Title: Systematic Review of Antibiotic Resistance of Public Health Importance in Environments near Swine Operations, **NPB Project #13-260**

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Industry Summary

Systematic reviews are a rigorous knowledge synthesis technique first developed in the health sciences to summarize information from numerous randomized trials examining the clinical efficacy of an intervention. In contrast to a narrative review, this method permits the evaluation of all available evidence on the question of interest using a standardized process. Because of varied opinions regarding the evidence for sources of antibiotic resistance found in the environment, this app9roach is ideally suited to this type of topic. We conducted a systematic review to address the following question: : Is the prevalence or concentration of antibiotic resistant bacteria (ARB) or resistance genes (ARG) in soil, water, air or free-living wildlife higher in close proximity to, or downstream from, known or suspected sources compared to areas more distant from or upstream from these sources? The total number of studies that were evaluated was 4,524, and from these, 60 were included in the final evaluation (19 with ARG outcome and 41 with ARB outcome). Many of the studies provided little useful information due to a high level of bias in the study design. The systematic review found a large number of studies presenting qualitative evidence that proximity to or direction from point sources, particularly waste water treatment plants, may be associated with higher levels of antibiotic resistance; however, very few studies quantitatively characterize this effect or provide statistical inference to aid in interpretation. This systematic review provides a strong imperative to improve research methods in order to provide interpretable, quantitative information about the effect of point sources on resistance in the environment.

Keywords

Systematic Review; Antibiotic Resistance; Environment; Manure; Waste;

Scientific Abstract

A systematic review was conducted to examine the evidence on whether point sources are associated with an increase in antibiotic resistance in the adjacent environment. The review question was based on the Population, Exposure, Comparator, Outcome (PECO) framework as follows: ***Is the prevalence or concentration of antibiotic resistant bacteria or resistance genes (O) in soil, water, air or free-living wildlife (P) higher in close proximity to, or downstream from, known or suspected sources (E) compared to areas more distant from or upstream from these sources (C)?*** A comprehensive search strategy was created to capture all relevant, published literature. Criteria for two stages of eligibility screening were developed to exclude publications that were not relevant to the question, and determine if the study evaluated the association between a source and levels of resistance using an appropriate comparison group. A decision matrix was created which will permit consistent assessment of risk of bias due to sample selection bias, information bias, and confounding. A data extraction tool was developed for the project with the capacity to efficiently extract data on multiple outcomes from a single study. The systematic review found a large number of studies presenting qualitative evidence that proximity to or direction from point sources, particularly waste water treatment plants, may be associated with higher levels of antibiotic resistance; however, very few studies quantitatively characterize this effect or provide statistical inference to aid in interpretation. Some studies reported no effect of point sources on resistance measures, even qualitatively. Due to a lack of quantitative effect estimates, we are not able to assess possible impacts of publication bias using visual or other methods such as funnel plots. Therefore, we cannot rule out the possibility that some studies found to have null effects were not published in the scientific literature. As such, it is not possible to conclude whether or not point sources are associated with increases in resistance measures in the environment. However, these findings do support the plausibility of such an effect and indicate the appropriateness of further study on this issue. This systematic review provides a strong imperative to improve research methods in order to provide interpretable, quantitative information about the effect of point sources on resistance in the environment. We make the following recommendations for future research:

- Design more longitudinal studies (versus cross-sectional studies), to study changes over time
- Plan study design and data analysis to control for the effect of confounding due to other sources of resistant bacteria or resistance genes
- Quantify the association between point sources and outcomes using effect measures and report with appropriate statistical inference
- Enhance collaborative work between epidemiologists, microbiologists, ecologists, and other scientists, to provide expertise where needed

Introduction

The dissemination of antibiotic resistant bacteria (ARB) or associated antibiotic resistance genes (ARG) from anthropogenic sources into the environment is an area of growing concern with potential risks for human, animal and ecosystem health. Numerous studies have detected ARB and ARG in a variety of environmental sites across the globe, including seawater, fresh water, soil and air. Although antibiotic resistance in environmental bacteria has existed for billions of years and occurs in locations across the globe, some evidence suggests that the amount of resistance in environmental media increased dramatically in the last century, likely due to the extensive use of pharmaceutical antibiotics (Finley et al., 2013). Despite this, the degree to which increased levels of these resistance factors in the environment result from specific anthropogenic sources, such as human wastewater or animal agriculture, is an area of debate (Wooldridge, 2012, Woolhouse et al., 2015). Point sources, such as wastewater effluent pipes and agricultural waste lagoons, represent an important and definable contribution to this environmental

problem. Here we present a method of summarizing all published, scientific evidence on the effect of point sources on environmental resistance.

