that would affect the results (e.g. use of different microbiological methods between upstream and downstream samples). A study was classified as "low" risk of bias when consistency across the comparison groups made the introduction of systematic differences unlikely. Finally, "unclear" was used when there was not enough information provided in the study to assess the threat to internal validity.

A final qualitative (low, high and unclear) overall bias rubric was assigned to each study by considering the risk of bias from each domain after consensus was reached among the reviewers. In general, if a study had at least one of the three domains classified as high risk, the overall result was considered high risk of bias and the same applied for

unclear risk of bias. However, the overall decision was made on a case-by-case basis relying on the judgement of the three reviewers involved in the risk of bias assessment.

After the risk of bias assessment, data from all studies, including those that were deemed to be at high risk of bias, were extracted and synthesized. Data consisted of characteristics of the study (geographic location, publication year, spatial scale, sampling design, type of laboratory detection method used), the exposure (point source) and the outcome: ARB prevalence/proportion or ARB concentration. Any information available on statistical methods or modelling approaches used and effect measures (and variability) reported for the comparison of interest was also recorded. Data were entered into the same custom relational database albeit in a different table from the one used for the risk of bias assessment. Additionally, a summary of the most relevant findings for the comparison of interest from each individual study was recorded (Tables 2 and 3).

In contrast to the original protocol (Williams-Nguyen, Bueno, et al. 2016; Williams-Nguyen, Sallach, et al. 2016), the risk of bias assessment was conducted prior to data extraction. To minimize introduction of bias by conducting these steps in reverse order, the reviewers who assessed studies during the risk of bias stage did not review the same studies during data extraction and were blinded to the risk of

bias assessment decisions. Afterwards, a review team member uninvolved in either risk of bias assessment or data extraction validated all the extracted data.

3 | RESULTS

Literature searches retrieved a total of 5,247 records after deduplication. This total included those studies that used both culture-dependent methods to ascertain ARB and culture-independent methods for ARG. After the two screening stages (relevance and design), the number of records retained was 813 (relevance) and 75 (design). Forty-seven of these 75 articles used culture-dependent methods to ascertain ARB (Figure 1). The results for ARG outcomes are reported in a separate publication (Bueno et al., 2017).

For the overall risk of bias assessment, 11 studies were deemed to be at high risk of bias, 24 were at an unclear risk of bias, and 12 were at low risk for bias.

An example of a study considered at high risk of bias was Laroche, Pawlak, Berthe, Skurnik, and Petit (2009). In this study, neither sampling design nor analytic approaches were used to control for potential confounding due to potential point sources other than the wastewater
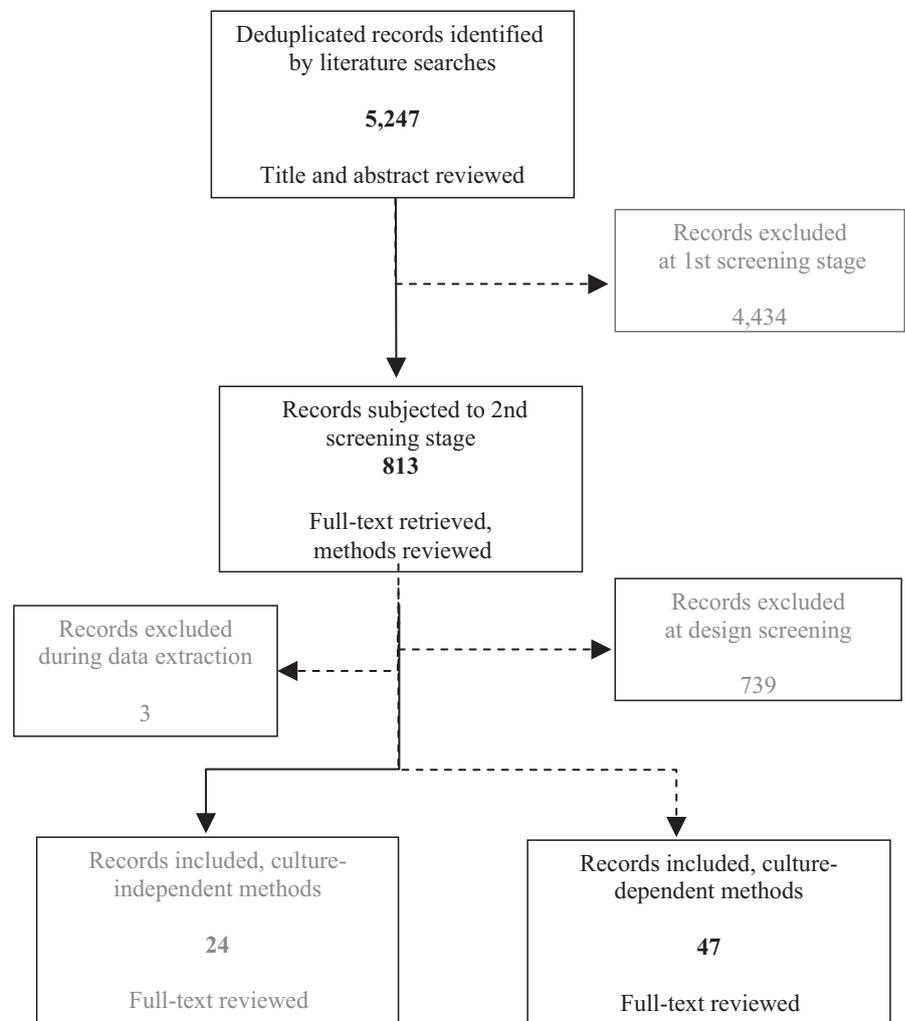

FIGURE 1 Flow chart summarizing the selection process for the studies included in the systematic review (the shaded boxes depict the articles excluded from the process and the records for the ARG outcome, not assessed in this manuscript)

Deduplicated records identified by literature searches

5,247

Title and abstract reviewed

Records excluded at 1st screening stage

4,434

Records subjected to 2nd screening stage
813

Full-text retrieved, methods reviewed

Records excluded during data extraction

3

Records excluded at design screening

739

Records included, culture-independent methods

24

Full-text reviewed

Records included, culture-dependent methods

47

Full-text reviewed

treatment plant (WWTP) of interest that could have influenced the results over the large spatial scale of this study (58 km). Several other WWTP with potential confounding influence were documented in the study (Laroche et al., 2009).

An example of a low risk of bias study was Harnisz, Korzeniewska, and Gołaś (2015). In this study, sampling was conducted the same way across all sites, the outcome was ascertained consistently at all sites, and the small spatial scale (400 m) made it unlikely for other point sources to influence the results (Harnisz et al., 2015).

Finally, an example of an unclear risk of bias study was Sulzner, Kelly, Smith, and Johnson (2014). In this study, turkey vultures (Cathartes aura) were sampled at a location near livestock and at a location far from livestock. The study did not provide enough information to ascertain possible exposure of sampled birds to other point sources of ARB, a plausible issue given that turkey vultures can fly long distances. Also, there was lack of information about sampling procedure consistency between the sites (Sulzner et al., 2014).

Of the 47 studies, 46 were written in English and one in Portuguese (Fuentefria, Ferreira, Gräf, & Corção, 2008), which was translated by the review team. The geographic location of the studies was diverse: China ($n = 7$), the United States ($n = 7$), Poland ($n = 6$), France ($n = 4$), Germany ($n = 4$), Spain ($n = 3$), Brazil ($n = 2$), Austria ($n = 1$), Canada ($n = 1$), Croatia ($n = 1$), Ireland ($n = 1$), Japan ($n = 1$), South Korea ($n = 1$), Mexico ($n = 1$), the Netherlands ($n = 1$), Portugal ($n = 1$), Romania ($n = 1$), South Africa ($n = 1$), Switzerland ($n = 1$), Taiwan ($n = 1$), and one study included two countries: Saudi Arabia and the UK (Alharbi, 2012). Date of publication ranged from 1995 to 2016, with the highest number of publications in 2015 ($n = 11$). The spatial scale for the sampling frame ranged from 40 m (Li, Song, et al, 2015) to 450 km (Sulzner et al., 2014); six studies did not report a specific distance.

