FINAL REPORT

Rapid Evidence Assessment

The relative risks to human health from the occurrence of antimicrobial resistance and antibiotic resistance genes in the aquatic environment

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Centre for Evidence Based Agriculture

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The relative risks to human health from the occurrence of antimicrobial resistance and antibiotic resistance genes in the aquatic environment

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Executive Summary

Background

The emergence of antibiotic resistant bacteria (ARB) of clinical significance, poses a global threat to humans. Natural aquatic environments are increasingly being recognised as not only as reservoirs of clinically important ARB, antimicrobial resistance (AMR) and antibiotic resistance genes (ARGs) but also as potential pathways for human exposure. Aquatic pathways by which humans can become exposed include drinking water, water based recreational activities, consumption of shellfish and irrigated crops. A better understanding of the pathways that are most responsible for disseminating 'resistance' from the aquatic environment into humans is needed so that relevant authorities are better placed to develop effective strategies for dealing with the risks.

Methods

A Rapid Evidence Assessment (REA) was used to review the evidence base for the primary question: 'What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans?' Two secondary questions were also addressed 1) 'What are the factors affecting the transmission of AMR and ARGs from the water environment into humans?' 2) 'What evidence is there that exposure to the pathways is contributing to clinical infections that cannot be treated with existing antibiotics?' A protocol developed a priori by the review team and steering group was used to lead the review process.

Key findings

Forty eight articles, comprising 49 studies were included in the REA, consisting of a mixture of observational and clinical case studies. Twenty seven studies were relevant to the primary question, and 24 for secondary question 1. Two studies were relevant to both the primary and secondary questions. No evidence was found for secondary question 2, but studies included in the REA were used to discuss this question. Pathways reported included drinking water, water sports, contact with sea, brackish or fresh water, irrigation water, irrigated crops, contact with aquaria and marine mammals and consumption of seafood.

Tetracycline resistance and integrase genes, together with a diverse range of ARB including *Aeromonas* sp. (in particular *Aeromonas hydrophila*), *Campylobacter jejuni*, *Escherichia* coli, *Enterococcus* sp., *Enterobacter* sp., *Legionella pneumophila*, *Pseudomonas* sp., *Salmonella sp*. (including *Salmonella enterica* and *Salmonella* Java), *Staphylococcus* sp. (including *Staphylococcus aureus*), *Vibrio* sp. (including *Vibrio parahaemolyticus* and *Vibrio vulnificus*), and *Yersinia ruckeri*.

The earliest study dated from 1975 and the most recent from 2017 and the majority of studies from the US, followed by Poland, Spain, UK, France, Australia, Canada, Chile, Norway, Mexico, Turkey, Portugal, Italy, Germany, Netherlands and Sweden.

'What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans?'

Observational and clinical case studies included in this REA indicated a potential but not direct causal relationship between exposure to ARB/ARG through aquatic pathways and colonisation/infection in humans. The lack of direct causal evidence does not mean that

transmission is not occurring, but rather that this type of evidence is difficult to obtain as studies that generate this data are often too complex to undertake, cost prohibitive or would be unethical to perform. The studies included were highly heterogeneous and low in volume. It was therefore not possible to identify which pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans.

Five studies linked colonisation/infection with consumption of drinking water. The first study described a case where a boy from Sweden became ill after drinking well water contaminated with *A. hydrophilia* (Krovacek *et al.*, 1989), the second study investigated an outbreak of *S. enterica* serovar Typhi that was thought to originate from sewage contaminated tap water in Turkey (Bayram *et al.*, 2011). An observational case-controlled study indicated that in England and Wales travel-associated ciprofloxacin-resistant infection cases are independently associated with travel to Spain, Portugal or Cyprus and the consumption of chicken or bottled water (The Campylobacter Sentinel Surveillance Scheme Collaborators, 2002). Finally, two observational cross-sectional studies showed risk of carriage of resistant *E. coli* was greater for users of water contaminated with resistant *E. coli* (Coleman *et* al., 2012) and that antibiotic resistance patterns in bacteria isolated from mineral waters for sale in Germany were similar to clinical isolates (Rosenberg & Hernandez Duquino 1989).

Seven studies were found linking contact with sea, brackish or fresh water with colonisation/infection. Five clinical case studies reported antibiotic resistant infection with *Vibrio* sp or *Aeromonas* sp. following either ingestion or wound exposure to sea, brackish or freshwater (Fernandez & Pankey, 1975; Joseph *et al.*, 1979; Kelly *et al.*, 1980; Goncalves *et al.*, 1992). A case control study indicated that recreational swimming in freshwater is a risk factor for community-acquired urinary tract infections caused by extended spectrum beta-lactamases (ESBL) – producing *E. coli* (Søraas *et al.*, 2013) and a cross-sectional study that showed regular surfers were at an increased risk of being faecal carriers of cefotaxime-resistant *E. coli* compared to non-surfers (Leonard 2016 unpublished).