Systematic reviews are a rigorous knowledge synthesis technique first developed in the health sciences to summarize information from numerous randomized trials examining the clinical efficacy of an intervention (Sargeant and O'Connor, 2014b). In contrast to a narrative review, this method permits the evaluation of all available evidence on the question of interest using a standardized process. A documented search strategy gathers all published literature on a predefined question, and identified publications are screened for relevance to this question. For included studies, risk of bias to the internal validity of the study is assessed, relevant data are extracted and the overall evidence is summarized. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement, published in 2009, proposed reporting standards for systematic reviews to ensure thorough and consistent reporting of systematic review activities (Moher et al., 2009). The PRISMA statement recommends creating and making accessible a predefined protocol describing how the systematic review will be conducted.

Here we describe the protocol for a systematic review of available evidence on the subject of whether environmental levels of bacterial antibiotic resistance are higher in close proximity to point sources compared to areas more distant from those sources. Specifically, this protocol provides a way to rigorously evaluate the state of evidence on the following question: ***Is the prevalence or concentration of antibiotic resistant bacteria (ARB) or resistance genes (ARG) in soil, water, air or free-living wildlife higher in close proximity to, or downstream from, known or suspected sources compared to areas more distant from or upstream from these sources?***

Objectives:

The objective of the proposed systematic review methodology is to rigorously summarize the current evidence for an association between point sources and environmental levels of antibiotic resistance. The process will result in a clearer understanding of the state of knowledge about this question and identification of data gaps. The specific question we will address is:
Is the prevalence or concentration of antibiotic resistant bacteria (ARB) or resistance genes (ARG) in soil, water, air or free-living wildlife higher in close proximity to, or downstream from, known or suspected sources compared to areas more distant from or upstream from these sources?

Materials and Methods

First, a team was assembled. Each one of its members brought specific skills, and the different tasks and responsibilities for the project were assigned accordingly. Then, a protocol was developed by one of the team members to establish the components and the criteria for each step of the review process. In order to develop this protocol, the team members had numerous meetings to figure out the best approach, and to clearly define the review question. The specific review question of concern was: *Are environmental levels of antibiotic resistance higher in close proximity to, or downstream from, known or suspected sources compared to areas more distant from or upstream from these sources?*

A comprehensive search of the electronic databases PubMed, CABI, and Scopus was conducted on October 14th 2014. The specific search strategy chosen for PUBMED 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

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("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]

There was no exclusion for citations in other languages other than English, nor a specific date range, or geographic area, including any study from any location worldwide.

In addition to the electronic literature searches, hand searches of the references listed in relevant narrative reviews and key research articles were also conducted to ensure all relevant peer reviewed literature was assessed.

All citations were exported into the EndNote reference management software package (Thomson Reuters) and were screened in two phases for relevance to the review question. First, the titles and abstracts were reviewed and were excluded a) if they were not primary research (e.g., review article or conference proceedings), b) if no environmental samples were collected (air, water, soil, or biological samples from wildlife), or c) if the study did not report prevalence or concentration of bacterial antibiotic resistance factors (bacteria or genes). The second phase of the screening evaluated the quality of the study design, and just the methods section was reviewed. Studies were excluded if a) they did not report proximity to, or direction from (in the case of unidirectional flow) a suspected source of resistance elements, or b) there was no comparison group. A Microsoft Excel spreadsheet was used to record the results from the screening process. Then, all the studies that remained after the screening process were assessed for potential biases. A rubric consisting of low (plausible bias unlikely to seriously alter the results), unclear (plausible bias that raises some doubt about the results), and high (plausible bias that seriously weakens confidence in the results) was used. Studies were independently reviewed by two team members for screening and risk of bias assessment. A Microsoft Access database, created by Dr. Singer for this project specifically, was used to record the results from the bias assessment. The same database was used to extract relevant data from the studies that were finally included, which was the next step in the methods. They were divided in two outcomes types: ARG, and ARB. All extracted data was checked against the original study by a different reviewer to ensure accuracy. The extracted data consisted of: study design, source (exposure) type, sampling details, comparison of interest, statistical analysis used, effect measures reported, and information related to the outcome (either ARG or ARB). If no effect measure was presented, qualitative evidence pertaining the systematic review question was extracted when available.