Most studies investigated point sources of human waste ($n = 35$), primarily human wastewater treatment plants ($n = 26$). The remaining studies assessed animal agriculture ($n = 12$), both aquaculture ($n = 3$) and terrestrial agriculture ($n = 9$). Across all studies, surface water was the most common type of environmental media sampled ($n = 36$), followed by sediment ($n = 5$), groundwater ($n = 4$), air ($n = 2$), soil ($n = 2$), biofilm ($n = 1$), wildlife ($n = 1$) and shellfish ($n = 1$). Five studies collected two sample types. Descriptive information about each study can be found in Table 1.

Eight of the 47 studies reported more than one bacterium outcome. In those studies where the outcome was detailed at the bacterium species level, the most common bacteria species were Escherichia coli (E. coli) ($n = 18$), Pseudomonas aeruginosa ($n = 3$), Enterococcus faecalis ($n = 1$), Enterococcus faecium ($n = 1$) and Staphylococcus aureus ($n = 1$). Some studies only reported the outcome at the genus level: Enterococcus ($n = 5$), Aeromonas ($n = 1$), Klebsiella ($n = 1$), Pseudomonas ($n = 2$) or at the family level: Enterobacteriacea ($n = 4$) and Rhodospirillacea ($n = 1$). Sixteen studies presented their results at a broader level of bacterial classification: heterotrophic ($n = 8$), coliforms ($n = 5$), Gram-negative ($n = 2$), Gram-positive ($n = 1$), Cytophage–flavobacterium ($n = 1$), oligotrophic ($n = 1$) and copiotrophic ($n = 1$). Two studies did not specify the bacterial outcome: one reported the proportion of the total resistant bacterial community (Li, Qi, Yang, Zhang, & Yu, 2011), and the other one reported oxytetracycline-resistant total culturable organisms (Kerry et al., 1995). Three studies also reported either the proportion of phenotypically resistant isolates with selected resistance genes (Marinescu, Marutescu, Savin, & Lazar, 2015; Zhang et al., 2015) or the relative gene abundance among resistant isolates (Hsu et al., 2014).

The ascertainment of ARB was carried out by disc diffusion in 40 studies, while other methods used in the remaining studies were dilution method ($n = 5$) and a plating method ($n = 1$). The Clinical and Laboratory Standards Institute (CLSI) guidelines were used for antimicrobial susceptibility in 28 studies, followed by The French National Guidelines ($n = 3$), Deutsches Institut für Normung or DIN ($n = 3$), Comite′ de l'Antibiogramme de la Societe′ Francaise de Microbiologie or CA-SFM ($n = 2$), defined by the manufacturer ($n = 2$), by the study ($n = 2$) and European Committee on Antimicrobial Susceptibility or EUCAST ($n = 1$). There were six studies where the guidelines were not reported.

With reference to statistical methods and modelling approaches, nine studies conducted statistical analysis to compare the prevalence/proportion of ARB between sites with reference to a single-point source, and one study used a mixed-model approach to describe the effect of multiple point sources on the proportion of resistant E. coli (Rees et al., 2015). None of the studies, however, reported effect measures, which quantify the strength of the effect of the point sources under investigation on the prevalence or concentration of ARB in the natural environment.

For the studies assessing a single-point source, Akiyama, Asfahl, and Savin (2010) compared the proportion of antibiotic-resistant E. coli as well as resistant total coliforms between sites upstream and downstream from a WWTP using split-plot ANOVA (Akiyama et al., 2010). Li et al. (2010) compared the prevalence of resistant heterotrophic bacteria between sites upstream and downstream from a WWTP with a Mann–Whitney U test (Li et al., 2010). Schreiber and Kistemann (2013) compared the proportion of resistant Rhodospirillaceae between upstream and downstream sites from WWTP using Cramer V correlation (Schreiber & Kistemann, 2013). Koczura et al. (2012) compared the proportion of resistant E. coli between upstream and downstream sites from a WWTP using a Pearson chi-square test (Koczura et al., 2012). Sulzner et al. (2014) used this same test to compare the prevalence of resistant E. coli in turkey vultures (Cathartes aura) between two sites (one near a sheep flock and another site far away from it; Sulzner et al., 2014). Kotlarska, Łuczkiewicz, Pisowacka, and Burzyński (2015) compared the prevalence of resistant E. coli between upstream and downstream sites from a WWTP with Fisher's exact test (Kotlarska et al., 2015), and Sapkota, Curriero, Gibson, and Schwab (2007) used the same statistical test to compare the proportion of resistant Enterococcus spp. at sites upstream and downstream from a swine farm (Sapkota et al., 2007). Fisher's exact test was also used by West, Liggit, Clemans, and Francoeur (2011) to compare the prevalence of resistant faecal coliforms upstream and downstream from a WWTP (West et al., 2011). Marinescu et al. (2015) compared the proportion of resistant Gram-negative bacteria between upstream and

TABLE 1 Descriptive information for each one of the 47 studies included in this systematic review. WWTP: Wastewater treatment plant

Citation	Country/s	Spatial scale	Source type	Environmental media/system
Abia, Ubomba-Jaswa, and Momba (2015)	South Africa	50 km	Human waste (WWTP)	Surface water/river
Alharbi (2012)	Saudi Arabia, UK	2 km	Human waste (Hospitals)	Soil
Akiyama and Savin (2010)	United States	2 km	Human waste (WWTP)	Surface water/river
Amador, Fernandes, Prudêncio, Barreto, and Duarte (2015)	Portugal	1 km	Human waste (WWTP)	Surface water/river
Anderson et al. (2014)	United States	0.6 km	Animal agriculture (Poultry processing plant)	Surface water/river
Blaak et al. (2014)	The Netherlands	1–2 km	Human waste (WWTP)	Surface water/river
Czekalski et al. (2012)	Switzerland	3.2 km	Human waste (WWTP)	Sediment, surface water/lake
Fincher et al. (2009)	United States	2–3 km	Human waste (Urban area)	Surface water/river
Fuentefria et al. (2008)	Brazil	Not reported	Human waste (Hospital)	Surface water/river
Fuentefria et al. (2011)	Brazil	1.4 km	Human waste (Hospital)	Surface water/river
Gallert, Fund, and Winter (2005)	Germany	430 m	Human waste (Leaky sewer)	Groundwater
Goni-Urriza et al. (2000)	Spain	30 km	Human waste (Sewage dump)	Surface water/river
Gordon et al. (2007)	France	2 km	Aquaculture (Fish farm)	Sediment/river
Harnisz (2013)	Poland	2 km	Human waste (WWTP)	Surface water/river
Harnisz et al. (2015)	Poland	400 m	Aquaculture (Fish farm)	Surface water/river
Hsu et al. (2014)	Taiwan	1 km	Animal agriculture (Swine farm)	Groundwater, surface water/river
Kerry et al. (1995)	Ireland	Unclear	Aquaculture (Fish farm)	Sediment/ocean
Koczura et al. (2012)	Poland	Not reported	Human waste (WWTP)	Surface water/river
Kotlarska et al. (2015)	Poland	3 km	Human waste (WWTP)	Surface water/river
Laroche et al. (2009)	France	58 km	Human waste (WWTP)	Surface water/river
Laube et al. (2014)	Germany	0.15 km	Animal agriculture (Poultry)	Air
Leclercq et al. (2013)	France	4 km	Human waste (WWTP)	Surface water/river
Li et al. (2009)	China	35 km	Human waste (WWTP treating water from Penicillin G production plant)	Surface water/river
Li et al. (2010)	China	25 km	Human waste (WWTP receiving waste from oxytetracycline production plant)	Surface water/river
Li et al. (2011)	China	35 km	Human waste (WWTP receiving Penicillin G production wastewater)	Surface water/river
Li, Song, et al. (2015)	China	40 m	Animal agriculture (Swine farm)	Surface water/river
Li, Atwill, et al. (2015)	United States	Not reported	Animal agriculture (Dairy farm)	Groundwater
Marinescu et al. (2015)	Romania	1.2 km	Human waste (WWTP)	Surface water/river
Marti et al. (2014)	Spain	200 m	Human waste (WWTP)	Biofilm, sediment/river
Mondragón et al. (2011)	Mexico	Not reported	Human waste (Sewage site)	Surface water/river
Oberle, Capdeville, Berthe, Budzinski, and Petit (2012)	France	4 km	Human waste (WWTP)	Surface water/river
Oh et al. (2009)	South Korea	Not reported	Human waste (WWTP)	Surface water/river
Osińska, Harnisz, and Korzeniewska (2016)	Poland	1.2 km	Human waste (WWTP)	Surface water/river
Reinthaler et al. (2003)	Austria	200 m	Human waste (WWTP)	Surface water/river
Rees et al. (2015)	Canada	10 km	Human waste (Urban area)	Shellfish/river