Table 1. Eligibility criteria for inclusion of studies

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Population:	Soil, water, air or free-living wild animal samples
Exposure:	Locations nearer to a potential point source of antibiotic resistance, or downstream/down-gradient from such a source in a unidirectional system
Comparator:	Locations distal to such a source, or upstream/up-gradient from such a source in a unidirectional system
Outcomes:	Prevalence or concentration of ARB or ARG
Study design:	Experimental and observational studies

Results

The total number of studies that were evaluated was 4,524. From those, 690 made it through relevance screening, and 60 were included after the design screening process (19 with ARG outcome and 41 with ARB outcome) (**Figure 1**). The majority of the studies were of cross-sectional design. Of the 60 included studies, 28% were judged to be at low risk of presenting substantially biased estimates of the effect of interest, namely the effect of point source(s) on the frequency or concentration of bacterial resistance in environmental media. Of these same 60 studies, 23% were judged to be at high risk of presenting biased estimates. For 49% of these studies the risk of bias could not be determined from the available information.

The data extracted from each study was stratified by outcome (ARG and ARB) and can be found in Tables 2 (ARG) and 3 (ARB). Some of the main highlights were:

- Most studies evaluated human waste (mainly wastewater treatment plants) as a point source, followed in frequency by terrestrial agriculture, urban, and aquaculture. Samples consisted mainly of superficial water, then sediment, ground water, soil, and biofilm. There were no studies that included samples taken from wildlife or from air.
- Only one study reported an effect measure (risk difference). None of the studies reported statistical analysis to evaluate the association of interest between a point source and the ARG levels in the environmental samples. Thus, no quantitative data was extracted, and only qualitative information was assessed, as shown in the results tables.
- The main ARG evaluated were *tet* and *sul* genes (resistance to tetracyclines and sulfonamides, respectively)
- For ARB, there was a wide diversity of bacteria evaluated, including *E.coli*, *Aeromonas, Listeria monocytogenes*, *MRSA, Salmonella*, and *Staphylococcus spp*. Most studies used the disk diffusion method to test for antibiotic resistance. The most commonly followed guidelines were CLSI, but there were some studies that followed national guidelines according to the country where the study was conducted (e.g. French National Guidelines). There was a variety of antibiotics tested with no consistent panel across studies.

Figure 1. Flowchart describing inclusion and exclusion of studies.
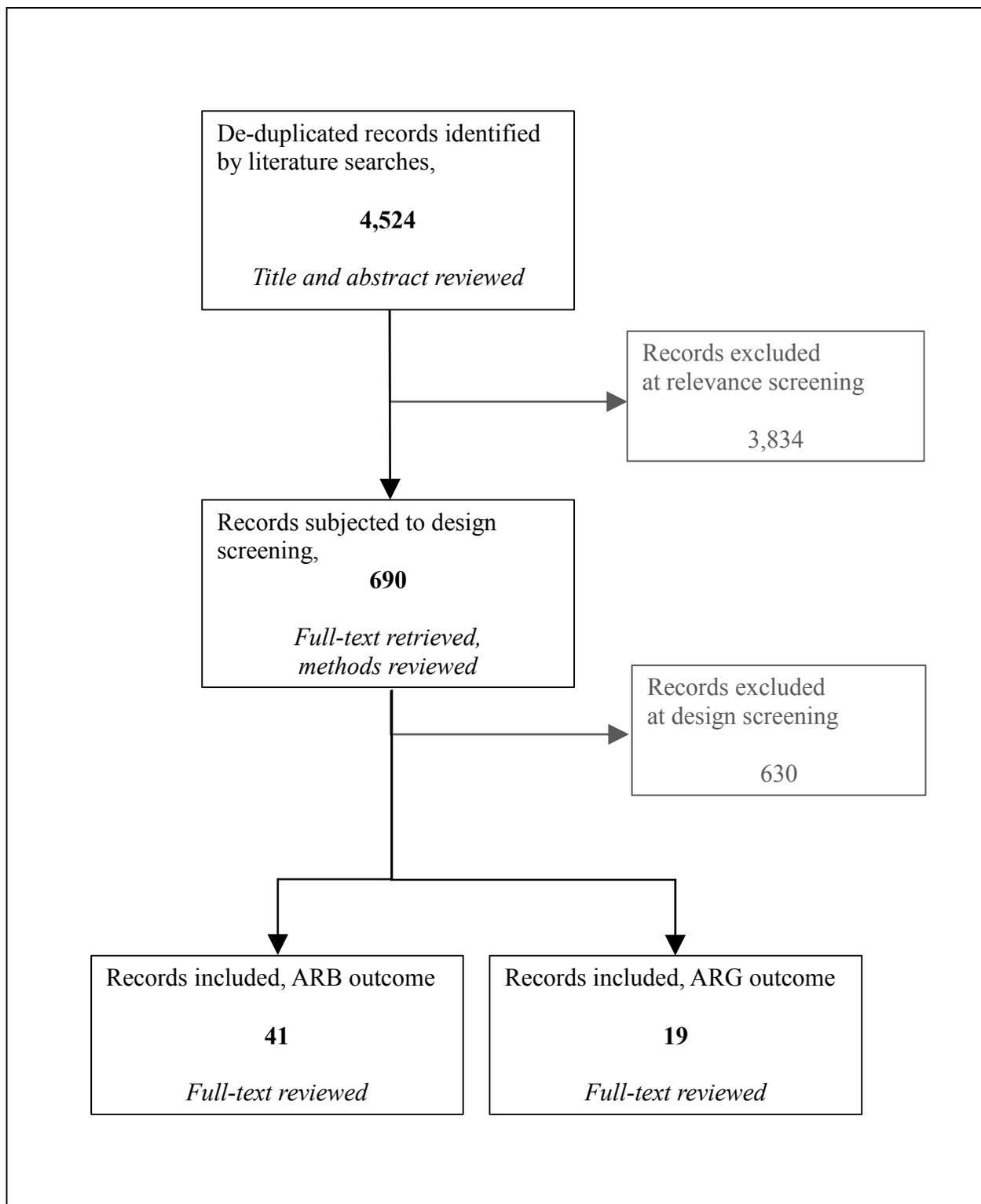
Discussion

The systematic review found a large number of studies presenting qualitative evidence that proximity to or direction from point sources, particularly waste water treatment plants, may be associated with higher levels of antibiotic resistance; however, very few studies quantitatively characterize this effect or provide statistical inference to aid in interpretation. Some studies reported no effect of point sources on resistance measures, even qualitatively. Due to a lack of quantitative effect estimates, we are not able to assess possible impacts of publication bias using visual or other methods such as funnel plots. We cannot rule out the possibility that some studies found to have null effects were not published in the scientific literature. As such, it is not possible to conclude whether or not point sources are associated with increases in resistance measures in the environment. However, these findings do support the plausibility of such an effect and indicate the appropriateness of further study on this issue.

Recommendations for research

This systematic review provides a strong imperative to improve research methods in order to provide interpretable, quantitative information about the effect of point sources on resistance in the environment. We make the following recommendations for future research:

- Design better studies that are capable of quantifying the impact of specific sources on environmental resistance levels. Different study designs can address different types of questions, and the choice of study design should be made carefully. For example, a longitudinal study (over time) can help establish temporal changes but might still lack the capacity to determine if distance is having an effect on resistance levels. If temporal data are collected, these data should be reported in full, not collapsed into a single estimate where the temporal component is lost.
- Study designs should carefully address the possible sources of resistance within the study area and control for these potential confounders. Without knowledge of the influence that other sources within the study area have on resistance, it is very difficult to discern the impact of the focal point source on resistance.
- The scale of the study should be carefully considered prior to implementation. The spatial scale of the study should reflect the precision of the results that are desired and the overall effect that the point sources are believed to have on environmental resistance levels.
- Studies should be measuring and then analyzing the distance that each sampling site is located from other potential sources (e.g. see Prudent et al.). This approach is one way of controlling for potential confounders.
- Proper epidemiological effect measures that can quantify the association between point sources and resistance outcomes need to be employed. Appropriate statistical methods should be standard for these types of studies.