(Continues)

TABLE 1 (Continued)

Citation	Country/s	Spatial scale	Source type	Environmental media/system
Sadowy and Luczkiewicz (2014)	Poland	37 km	Human waste (WWTP)	Surface water/river, ocean
Sapkota et al. (2007)	United States	500 m	Animal agriculture (Swine farm)	Groundwater, surface water/river
Schreiber and Kistemann (2013)	Germany	0.16 km	Human waste (WWTP)	Surface water/river
Sidrach-Cardona, Hijosa-Valsero, Marti, Balcázar, and Becares (2014)	Spain	1.5 km	Human waste (WWTP, Antibiotic-production plant)	Sediment, surface water/river
Sulzner et al. (2014)	United States	450 km	Animal agriculture (Sheep flock)	Wildlife (Turkey vultures)
Suzuki et al. (2013)	Japan	12 km	Human waste (Urban area)	Surface water/river
Topić Popović et al. (2015)	Croatia	20 km	Human waste (WWTP)	Surface water/river
von Salviati et al. (2015)	Germany	150 m	Animal agriculture (Swine farm)	Air
West et al. (2011)	United States	2–3 km	Human waste (WWTP)	Surface water/river
Xu, Chen, Wang, Yang, and Zhao (2012)	China	Not reported	Human waste (WWTP)	Surface water/river
Yao et al. (2011)	China	50 km	Animal agriculture (Swine farm)	Soil
Zhang et al. (2015)	China	17.6 km	Human waste (WWTP)	Surface water/river

downstream sites from a WWTP with one-way ANOVA (Marinescu et al., 2015).

Of these nine studies, six found a significant relationship for some of their comparisons (Akiyama et al., 2010; Koczura et al., 2012; Kotlarska et al., 2015; Li et al., 2010; Marinescu et al., 2015; Sapkota et al., 2007), and three of them did not (Schreiber & Kistemann, 2013; Sulzner et al., 2014; West et al., 2011). The study that used a mixed-model approach to describe the effect of multiple point sources on the proportion of resistant *E. coli* in shellfish found that resistant isolates were significantly closer to point sources compared to non-resistant isolates (Rees et al., 2015).

In the section that follows, results are summarized for each group of point sources investigated (human waste and animal agriculture) and by the type of comparison made (upstream vs. downstream or upwind vs. downwind in unidirectional systems or based on distance from the source).

3.1 | Human waste (*n* = 35)

From the 35 studies evaluating human waste, 26 assessed WWTP; three evaluated the effluent from hospitals, three urban areas and three evaluated sewage sites (one of them defined as a leaky sewer).

Among the 26 that assessed WWTP, three compared ARB outcomes based on distance and the rest (*n* = 23) in unidirectional systems. Of the three studies based on distance, evidence was inconclusive, meaning these studies did not find a clear impact of the source on environmental ARB levels (Czekalski, Berthold, Caucci, Egli, & Bürgmann, 2012; Kotlarska et al., 2015; Sadowy & Luczkiewicz, 2014). Of the studies in unidirectional systems, eight reported higher prevalence/proportion downstream compared to

upstream, one study reported higher levels upstream compared to downstream (Zhang et al., 2015), and the remaining (*n* = 14) reported no association or clear trend.

From the three studies that evaluated the effluent from hospitals, two were in unidirectional systems (i.e. rivers) and one was based on distance from the source (hospital). From the two studies in rivers, one reported a higher proportion of resistant isolates downstream compared to upstream (Fuentefria et al., 2008), and the other reported conflicting evidence (Fuentefria, Ferreira, & Corção, 2011). The study based on distance showed no evidence between distance to hospitals and concentration of methicillin-resistant *Staphylococcus aureus* (MRSA) in soil samples (Alharbi, 2012). Of the three studies assessing urban areas, one evaluated the effect of multiple point sources (Rees et al., 2015). This study, based on distance, found that resistant isolates were significantly greater closer to point sources. The other two studies reported inconclusive evidence for an effect of the urban area on the prevalence of ARB in unidirectional systems (Fincher, Parker, & Chauret, 2009; Suzuki, Kajii, Nishiyama, & Iguchi, 2013). One study evaluated the impact of a sewage dump and found higher levels downstream compared to upstream (Goni-Urriza et al., 2000), and one study evaluated a sewage site (Mondragón et al., 2011), reporting inconclusive evidence. One study that evaluated the impact of a leaky sewer on groundwater based on distance found no evidence for increase in ARB levels.

3.2 | Animal agriculture (*n* = 12)

Of these 12 studies, three assessed aquaculture and nine terrestrial agriculture. Among the three studies that assessed the impact of aquaculture, two were conducted in unidirectional systems (i.e. rivers),

and one was based on distance from a fish farm. Specifically, Harnisz et al. (2015) found inconclusive evidence for the prevalence of resistant heterotrophic bacteria between upstream and downstream sites from a freshwater trout farm (Harnisz et al., 2015). Gordon et al. (2007) found a higher proportion of resistant *Aeromonas* spp. downstream compared to upstream from freshwater fish farms (Gordon et al., 2007). Kerry et al. (1995) found a higher proportion of resistant bacteria in sites closer to the farms compared to sites farther away (Kerry et al., 1995).

For terrestrial agriculture (*n* = 9), three studies made comparisons based on distance and the remaining (*n* = 6) were made in unidirectional systems. Among the studies based on distance, Li, Atwill, et al. (2015) found no evidence of impact of dairy farms on the prevalence of resistant *E. coli* (Li, Atwill, et al., 2015). Sulzner et al. (2014) found a higher prevalence (albeit not significant) in turkey vultures closer to a sheep flock compared to a site far away (Sulzner et al., 2014); and Yao et al. (2011) reported a higher proportion of resistant *Enterobacteriacea* in sites closer to a swine farm compared to sites more distant (Yao et al., 2011). For the studies that made their comparisons in unidirectional systems, von Salviati, Laube, Guerra, Roesler, and Friese (2015) reported no difference in the proportion of ESBL between upwind and downwind from swine barns (von Salviati et al., 2015), while Anderson, McCauley, Lewis, and Liao (2014) showed no trend for counts of tetracycline-resistant *E. coli* between upstream and downstream sites from a poultry processing plant (Anderson et al., 2014). Laube, Friese, Salviati, Guerra, and Rosler (2014) showed a potential trend of higher proportion of *E. coli* harbouring bla^{TEM-1} downwind from broiler chicken farms compared to upwind samples (Laube et al., 2014). Li, Atwill, et al. (2015) reported higher proportion of ESBL downstream compared to upstream from a swine farm (Li, Song, et al, 2015). Sapkota et al. (2007) reported mixed findings: they found no trend for the proportion of resistant *Enterococcus* spp. in groundwater upgradient and downgradient from a swine farm, but found a higher proportion of resistant *Enterococcus* spp. in surface water in downstream sites compared to upstream sites for most of the antibiotics tested (Sapkota et al., 2007). Finally, Hsu et al. (2014) found an effect of a swine farm on the prevalence of resistant heterotrophic bacteria both in groundwater and in surface water (Hsu et al., 2014). Also, among the resistant heterotrophic isolates, they found a possible effect of the swine farm on relative abundance for *sul1* and *sul2* genes. For more details on the results for individual studies, refer Tables 2 and 3 (human waste and animal agriculture, respectively).

4 | DISCUSSION

The available evidence on the impact of point sources on increases in ARB in the natural environment was evaluated and synthetized in this systematic review. Over the past decade, the number of studies looking at the role of the natural environment on the spread of ARB has increased dramatically. This is reflected in the fact that the highest number of articles that were included in the final pool of this systematic review was published in 2015.

Despite an increase in studies in the last few years assessing ARB in the environment, our specific goal was to identify those studies that were able to measure an effect (or impact) using ecological or epidemiological approaches/tools. However, most studies were considered to be unclear for risk of bias, primarily because these studies did not provide enough information about potential confounders (especially the introduction of ARB, ARG and/or antibiotics from other sources in the same system) that could have biased the relationship between proximity to a point source and prevalence/concentration of ARB in the environment. Studies that evaluated sources in a large spatial scale and did not address the influence from additional sources in that scale were most likely to be considered at high risk of bias. With larger spatial scales, there is a more plausible introduction of ARB, ARG or antibiotics from sources other than the source of interest into the system, which would influence the results.

As mentioned in the Materials and Methods section, the risk of bias assessment was conducted in a different order from what the protocol had established. However, the authors do not believe this affected or biased the process given that all studies, including the ones considered at high risk of bias, were included in the data extraction, and different reviewers evaluated the same study at the different screening stages. Additionally, although the risk of bias assessment used a qualitative rubric and the choice of bias category (low, unclear, high) was based on reviewer judgement, the quality of designations was assured by consensus conflict resolution process between the three reviewers.

Wastewater treatment plants were the most common point source evaluated. They have been more extensively studied and have been identified as places of antibiotic resistance emergence due to the favourable conditions and the mixing of bacteria, nutrients and antibiotics (Bouki, Venieri, & Diamadopoulos, 2013; Rizzo et al., 2013). However, there are still knowledge gaps and concern about the fate of antibiotics and ARB in WWTP and how that might ultimately impact human health and ecological processes (Kim & Aga, 2007).

In this systematic review, we found that most studies reported higher levels of ARB in sites downstream from the WWTP compared to upstream sites, or near the WWTP compared to sites far away. However, the evidence documented in this systematic review was primarily descriptive and often did not fully account for possible alternate explanations, and thus, it is not possible to quantify the impact of point sources on increases in ARB in the environment.

Studies evaluating animal agriculture as the point source were less abundant than human waste studies. Although animal agriculture (both terrestrial and aquaculture) point sources were documented to disseminate ARB into the natural environment, there was inconclusive evidence of an impact of these sources on increased levels of ARB in environmental media. As antimicrobial use practices change in animal agricultural settings (FDA, 2015), longitudinal studies at consistent sites would be helpful to determine whether changes in ARB emission from specific point sources and associated effects on the levels of ARB in the receiving environment can be observed.