Challenges

One of the main challenges during the screening process of this project was to retrieve older citations, but this is a common challenge with these types of reviews. Many of the papers were in different languages (including Russian, Polish, Chinese, and German, among others), and this required an effort to translate them into English. Finally, the scope of the review question was broad, which led to the inclusion of heterogeneous studies which were difficult to compare with each other.

I.   Publications / Presentations

Both the Systematic Review protocol and the preliminary results for the ARG were presented at the 6th Symposium on Antimicrobial Resistance in Animals and the Environment (July 2015; Tours, France). They will also be presented at the International Symposia on Veterinary Epidemiology and Economics (November 2015, Merida, Mexico).

Williams-Nguyen , J., Bueno, I., Sargeant, J., Nault, A., Hwang, H., and Singer, R. "Application of systematic review methodology to dissemination of antibiotic resistance from anthropogenic sources into the environment." 6th Symposium on Antimicrobial Resistance in Animals and the Environment, July 2015, Tours, France

Bueno, I., Williams-Nguyen, J., Sargeant, J., Nault, A., Hwang, H., and Singer, R. "Dissemination of antibiotic resistance genes from Human and agricultural sources into the environment: A systematic review of the evidence." 6th Symposium on Antimicrobial Resistance in Animals and the Environment, July 2015, Tours, France

Both the protocol and the ARG results will be submitted for publication by October 1, 2015 to the journal Veterinary Microbiology, Special Edition (ARAE 2015), and the ARB results will be submitted to a different journal, as yet to be determined.

Table 2. Data extracted from studies with ARG as the outcome (n=19)

Citation	Country	Spatial scale	Source type	Sample type	Applicable comparison	Gene(s)	Overall risk of bias	Findings
Czekalski, N., et al. (2014)	Switzerland	4 km	Human waste	Sediment	Distance	*sul1, sul2 tetB, tetM, tetW qnrA*	Unclear	sul1, sul2, tetB: Detected at all sampling sites, even the remote ones from the source. tetM, tetN: Decreased with distance from the source qnrA: Detected only at source.
Hong, P. Y., et al. (2013)	United States	15 m	Terrestrial agriculture (Swine)	Ground water	Direction	*tetZ, tetQ intl1, intl2*	Unclear	Concentration of ARG was higher near the source compared to more distant points.
Khan, G. A., et al. (2013)	Pakistan	20 km	Urban (city)	Sediment	Direction and Distance	*sul1, dfrA1, ermB, tetA, tetB, intl1*	Unclear	Higher concentration of ARG downstream from the source compared to the source and upstream from it.

Kristiansson, E., et al. (2011).	India	20 km	Human waste	Sediment	Direction and Distance	*sul2, strA, strB, qnrS*	Unclear	sul2, strA and strB with higher levels downstream compared to upstream and at the source; qnrS lower levels downstream compared to upstream and at the source.
Leclercq, R., et al. (2013).	France	4 km	Human waste	Surface water	Direction	*mefA, tetM, ermB*	Low	Higher levels at the source compared to downstream.
Marti, E., et al. (2013)	Spain	200 m	Human waste	Sediment	Direction and Distance	*qnrA, qnrB, qnrS, bla^{TEM}, bla^{CTX-M}, bla^{SHV}, ermB, sul1, sul2, tetO, tetW*	Unclear	Higher levels at the source compared to downstream, and the lowest levels of ARG were found upstream; some genes only found at the source.
Pei, R., et al. (2006)	United States	50 km	Urban (city)	Sediment	Direction	*sul1, sul2, tetO, tetW*	Unclear	Higher concentration of sul genes compared to tet genes. Higher concentration downstream from the source compared to upstream.

Reference	Country	Distance	Source	Sample	Study Type	Genes	Risk	Findings
Tamminen, M., et al. (2011)	Finland	1 km	Aquaculture	Sediment	Distance	*tetA, tetC, tetE, tetG, tetM, tetH, tetW*	Low	Genes only found at the farm sites, but not outside the farm sites.
Uyaguari, M. I., et al. (2011).	United States	100 km	Human waste	Surface water Sediment	Direction	*bla$_{M-1}$*	Unclear	Higher concentration at the source compared to downstream and upstream sites. Higher level of *bla$_{M-1}$* detected in the sediments compared to water.
Zhang, S., et al. (2013)	China	<50 km	Urban (city)	Surface water	Direction	*dfrA1, bla^{TEM}, cmlA5, sul2, tetA, ermB, bla^{OXA1}, tetG, strA*	High	When detected (blaTEM, sul2, ermB, dfrA1), higher concentration downstream from the source than upstream.
LaPara, T. M., et al. (2011).	United States	8 km	Human waste	Sediment	Direction	*tetA, tetX, tetW, intl1*	Unclear	Higher concentration of genes at the source outflow compared to other sites.
Li, L., et al. (2013)	China	Unclear	Terrestrial agriculture (swine)	Surface water, Soil	Direction	*lnu(F), erm(A), lnu(A), lnu(D), vgaA, vgaC, vgaD, vgaE*	Unclear	Higher concentration at the source site compared to downstream sites.

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Böckelmann, U., et al. (2009)	Spain	1 km	Human waste	Ground water, Surface water	Direction	*tetO, ermB*	Unclear	Higher concentration at the source site compared to downstream sites.
Castiglioni, S., et al. (2008)	Italy	100 km	Human waste	Surface water, Sediment	Direction	*marA*	Unclear	Higher levels downstream compared to source and upstream.
Czekalski, N., et al. (2012).	Switzerland	4 km	Human waste	Surface water	Distance	*sul1, sul2*	Low	No difference between the source and downstream sites.
Hsu, J. T., et al. (2014)	Taiwan	800 m	Terrestrial agriculture (Swine)	Surface water	Direction and Distance	*sul1, sul2, sul3*	Unclear	Sul1 and sul2: Higher level downstream compared to upstream; sul3 not detected.
Jia, S., et al. (2014)	China	10 km	Terrestrial agriculture (swine)	Surface water	Direction	*tetC, tetX, tetQ, tetW, tetO, tetM*	High	Decreased levels of gene abundance at points further from the source.
Stalder, T., et al. (2014)	Not reported	5 km	Human waste	Surface water	Direction and Distance	*intl1, intl2, intl3*	Low	No difference reported across sites.
Pruden, A., et al (2012)	United States	>50 km	Human waste, CAFOs	Surface water	Direction and Distance	*sul1, tetW*	Low	No trend reported for gene abundance.

Table 3. Data extracted from studies with ARB as the outcome (n=41)

Citation	Country	Spatial scale	Source type	Sample type	Applicable Comparison	Type of bacteria	Overall risk of bias	Findings
Al-Bahry, S. N., et al. (2009)	Oman	10 km	Human waste	Water, Fish	Distance	Gram negative (*Salmonella spp*)	Low	For both water and fish samples, isolates were resistant to ampicillin, followed by carbenicilin, but less to other antibiotics.
Dzierzanowska, D., et al. (1977)	Poland	7 km	Urban (city)	Water	Direction and Distance	Gram negative	Unclear	No resistant bacteria upstream from the source detected; highest level near the source.
Goni-Urriza, M., et al. (2000)	Spain	30 km	Human waste	Water	Direction and Distance	Gram negative (*Aeromonas spp*, *Enterobacteriacea*)	High	There was an increase of resistant bacteria percentage downstream from the source compared to upstream, especially with beta-lactams and tetracycline.