TABLE 2 Findings for the studies included in this systematic review that assessed human waste (WWTP, industrial, urban areas) as a point source, organized by risk of bias (from low to high) ($n = 35$)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Akiyama and Savin (2010)	Proportion of antibiotic-resistant *E. coli* isolated from river surface water and from sediment 20 m upstream (M1) compared to 640 m downstream (M2), and 2,000 m downstream (M3) of a WWTP discharge point over a 2-year sampling campaign	Low (small spatial scale)	Disc diffusion (CLSI)/ three antibiotics (1st year of sampling), six antibiotics (2nd year)	In surface water, significantly higher proportion of resistant *E. coli* downstream (M2, M3) compared to M1 during the 1st year of sampling. In the 2nd year, proportion at M2 significantly higher than at M1 only one-third of sampling times, and M3 not significantly different than M1. In sediment, significantly higher proportion at M2 compared to M1 for ampicillin and trimethoprim, but higher at M1 compared to M2 for tetracycline; for sulfamethoxazole, there was no difference. Statistical inference conducted using split-plot ANOVA ($p < .05$). Effect estimates were not provided
Akiyama and Savin (2010)	Proportion of antibiotic-resistant total coliforms isolated from river surface water and sediment 20-m upstream (M1) compared to 640-m downstream (M2), and 2,000 m downstream (M3) of a WWTP discharge point over a 2-year sampling campaign	Low (small spatial scale)	Disc diffusion (CLSI)/ three antibiotics (1st year of sampling), six antibiotics (2nd year)	In surface water, no significant difference between upstream and downstream. In sediment, higher proportion at M2 compared to M1 for sulfame-thoxazole and trimethoprim-resistant coliforms, but no significant difference for ampicillin and tetracycline. Statistical inference conducted using split-plot ANOVA ($p < .05$). Effect estimates were not provided
Leclercq et al. (2013)	Proportion of antibiotic-resistant *Enterococci* isolated from river surface water upstream from a WWTP (site 6) compared to a river site immediately downstream from the WWTP (site 5)	Low (small spatial scale)	Disc diffusion (CA-SFM)/12 antibiotics	Overall, higher proportion of resistant bacteria downstream compared to upstream. Effect estimates and accompanying statistical inference were not provided
Li et al. (2010)	Prevalence of resistant heterotrophic bacteria isolated from river surface water 5 km upstream from a WWTP treating water from an oxytetracycline production plant compared to a site 20 km downstream from the WWTP	Low (authors acknowledge the WWTP is the only source in the spatial scale they compared)	Disc diffusion (CLSI)/10 antibiotics	Significantly higher prevalence at downstream site compared to upstream site (Mann–Whitney U test, $p < .01$). Effect estimates were not provided
Marti et al. (2014)	Proportion of resistant *Pseudomonas* spp., *Cytophage–Flavobacterium* spp., and coliforms isolated from river sediment and biofilm collected over 2 campaigns (June and September) 100 m upstream from a WWTP compared to a site 100 m downstream from the WWTP	Low (small spatial scale)	Disc diffusion (CLSI)/ Ciprofloxacin	For all types of bacteria types in sediment samples: higher proportion downstream in June and higher upstream in September. Same trend in biofilm samples, except for *Pseudomonas* spp. isolates (higher proportion upstream both time periods). Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/Antibiotics	Relevant findings
Osińska et al. (2016)	Prevalence of fluoroquinolone-resistant bacteria (FQRB) isolated from river surface water 600 m upstream from a WWTP compared to resistant isolates at 600 m downstream from the WWTP	Low (small spatial scale)	Disc diffusion (EUCAST)/nine antibiotics	Higher prevalence downstream compared to upstream (40% vs. 32%). Effect estimates and accompanying statistical inference were not provided
Schreiber and Kistemann (2013)	Proportion of resistant *Rhodospirillaceae* isolated from surface water 80 m upstream compared to 80 m down-stream from a WWTP	Low (small spatial scale)	Disc diffusion (DIN 58940-3)/nine antibiotics	Resistance in *Rhodospirillaceae* did not appear to be associated with wastewater discharge. There was no statistical correlation between resistance levels at the sampling sites and influence of wastewater (Cramer V correlation). Effect estimates were not provided
Sidrach-Cardona et al. (2014)	Prevalence of resistant *E. coli* isolated from river surface water and from sediment 50 m upstream from a WWTP (site 3), a site immediately downstream from the WWTP (site 4), a site about 50 m downstream (site 5) and a site 500 m downstream from the WWTP (site 6)	Low (unlike introduction of any type of bias)	Disc diffusion (NCCLS)/seven antibiotics	For most antibiotics, prevalence of resistant *E. coli* was higher at downstream sites compared to upstream sites in both sediment and water samples for azithromycin, doxycycline, streptomycin and tetracycline. Prevalence was higher upstream compared to downstream for ampicillin in both sediment and water samples. For penicillin and erythromycin, there was a 100% prevalence at all sites in both types of samples. Effect estimates and accompanying statistical inference were not provided
Sidrach-Cardona et al. (2014)	Prevalence of resistant *E. coli* isolated from river surface water and from sediment 50 m upstream from an antibiotic-production plant (APP) and at a site 100 m downstream from the APP	Low (unlike introduction of any type of bias)	Disc diffusion (NCCLS)/seven antibiotics	Prevalence of resistant *E. coli* in water samples was either higher downstream, or the same for both sites except for doxycycline, tetracycline and streptomycin, where it was higher upstream. In sediment samples, prevalence tended to be either higher or the same upstream and downstream, except for doxycycline. Effect estimates and accompanying statistical inference were not provided
Alharbi (2012)	Concentration of *MRSA* isolated from soil compared at different distances (10 m, 500 m and 2,000 m) from hospital facilities in both Saudi Arabia and the UK	Unclear (not enough information provided about sample collection and potential influence of other sources)	Disc diffusion/(not reported)/Methicillin	Qualitative analysis did not suggest a relationship between distance to the hospital facilities and concentration of *MRSA* in soil samples at any of the sites. Effect estimates and accompanying statistical inference were not provided
Amador et al. (2015)	Proportion of resistant *Enterobacteriaceae* bacteria isolated from river water collected 500 m downstream (DRW) compared to 500 m upstream (URW) of WWTP discharge	Unclear (lack of information regarding other potential sources)	Disc diffusion (CLSI)/13 antibiotics	Evidence is conflicting for an impact of the WWTP on the proportion of resistant *Enterobacteriaceae* in river water based on qualitative comparison. Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Czekalski et al. (2012)	Proportion of several types of resistant bacteria isolated from lake sediment and lake surface water immediately adjacent to STEP (WWTP outfall site) compared to measurements at DP (3.2 km away from STEP)	Unclear (insufficient information about possible confounding due to lake depth)	Disc diffusion (DIN, EUCAST)/Three combinations of antibiotics at inhibitory concentrations: (i) sulfamethoxazole, trimethoprim and streptomycin (ii) norfloxacin and ceftazidime, (iii) clarithromycin and tetracycline	In surface water, the proportion resistant for all bacteria type was low (<1%) or absent at both lake sites. In sediment, the proportion resistant was low (<1%) or absent at both lake sites except for antibiotic combination a), but the evidence of any effect was inconclusive. Effect estimates and accompanying statistical inference were not provided
Fincher et al. (2009)	Prevalence of resistant *E. coli* O157:H7 isolated from river surface water compared between the most upstream site (site 1), a site just upstream from a city (site 2), and downstream from the city (site 3)	Unclear (not enough information provided about the potential impact from farms near the city)	Disc diffusion (manufacturer)/eight antibiotics	There was little support for an effect of the city on the prevalence of resistant *E. coli* 0157:H7 in water samples. At site 3, there were 2/21 (9.5%) isolates that showed resistance to three antibiotics at the same time compared to 0 at site 2 and 1/21 (4.8%) at site 1. For isolates resistant to two antibiotics, site 3 had 9.5% compared to 0 at the upstream sites. For isolates resistant to just 1 antibiotic, there was a 9.5% prevalence at site 2 compared to 0 at sites 1 and 3. Effect estimates and accompanying statistical inference were not provided
Fuentefria et al. (2008)	Proportion of resistant *Pseudomonas aeruginosa* isolated from surface water compared between point B (upstream from a hospital) and point C (downstream from the hospital effluent). No distance between these sites was reported	Unclear (no information provided about distance between sampling locations)	Disc diffusion/(CLSI) 11 antibiotics	Overall, proportion of resistant *Pseudomonas aeruginosa* was higher downstream compared to upstream (55.6% vs. 22.7%). Effect estimates and accompanying statistical inference were not provided
Fuentefria et al. (2011)	Proportion of resistant *Pseudomonas aeruginosa* isolated from surface water compared between a site 800 m upstream (W1) and 600 m downstream (W2) from the discharge of HSVP hospital	Unclear (not enough information provided about other potential sources)	Disc diffusion (CLSI)/11 antibiotics	Higher proportion of resistant *Pseudomonas aeruginosa* upstream compared to downstream for imipenem (20.8% vs. 5.5%), but higher downstream compared to upstream for meropenem (22.2% vs. 0%). Not detected for the other antibiotics. Effect estimates and accompanying statistical inference were not provided
Fuentefria et al. (2011)	Proportion of resistant *Pseudomonas aeruginosa* isolated from surface water compared between a site 100 m upstream (W3) and 100 m downstream (W4) from HDP hospital	Unclear (not enough information provided about other potential sources)	Disc diffusion (CLSI)/11 antibiotics	Higher prevalence upstream compared to downstream for both imipenem (97.7% vs. 96.7%) and meropenem (2.3% vs. 0%). Not detected for the other antibiotics. Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Gallert et al. (2005)	Proportion of resistant *Enterococci*, faecal coliforms and *Pseudomonads* isolated from groundwater down-stream from a leaky sewer at different distances from the source (1, 1.8, 2, 15 m) and at 410 m away (reference site)	Unclear (not enough information provided about other potential sources)	Disc diffusion (guidelines not reported)/14 antibiotics	No evidence for an effect of the leaky sewer on the proportion of any of the types of resistant bacteria by distance. Effect estimates and accompanying statistical inference were not provided
Harnisz (2013)	Prevalence of resistant heterotrophic bacteria isolated from surface water 600 m upstream from a WWTP compared to a site 600 m downstream from the WWTP	Unclear (not enough information provided about other potential sources)	Disc diffusion (CLSI)/six antibiotics	All resistant isolates showed higher prevalence downstream compared to upstream except for CEF^R (28% downstream vs. 47% upstream). ENR^R and DOX^R were barely detected. Effect estimates and accompanying statistical inference were not provided
Koczura et al. (2012)	Proportion of resistant *E. coli* isolated from river surface water upstream from a WWTP compared to a downstream site from the WWTP (no specific distance reported)	Unclear (not enough information provided about the spatial scale of the study)	Disc diffusion (CLSI)/27 antibiotics	For most resistant *E. coli* isolates, there was a higher proportion downstream compared to upstream except for isolates resistant to amikacin and streptomycin that had a higher prevalence upstream compared to downstream. For some antibiotics, there was a statistically significant relationship (Pearson chi-square test, $p < .05$), but effect estimates were not provided
Li et al. (2009)	Prevalence of resistant heterotrophic bacteria isolated from river surface water at a point downstream 30 km from a WWTP treating water from a Penicillin G production plant, and an upstream point 5 km from the WWTP	Unclear (large spatial scale but authors acknowledge being not aware of any other point sources in that area)	Disc diffusion (CLSI)/18 antibiotics	Prevalence downstream higher than upstream for all antibiotics except for ciprofloxacin and levofloxacin. Effect estimates and accompanying statistical inference were not provided
Li et al. (2011)	Proportion of resistant isolates from different bacteria phyla from river surface water 5 km upstream compared to a site 30 km downstream from a WWTP treating penicillin G wastewater	Unclear (not enough information provided about potential effect of other sources)	Disc diffusion (guidelines not reported)/Ampicillin	Higher proportion found downstream compared to upstream (about 65% of isolates resistant to ampicillin vs. <10% upstream). Effect estimates and accompanying statistical inference were not provided
Li et al. (2011)	Proportion of resistant isolates from different bacteria phyla at an upstream site (5 km from a WWTP that receives oxytetracycline production wastewater) compared to a downstream site (20 km from the WWTP)	Unclear (not enough information provided about potential effect of other sources)	Disc diffusion (guidelines not reported)/ oxytetracycline	Higher proportion found downstream compared to upstream (about 55% of isolates resistant to oxytetracy-cline vs. <5% upstream). Effect estimates and accompanying statistical inference was not provided
Oberle et al. (2012)	Proportion of resistant *E. coli* isolated from river surface water at a site upstream from a WWTP compared to a river site immediately downstream from the WWTP discharge point	Unclear (not enough information provided about potential effect of agricultural sources and untreated waste in the area)	Disc diffusion (French National Guidelines)/11 antibiotics	Higher proportion of resistant *E. coli* downstream compared to upstream for isolates that were resistant to at least 1 antibiotic (26% vs. 22.2%) and for multidrug resistance (14% vs. 11.1%). Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Reinthaler et al. (2003)	Proportion of resistant *E. coli* isolated from river surface water at a site 100 m upstream from a WWTP (A) compared to a site 100 m downstream from WWTP (A)	Unclear (no information provided about locations and potential influence from other sources)	Disc diffusion (CLSI)/24 antibiotics	Proportion of resistant *E. coli* higher upstream compared to downstream for the majority of antibiotics except for tetracycline and trimethoprim/ sulfamethoxazole, where the proportion was higher downstream compared to upstream. Effect estimates and accompanying statistical inference were not provided
Reinthaler et al. (2003)	Proportion of resistant *E. coli* isolated from river surface water at a site 100 m upstream from a WWTP (B) compared to a site 100 m downstream from WWTP (B)	Unclear (no information provided about locations and potential influence from other sources)	Disc diffusion (CLSI)/24 antibiotics	Proportion of resistant *E. coli* higher downstream compared to upstream for the majority of antibiotics except for piperacillin, nalidixic acid, tetracycline and nitrofurantoin, where proportion was higher upstream compared to downstream. Effect estimates and accompanying statistical inference were not provided
Reinthaler et al. (2003)	Proportion of resistant *E. coli* isolated from river surface water at a site 100 m upstream from a WWTP (C) compared to a site 100 m downstream from WWTP (C)	Unclear (no information provided about locations and potential influence from other sources)	Disc diffusion (CLSI)/24 antibiotics	No clear evidence for an impact of WWTP in river samples, being the proportion of resistant *E. coli* similar upstream and downstream. Effect estimates and accompanying statistical inference were not provided
Rees et al. (2015)	Proportion of resistant *E. coli* isolated from shellfish at different sampling points as the continuous exposure defined as standardized seaway distance to the nearest human point source	Unclear (not enough information about the other sources in the area)	Microdilution (CLSI)/14 antibiotics	In general, the mixed-model found that ARB positive isolates had a lower proportion of agricultural land adjacent compared to non-ARB isolates. Overall, resistant isolates were significantly closer to human point sources. Mean seaway distance to nearest human source in ARB isolates (and standard deviation) was 1247 ± 802.0 (n = 6), and the mean seaway distance in non-resistant isolates was as follows: 4286.6 ± 3302.1 (n = 16). The model accounted for the proportion of surrounding land in agricultural use and for correlation due to sampling, site, month and shellfish species. Effect estimates were not provided
Sadowy and Luczkiewicz (2014)	Prevalence of resistant *Enterococcus faecalis* isolated from surface water from a site near the marine outfall of a WWTP compared to a river site (farther from the WWTP)	Unclear (not enough information about other potential sources)	Disc diffusion (CLSI)/11 antibiotics	Prevalence of resistant *E. faecalis* higher at the marine outfall site compared to the river site, except for quinupristin–dalfopristin (prevalence at the river site higher). Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Sadowy and Luczkiewicz (2014)	Prevalence of resistant *Enterococcus faecium* isolated from surface water from a site near the marine outfall of a WWTP compared to a river site (farther from WWTP)	Unclear (not enough information about other potential sources)	Disc diffusion (CLSI)/11 antibiotics	There was little support for an effect of the WWTP on the prevalence of resistant *E. faecium*. There were no resistant isolates for most of the antibiotics tested at both sampling sites. Only ciprofloxacin (not detected at the marine outfall and 40% prevalence at the river site) and erythromycin (100% marine site vs. 20% river site) were detected. Effect estimates and accompanying statistical inference were not provided
Topić Popović et al. (2015)	Proportion of resistant heterotrophic bacteria isolated from river surface water compared between a site downstream (site 9) from a WWTP and a reference site upstream from the WWTP (site 1)	Unclear (not enough information provided about sampling design and influence of confounders)	Disc diffusion (manufac-turer's guidelines)/ Oxytetracycline	There were no resistant isolates in site 1 (reference) and 100% resistant isolates in site 9 (downstream). Effect estimates and accompanying statistical inference were not provided
West et al. (2011)	Prevalence of resistant faecal coliforms isolated from surface water upstream and downstream from a WWTP (1–1.5 km from the WWTP in each direction)	Unclear (information provided about presence of other sources but unknown impact at the spatial scale of the study)	Disc diffusion (Study defined)/5 antibiotics	For ampicillin resistance, prevalence of resistant coliforms (%) was higher downstream compared to upstream. For MDR, prevalence (%) was lower downstream compared to upstream. However, neither of these differences were statistically significant (Fisher's exact test; α = 0.05). Effect estimates were not provided
Xu et al. (2012)	Proportion of resistant coliform bacteria isolated from river surface water upstream and downstream from a WWTP (no distance reported)	Unclear (not enough information provided about spatial scale)	Microbroth dilution (CLSI)/7 antibiotics	Higher proportion downstream compared to upstream for all antibiotics tested, except for ciprofloxacin. Effect estimates and accompanying statistical inference were not provided
Zhang et al. (2015)	Proportion of resistant faecal coliforms isolated from river surface water from a downstream site (site E) compared to an upstream site (site D), 200 m upstream from a WWTP	Unclear (site D likely heavily influenced by other urban outfalls of sewage and storm water, but not enough information provided)	Plating method (CLSI)/ Tetracycline	Qualitatively, proportion of tet-resistant coliforms lower downstream compared to upstream. Effect estimates and accompanying statistical inference were not provided
Zhang et al. (2015)	Among phenotypically tetracycline-resistant faecal coliforms isolated from river water, proportion of isolates with selected resistance genes from a site downstream (E) of a WWTP compared to proportion at site 200-m upstream (site D)	Unclear (site D likely heavily influenced by other urban outfalls of sewage and storm water, but not enough information provided)	*tetA* *tetB* *tetC* *tetG* *tetK* *tetM* *tetO* *tetQ* *tetX*	Qualitatively, evidence did not support an effect of the WWTP on the proportion of tetracycline-resistant faecal coliforms carrying specific resistance genes downstream. No apparent difference in proportion of isolates carrying *tetA*, *tetC*, *tetQ*, *tetX* between downstream and upstream sites. Proportion carrying *tetB*, *tetK* lower downstream. Results for *tetG*, *tetM*, *tetO* not reported. Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Abia et al. (2015)	Prevalence of resistant *E. coli* isolated from river surface water compared across several sites: DAS (downstream of Daspoort WWTP), AP1 (downstream of DAS), AP6 (downstream of AP1), A5 (downstream of AP6—tributary), A4 (downstream of A5), A3 (downstream of A4), Rooiwal WWTP (downstream of AP1), AP2 (downstream of Rooiwal WWTP), AP7 (downstream of AP2 and upstream of Temba WWTP), Babelegi WWTP, AP8 (downstream of Babelegi) and AP9 (downstream of AP8)	High (large spatial scale with several WWTP and other sources present; no control for confounding)	Disc diffusion (CLSI)/ Chloramphenicol	No clear trend found. Effect estimates and accompanying statistical inference were not provided
Blaak et al. (2014)	Prevalence of ESBL-producing *E. coli* isolated from surface water upstream from a WWTP, at the discharge point, and downstream from four different WWTP in four different regions, each one of the WWTP located 1–2 km from the downstream site	High (likely influence from other sources)	Disc diffusion (CLSI)/12 antibiotics	Across the four regions, prevalence was highest at the upstream sites compared to the discharge points and to the downstream sites for five antibiotics: ceftazidime, ciprofloxacin, tetracycline, streptomycin and chloramphenicol. Prevalence was highest at the discharge point compared to upstream and downstream sites for nalidixic acid, gentamycin, trimethoprim and sulfisoxazole. Prevalence was highest at the downstream site compared to the discharge point and the upstream site for co-amoxiclav, and the same prevalence (100%) at the downstream and discharge point for ampicillin (vs 98% for the upstream site), and a 100% at all sites for cefotaxime. Across the four regions, the prevalence of MDR among ESBL-producing isolates was: 63% at the WWTP discharge point; 62% upstream; and 41% at the downstream. Effect estimates and accompanying statistical inference were not provided
Goni-Urriza et al. (2000)	Proportion of resistant *Enterobacteriacea* isolated from river surface water compared between a sewage dump (Kp 0), a site upstream (Azazuri, at 0.5 km), and 5 sites downstream at 10, 16, and 30 km distance	High (likely influence from other sources)	Disc diffusion (French National guidelines)/22 antibiotics	Higher proportion downstream compared to upstream. Greatest increase with beta-lactams (0% upstream to 20.5% downstream) and tetracycline (12.5% to 24.3%). Effect estimates and accompanying statistical inference were not provided
Goni-Urriza et al. (2000)	Proportion of resistant *Aeromonas* spp. isolated from river surface water compared between a sewage dump (Kp 0), a site upstream (Azazuri, at 0.5 km) and 5 sites downstream. at 10, 16 and 30 km distance	High (likely influence from other sources)	Disc diffusion (French National guidelines)/22 antibiotics	Higher prevalence downstream compared to upstream. Tetracyclines had the highest difference (0% to 27.5%) and cotrimoxazole (0–26.6%). Effect estimates and accompanying statistical inference were not provided