Gordon, L., et al. (2007)	France	2 km	Aquaculture	Sediment	Direction	Gram negative (*Aeromonas spp*)	High	*Aeromonas spp-resistant to Florfenicol, Oxytetracycline, and Oxolinic acid found downstream from the source and not found upstream.*
Hess, S. and C. Gallert (2014).	Germany	Unclear	Human waste	Surface water	Direction	Gram positive (*Staphylococcus*)	Unclear	The percentage of antibiotic resistant Staphylococci decreased during the wastewater treatment process, and it was lower in the surface river water, except for erythromycin and clindamycin which was a higher percentage compared to the source.
Leclercq, R., et al. (2013).	France	4 km	Human waste	Water	Direction	Gram positive (*Enterococci*)	Low	Higher percentage of antibiotic resistant bacteria at the source compared to downstream.

Oberle, K., et al. (2012)	France	4 km	Human waste	Water	Direction	Gram negative *(E.coli)*	Unclear	The percentage of *E.coli* resistant to at least 1 antibiotic was 44.9% at the source, and 26% downstream from the source in the river. A similar trend was reported for multidrug resistant *E.coli.*
Oh, H., et al. (2009)	South Korea	Large	Human waste	Water	Direction	Oligotrophic/Heterotrophic	High	The general trend was a lower percentage of resistant bacteria in the river samples compared to the source, which was upstream from the river.
Blaak, H., et al. (2014)	Netherlands	2 km	Human waste	Water	Direction	Gram negative *(E.coli)*	High	Higher percentage of resistant bacteria at the source compared to upstream and downstream.
Chen, B., et al. (2011)	China	<20 km	Urban (city)	Surface water	Direction and Distance	Gram negative *(E.coli)*	High	No relevant data to be reported.

Fincher, L. M., et al. (2009)	United States	3 km	Urban (city)	Surface water	Direction	Gram negative (*E.coli*)	Unclear	There was resistant bacteria found upstream and downstream from the source with no significant differences.
Harnisz, M. (2013)	Poland	2 km	Human waste	Water	Direction and Distance	Heterotrophic	Unclear	Higher percentage of resistant bacteria downstream compared to upstream and to the source.
Kerry, J., et al. (1995)	Ireland	5 km	Aquaculture	Sediment	Distance	Unclear	Low	Higher percentage of resistance at the farm sites compared to sites further away from the farms (at least 5 km).
Laroche, E., et al. (2009)	France	60 km	Human waste	Surface water	Direction	Gram negative (*E.coli*)	High	Higher percentage of resistant bacteria downstream compared to upstream across all time points of sampling.

Laube, H., et al. (2014).	Germany	0.15 km	Terrestrial agriculture (Poultry)	Air	Direction and Distance	Gram negative (*E.coli* harboring bla^{TEM1})	Low	10% of the air samples downwind were positive for ESBL/Ampc-producing *E.coli* compared to 5% of the samples upwind.
Li, D., et al. (2009).	China	35 km	Human waste	Surface water	Direction and Distance	Heterotrophic	High	Higher percentage for downstream samples compared to upstream for all the antibiotics tested.
Li, D., et al. (2010)	China	25 km	Human waste	Surface water	Direction and Distance	Heterotrophic	Low	Higher percentage for downstream samples compared to upstream for all the antibiotics tested.
Middleton, J. H. and J. D. Salierno (2013)	United States	0.38 km	Human waste	Surface water	Direction and Distance	Thermotolerant fecal coliforms	Low	No percentage of resistance reported.
Schreiber, C. and T. Kistemann (2013).	Germany	0.16 km	Human waste	Surface water	Direction and Distance	Gram negative *Rhodospirillaceae*	Low	Resistance found in *Rhodospirillaceae* did not appear to be related to the source (wastewater discharge).

Soni, D. K., et al. (2013)	India	3 km	Human waste	Surface water	Direction and Distance	Gram positive *(Listeria monocytogenes)*	Unclear	There were no bacteria detected upstream, so there was no comparison of resistance.
West, B. M., et al. (2011)(2014).	United States	3 km	Human waste	Surface water	Direction and Distance	Gram negative (Fecal coliforms*)*	High	Risk difference for Ampicilin was reported as 8.1% lower for upstream compared to downstream.
Yao, Q., et al. (2011).	China	50 km	Terrestrial agriculture (swine)	Soil	Distance	Gram negative *(Enterobacteriacea)*	High	Higher percentage of resistance near the farms compared to sites distant from the farm (45.2% vs 33.9%).
Akiyama, T. and M. C. Savin (2010).	United States	2 km	Human waste	Surface water, Sediment	Direction and Distance	Gram negative *(E.coli;* Total coliforms)	Low	Significantly higher percentage of resistant *E.coli* downstream compared to upstream in one sampling campaign, but not in the next sampling campaign. No difference for total coliforms.