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Kotlarska et al. (2015)	Prevalence of resistant *E. coli* isolated from surface water from a WWTP discharge point (marine outflow) of WWTP (A) compared to a river site 2.5 km away	High (river likely impacted by numerous upstream activities)	Microdilution (EUCAST)/17 antibiotics	For most antibiotics, marine outflow showed higher prevalence of resistant *E. coli* than at the river site, and it was statistically significant for ampicillin, trimethoprim–sulfamethoxazole, ciprofloxacin, and levofloxacin (Fisher's exact test, $p < .05$). Although non-statistically significant, there was a higher prevalence at the river site compared to the marine outflow for gentamicin and cefazolin. Effect estimates were not provided
Laroche et al. *(2009)*	Proportion of resistant *E. coli* isolated from river surface water upstream (KP202) compared to a site about 30 km downstream (KP 260) from a WWTP pooled across several time points	High (several other WWTP and other point sources that could influence the results)	Disc diffusion (French National guidelines)/16 antibiotics	There was a higher proportion of resistant *E. coli* downstream compared to upstream. For resistance to at least one antibiotic, the geometric mean across all sampling times was 49.6% (31.0–68.7) at KP260 (downstream) and 30.2% (16.0–50.0) at KP202 (upstream). For resistance to more than 1 antibiotic, the geometric mean of all sampling times was 28.1% at KP260 and 21.1% at KP202. Effect estimates and accompanying statistical inference were not provided
Marinescu et al. (2015)	Proportion of resistant Gram-negative bacteria (several species but not differentiated in the analysis by sites) isolated from river surface water 1,000 m upstream and 200 m downstream from a WWTP	High (pooling lac+ and lac- isolates seems likely to introduce bias if lac +/− status is associated both with the particular sampling site and with resistance)	Disc diffusion (CLSI)/18 antibiotics	Higher proportion in the downstream site compared to the upstream site for all antibiotics, some of them statistically significant by one-way ANOVA ($p < .001$). There was no detection of resistant bacteria to ticarcillin and aztreonam. No effect estimates provided
Marinescu et al. (2015)	Among phenotypically resistant Gram-negative bacterial isolates (predominantly *Enterobacteriaceae*) from river water, proportion of isolates hosting selected resistance genes from river water sampled at a site 200 m downstream of a WWTP compared to a site 1,000 m upstream	High (Pooling lac+ and lac- isolates seems likely to introduce bias if lac +/− status is associated both with the particular sampling site and with resistance)	bla^{TEM} bla^{SHV} bla^{CTX-M} bla^{CMY} bla^{NDM} bla^{VIM} bla^{IMP} *dfrA1-aadA1* *intI1* *qnrA* *qnrB* *qnrS* *sul1* *sul2* *tetA* *tetB* *tetC* *tetD* *tetM*	Overall, there was some evidence for an association between WWTP impact and mechanism of resistance for some genes. For beta-lactamase genes, bla^{NDM} and bla^{CMY} were detected in 100% and 25%, respectively, of isolates that exhibited resistance to beta-lactams from the downstream samples compared to 0% isolates upstream, while the remaining *bla*-type genes were not detected in isolates at either site. For non-beta-lactamase genes, *tetD*, *sul1* and *qnrB* were detected in 100%, 20% and 100%, respectively, of isolates that exhibited acquired resistance phenotypes from the downstream samples compared to 0% isolates upstream, while the remaining genes were not detected in isolates at either site. Effect estimates and accompanying statistical inference were not provided for these comparisons

(Continues)

TABLE 2 (Continued)

Citation	Relevant comparison	Overall risk of bias	Detection method/ Antibiotics	Relevant findings
Mondragón et al. (2011)	Proportion of resistant *Enterococcus* spp. isolated from river surface water at a reference site (AT) and at a downstream site (PP) from the point source (treated sewage site, PL)	High (likely influence of other sources)	Disc diffusion (CLSI)/5 antibiotics	Higher proportion of resistant *Enterococcus* spp. downstream compared to the reference site for ampicillin, vancomycin and gentamicin, but higher at the reference site for kanamycin and 100% resistant for ciprofloxacin at both sites. Effect estimates and accompanying statistical inference were not provided
Oh et al. (2009)	Proportion of resistant oligotrophic and heterotrophic bacteria isolated from river surface water upstream (US1, US2) and downstream (DS1, DS2, DS3) from a WWTP	High (large spatial scale)	Disc diffusion, two different nutrients (R2A and LB), CLSI/ Tetracycline, Vancomycin	For tetracycline, there was no detection of resistant isolates. For vancomycin, there was a difference in the proportion depending on the nutrient used. For R2A, the proportion was highest at DS2 and lowest at US1. For LB, the proportion was highest at US1 and lowest at DS2. Effect estimates and accompanying statistical inference were not provided
Suzuki et al. (2013)	Proportion of resistant *Pseudomonas aeruginosa* isolated from surface water upstream, midstream (near a city), and downstream from the city (no distances specified)	High (upstream site influenced by other sources)	Microbroth dilution (CLSI)/10 antibiotics	For most antibiotics, the proportion of resistant *Pseudomonas aeruginosa* was 0 at all sites. For tetracycline and minocycline, the proportion was a 100% at all sites. For cefotaxime, there was a higher proportion upstream than midstream, and it was not detected downstream. For ceftazidime, the proportion was 2.6% downstream and there was no detection at the other sites. Effect estimates and accompanying statistical inference were not provided

WWTP, Wastewater treatment plant.

Across all studies in the review, resistant *E. coli* was the most frequently studied bacterium. This is not surprising given that this bacterium has been widely studied (including as a faecal indicator), some strains have the potential to be pathogenic given their versatility, broad host range and diversity of virulence factors, and some resistant strains are included among the most relevant antibiotic-resistant infections according to the CDC threat list (Blount, 2015; Kaper, Nataro, & Mobley, 2004). Sixteen of the 47 studies reported their results using very broad categories for ARB outcome (e.g. Gram-positive bacteria or heterotrophic bacteria). It is unclear whether this was due to the difficulty in identifying the specific bacterium of interest given that <1% of bacteria are culturable (Walsh, 2013) or because the bacterial species was not the focus of the study.

As noted in the results, even those studies that conducted statistical analysis did not report effect measures. Examples of effect measures include odds ratios or prevalence ratios (for ARB prevalence outcomes) and mean differences (for ARB concentration outcomes) (Williams-Nguyen, Bueno, & Singer, 2017). For instance, as a simplified example, the effect of a point source on ARB prevalence in an adjacent waterway could be defined as a prevalence ratio wherein the prevalence of ARB at a site 100 m downstream of the point source (e.g. 50%) is divided by the prevalence of ARB at a site 1,000 m downstream of the point source (e.g. 20%) yielding a ratio of 2.5. In some cases, the goal of the studies was not to look at the impact of a point source on the levels of ARB, but just to describe what was found in the environment. However, to gain an understanding of the relationship between point sources and levels of ARB in the environment, it is critical to incorporate ecological or epidemiological tools that permit quantitative estimates of effect.

Studying antimicrobial resistance in the environment is very challenging given the complexity of the ecological processes involved, the numerous factors that can affect an observed relationship, and the intrinsic difficulties of field work, to name just

TABLE 3 Findings for the studies included in the systematic review that had animal agriculture (both terrestrial and aquaculture) as a point source, presented by risk of bias (from low to high) (n = 12)