Alharbi, S. A. (2012).	UK	2 km	Hospital	Soil	Distance	Gram positive *(MRSA)*	Unclear	No obvious trend reported.
Anderson, B. W., et al. (2014).	United States	0.6 km	Terrestrial agriculture (Poultry processing plant)	Surface water	Direction	Gram negative *(Tetracycline-resistant E.coli)*	Unclear	No obvious trend reported.
Bayne, S., et al. (1983)	UK	0.8 km	Human waste	Surface water	Direction and Distance	Gram positive *(Streptococcus group D)*	Low	No obvious trend reported.
Czekalski, N., et al. (2012).	Switzerland	3.2 km	Human waste	Surface water, Sediment	Distance	Multiple	Low	Proportion of resistant less than 1% or absent at all sites.
Fuentefria, D. B., et al. (2011)	Brazil	1.4 km	Hospital	Surface water	Direction and Distance	Gram negative *(Pseudomonas aeruginosa)*	Unclear	Higher percentage of resistance downstream compared to upstream.
Fuentefria, D. B., et al. (2008).	Brazil	Unclear	Hospital	Surface water	Direction	Gram negative *(Pseudomonas aeruginosa)*	Unclear	77.3% of *P. aeruginosa* strains from upstream was pan-susceptible compared to 44.4% of *P. aeruginosa* strains from downstream.

Hsu, J. T., et al. (2014)	Taiwan	1 km	Terrestrial agriculture (swine)	Surface water	Direction	Gram negative, Gram positive	Unclear	Higher percentage of resistance downstream compared to upstream.
Koczura, R., et al. (2012)	Poland	Unclear	Human waste	Water	Direction	Gram negative (*E.coli*)	Unclear	Higher resistance at the source (50%) compared to upstream (33.3%) and downstream sites (26.7%).
Li, D., et al. (2011).	China	35 km	Human waste	Water	Direction and Distance	Not specified	Unclear	Higher percentage of resistance downstream (65%) compared to upstream (10%).
Marti, E., et al. (2014).	Spain	200 m	Human waste	Sediment	Direction and Distance	Gram negative (*Pseudomonas aeruginosa*)	Low	In general, higher resistance downstream compared to upstream sites.
Mondragón, V. A., et al. (2011).	Mexico	Unclear	Human waste	Water	Direction	Gram positive (*Enterococcus*)	Unclear	Higher percentage of resistance downstream compared to upstream and the source.
Reinthaler, F. F., et al. (2003).	Austria	200 m	Human waste	Water	Direction and Distance	Gram negative (*E.coli*)	Low	No difference between the sites.

Sapkota, A. R., et al. (2007)	United States	500 m	Terrestrial agriculture (swine)	Surface water	Direction and Distance	Gram positive (*Enterococcus*)	Unclear	0% resistance upstream compared to 18% at all downstream sites.
Schulz, J., et al. (2012)	Germany	400 m	Terrestrial agriculture (swine)	Soil	Direction and Distance	Gram positive (*MRSA*)	Unclear	Higher resistance downwind compared to upwind (using bootsocks).
Sidrach-Cardona, R., et al. (2014).	Spain	1.5 km	Industrial waste	Water	Direction and Distance	Gram negative (*E.coli*)	Unclear	Higher resistance downstream compared to upstream.
Suzuki, Y., et al. (2013).	Japan	12 km	Human waste	Surface water	Direction and Distance	Gram negative (*Pseudomonas*)	High	No difference between sites.
Xu, H., et al. (2012).	China	Unclear	Human waste	Surface water	Direction	Gram negative (Coliforms*)*	Unclear	Higher percentage of resistance downstream compared to upstream.
Gallert, C., et al. (2005)	Germany	430 m	Human waste	Ground water	Direction and Distance	Gram positive (*Enterococcus*)	Unclear	Higher resistance downstream compared to upstream.

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