Citation	Relevant comparison	Overall risk of bias	ARB/Detection method/Antibiotics	Relevant findings
Harnisz et al. (2015)	Prevalence of resistant heterotrophic bacteria (oxytetracycline OTC^R and doxycycline DOX^{R}isolates) from surface water upstream (URW) and downstream (DRW), about 200 m distance each from a fish farm effluent	Low (small spatial scale)	Disc diffusion (CLSI)/12 antibiotics	For OTC^R, prevalence was higher upstream compared to downstream, except for norfloxacin, enrofloxacin, trimethoprim/sulfamethoxazole and for tetracycline. For DOX^R, prevalence was higher downstream compared to upstream, except for cefotaxime and for those antibiotics where the prevalence was the same at both sites (amoxicillin+clavulanic acid and tetracycline). Effect estimates and accompanying statistical inference were not provided
Li, Atwill, et al. (2015)	Prevalence of resistant *E. coli* isolated from groundwater in wells near-dairy farms (<2.4 km) and from wells far from the farms (non-dairy)	Low (small spatial scale and no other likely sources influencing the comparison in that scale)	Sensititre (CLSI)/Not reported	At the sites near-dairy, from 5 isolates tested, 5 (100%) resistant to 1 antibiotic, 3 isolates MDR; for non-dairy sites, of 4 isolates, 4 resistant to 1 antibiotic, 2 MDR. Effect estimates and accompanying statistical inference were not provided
Li, Atwill, et al. (2015)	Prevalence of resistant *Enterococcus* spp. isolated from groundwater in wells near-dairy farms (less than 2.4 km) and from wells far from the farms (non-dairy)	Low (small spatial scale and no other likely sources influencing the comparison in that scale)	Sensititre (CLSI)/Not reported	At the sites near-dairy, of eight isolates, eight resistant to one antibiotic (100%), eight MDR; for non-dairy sites, six of six resistant to one antibiotic (100%), five MDR. Effect estimates and accompanying statistical inference were not provided
Sapkota et al. (2007)	Proportion of resistant *Enterococcus* spp. isolated from river surface water at one upstream pond (UG SW),about 200 m from a swine farm (SW), compared to a pool of 3 sites downstream: a site near the swine farm (DG SW1), a site 200 m downstream from it (DG SW2), and a site 100 m from SW2 (DG SW3)	Low (small spatial scale)	Disc diffusion (CLSI)/5 antibiotics	Proportion of resistant *Enterococcus* spp. higher downstream compared to upstream for all antibiotics and statistically significant (Fisher's exact test) for erythromycin (p-value: 0.02), except for clindamycin (100% upstream compared to 89% downstream, p-value: 0.76). Effect estimates were not provided
Sapkota et al. (2007)	Proportion of resistant *Enterococcus* spp. isolated from river groundwater at one upgradient well (UG GW), 300 m from SW, and at one downgradient well (DG GW), 200 m from SW	Low (small spatial scale)	Disc diffusion (CLSI)/5 antibiotics	Proportion of resistant *Enterococcus* spp. higher upgradient compared to downgradient for erythromycin (67% vs. 20%, p-value < .001) and vancomycin (10% vs. 0%, p-value: .15; Fisher's exact test). Proportion higher downgradient compared to upgradient for tetracycline (19% vs. 3%, p-value: .07), and clindamycin (100% vs. 0%, p-value < .001). Effect estimates were not provided
Hsu et al. (2014)	Prevalence of resistant heterotrophic bacteria isolated from river surface water at upstream sites (S1 and S2, separated by 200 m) and down-stream sites (S3 and S4) from a swine farm (S2 and S3 with a separation of 400 m)	Low (small spatial scale)	Disc diffusion (Not reported)/Sulphonamide	Prevalence was two orders of magnitude lower in upstream site S2 compared to downstream site S4. Effect estimates and accompanying statistical inference were not provided
Hsu et al. (2014)	Prevalence of resistant heterotrophic bacteria isolated from groundwater upstream (W1, between S1 and S2), near the swine farm (W2), and downstream (W3, located after S4)	Low (small spatial scale)	Disc diffusion (Not reported)/Sulphonamide	The highest prevalence was at W2, 3-fold higher compared to W1 (26.7% vs. 9.2%), followed by the downstream well W3 (19.98%). Effect estimates and accompanying statistical inference were not provided

(Continues)

TABLE 3 (Continued)

Citation	Relevant comparison	Overall risk of bias	ARB/Detection method/Antibiotics	Relevant findings
Hsu et al. (2014)	Among resistant heterotrophic isolates, the relative gene abundance in surface river water sampled at sites immediately (S3) and 400 m (S4) downstream of a swine farm compared to a site 400 m upstream (S2) of the farm. Relative gene abundance was defined: gene copies/16S rRNA gene copies	Low (small spatial scale)	*sul1* *sul2* *sul3*	The evidence suggests a possible effect of the swine farm on relative abundance for some *sul* genes. Relative abundance of *sul1* and *sul2* was greater downstream (S3, S4) compared to upstream; however, quantitative effect measures and statistical inference were not provided. The remaining gene, *sul3*, was not detected at any site
von Salviati et al. (2015)	Proportion of ESBL/AmpC-producing *E. coli* isolated from air samples 100 m upwind and 50 m downwind from swine barns	Low (small spatial scale)	Disc diffusion (CLSI)/six antibiotics (or combinations thereof)	Qualitatively, no difference between upwind and downwind proportion of resistant isolates. Effect estimates and accompanying statistical inference were not provided
Anderson et al. (2014)	Count of tetracycline-resistant *E. coli* isolated from surface water at 7 upstream sites and 4 downstream sites from the effluent of a poultry processing plant at different sampling dates	Unclear (other run-off present in the study area could impact the results but not enough information provided)	Disc diffusion (study defined)/ Tetracycline	High variability by sampling date, no clear trend. Effect estimates and accompanying statistical inference were not provided
Kerry et al. (1995)	Proportion of resistant bacteria (unspecified) isolated from sediment at fish Farm A and B and compared to sites C and D (at least 5 km away)	Unclear (not enough information to determine potential biases)	Disc diffusion (unspecified)/ Oxytetracycline	Proportion of resistant bacteria was higher at the farm sites A and B compared to further away (C and D). Effect estimates and accompanying statistical inference were not provided
Laube et al. (2014)	Proportion of *E. coli* harbouring *bla*^TEM−1 isolated from air samples 50 m downwind compared to 100 m upwind locations at 7 broiler chicken farms pooled across time points	Unclear (Other farms nearby could confound the results)	Disc diffusion (CLSI)/5 antibiotics	Proportion of *E. coli* harbouring *bla*^TEM−1 was 10% and 5 of air samples positive for ESBL/AmpC-producing *E. coli* on the 50 m downwind and the 100 m upwind side, respectively. Effect estimates and accompanying statistical inference were not provided
Li, Song, et al., (2015)	Proportion of ESBL-producing *E. coli* isolated from river surface water 10 m upstream and 20 m down-stream from a swine farm	Unclear (not enough information provided)	Disc diffusion (CLSI)/12 antibiotics	Proportion of ESBL-producing *E. coli* resistant to all 12 antibiotics was higher downstream than upstream (52.9% vs. 22.2%). Effect estimates and accompanying statistical inference were not provided
Sulzner et al. (2014)	Prevalence of resistant *E. coli* isolated from cloacal samples of turkey vultures (*Cathartes aura*) near a sheep flock (HREC) compared to samples distant from sheep at a more remote location (LHBCR)	Unclear (not enough information about other potential exposures for the turkey vultures)	Not reported (Not reported)/10 antibiotics	Overall prevalence of resistant *E. coli* was higher at HREC compared to LHBCR, but not statistically significant (Pearson chi-square; $p < .05$). Effect estimates were not provided
Gordon et al. (2007)	Proportion of resistant *Aeromonas* spp. isolated from river sediment upstream, immediately downstream from a fish farm effluent and at a site 100 m downstream from the fish farm	High (several other potential sources that discharge into the same river near the fish farm and no control for confounding)	Disc diffusion (CA-SFM)/3 antibiotics	No detection of resistant *Aeromonas* spp. upstream compared to a range of 0.01–25.9% at downstream sites (pooling both downstream sites). Effect estimates and accompanying statistical inference were not provided
Yao et al. (2011)	Proportion of resistant *Enterobacteriaceae* isolated from soil within 500 m radius of a swine farm and from a site 50 km away from the farm	High (large spatial scale with many other)	Disc diffusion (CLSI)/Amikacin	Proportion of resistant *Enterobacteriaceae* was higher at the site near the farm compared to the far away site (45.2% vs. 33.9%). Effect estimates and accompanying statistical inference were not provided

> **Box 1** Checklist summarizing recommendations for future AR environmental studies based on the results from the systematic review
>
> - Research question
>   - o Define question with PECO or other appropriate framework
> - Study design
>   - o Select adequate comparison groups
>   - o Maintain consistent sites for longitudinal studies
>   - o Sampling strategy/protocol
>   - o Choose appropriate spatial scale for question and planned data analysis
>   - o Consider temporal window
> - Laboratory analysis
>   - o Combination of molecular tools and culture-dependent methods
> - Data analysis plan and reporting
>   - o Provide detailed information about potential confounders
>   - o Use analytical methods to control for bias when needed (e.g. regression analysis, spatial analysis).
>   - o Report effect measures and measures of variability to quantify the effect of a source on levels of AMR in the environment/quantify the effect of a source on levels of AMR in the environment

a few. To advance the field and improve our understanding of the role of specific point sources on contamination of the natural environment, much more attention must be devoted to careful study design. Then, a combination of statistical methods such as regression analysis, spatial analysis, as well as identification of potential biases and approaches to address those biases to ultimately report effect measures along with measures of variability should be used. Effect measures are critical to be able to quantify the strength of the effect of the point source (an exposure) on the prevalence or concentration of ARB in the environment (the outcome). A summary of recommendations for future AR environmental studies is provided in Box 1.

Given the complexity of studying antimicrobial resistance in the environment, collaboration between ecologists, microbiologists, epidemiologists, soil scientists, hydrologists, civil engineers, agronomists and a variety of other disciplines is needed. Understanding the impact of specific point sources on the levels of ARB and ARG in the environment will be critical to be able to develop effective mitigation strategies to reduce the spread of AMR.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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