Three studies reported that serotypes of *Salmonella* sp. found in irrigation water were the same as those found in outbreaks of infection (Li *et al.*, 2014; Micallef *et al.*, 2012; Martinez *et al.*, 2017). Two articles by the same author also investigated occupational exposure to antibiotic resistant bacteria in irrigation workers compared to office workers, with one study indicating that spray irrigators are more likely to be carriers of antibiotic resistant CoNS and methicillin-resistant coagulase-negative staphylococci compared to controls (Goldstein *et al.*, 2014 & 2017).

Six studies were found linking seafood with infection. One study reported on an eel farmer who contracted antibiotic resistant *V. vulnificus* from his infected eels (Haenen *et* al., 2014) and a clinical case study reported on a man who spiked himself on a crab (shell) he was preparing to eat and developed colistimethate resistant *Vibrio* sp infection (Fernandez & Pankey 1975). Four studies, found a relationship between antibiotic resistant *V. parahaemolyticus* (Dauros *et* al., 2011; Hernandez-Diaz *et al.*, 2015), *E.coli* (Grevskott *et* al., 2017) and *Salmonella* serotype Paratyphi B (Martinez-Urtaza *et al.*, 2006) in environmental and clinical samples

Four articles investigated the risk that companion and wild animals in aquatic environments pose to the dissemination of resistance to humans. Three articles (Kirk *et al.*, 2006; OzFoodNet Working Group. 2004; Weir *et al.*, 2012 - a systematic review) reported on the

same antibiotic resistant *S.* Java infection outbreak in households with aquaria in Australia. Finally, an observational cross-sectional study at a cetacean rehabilitation centre indicated that there is a potential link between human and cetacean carriage of methicillin-resistant *S. aureus* (MRSA) (Hower *et al.*, 2013).

'What are the factors affecting the transmission of AMR and ARGs from the water environment into humans?'

Variables that lead to increased environmental exposure are to some extent independent of antimicrobial resistance and so this literature will not have been searched for and captured in this REA. Factors such as increased rainfall, season, sea surface temperature and tidal currents may increase the chance of environmental exposure, however, evidence included in this REA was very heterogeneous, and many of these factors are likely to be location specific therefore making it difficult to provide any blanket recommendations. Factors that increase the volume of water ingested by people (e.g. water sports such as swimming and surfing) may also increase likelihood of developing colonisation/infection status. Likewise, the health and immune status of the exposed individual might interact with these factors to mediate infection. Authors highlighted that people with pre-existing injuries/wounds or those that sustain them in aquatic environments, and people who are immunocompromised or chronically ill are at greatest risk of antibiotic resistant infection.

'What evidence is there that exposure to the pathways is contributing to clinical infections that cannot be treated with existing antibiotics?'

No evidence was found that exposure to aquatic pathways is contributing to clinical infections that cannot be treated with existing antibiotics.

Implications for policy, practice and research

Although there were no individual studies that directly showed pathways of resistance from the aquatic environment to humans, a combination of observational and case studies did indicate a potential causal relationship between exposure to ARB/ARG through aquatic pathways and colonisation/infection in humans. Factors that increase ingestion of water such as swimming and surfing, and the health, and immune status of people are likely to influence the risk of colonisation/infection.

Overall, the evidence collated for the primary and secondary questions was highly heterogeneous and low in volume making it impossible to identify which pathways are most responsible for transmission into humans and difficult to make blanket recommendations to reduce environmental exposure due to factors which are often location specific (e.g. tidal currents). The lack of direct causal evidence does not mean that transmission is not occurring but rather that this type of evidence is difficult to obtain.

The biology of AMR/ARB/ARG in the aquatic environment including the potential for transmission through aquatic pathways to humans is a highly complex subject. The evidence presented in this REA should be considered in combination with other evidence/components of the topic (e.g. occurrence, evolution, and increasing clinical antibiotic resistance) when considering implications for policy and practice. For example, a there is a large amount of

literature that shows the presence of diverse AMR, ARB and ARGs in aquatic pathways. Although not investigated as part of this review, this literature indicates that the human microbiome is linked with microbial communities in these environments. It is therefore possible for people coming into contact with these aquatic pathways to become colonised and either subsequently develop a drug-resistant infection, or pass on ARB/ARGs to other members of the community who are more vulnerable to developing drug-resistant infections (e.g. immunocompromised individuals). When the whole body of evidence is considered, it would indicate that a precautionary principle be followed when considering the implications of AMR/ARB/ARG contamination of the aquatic environment and potential transmission pathways.

Implications for policy and practice

Based on the findings of this REA there is a gap in the knowledge base to fully understand:

- What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans
- Which factors affect the transmission of AMR and ARGs from the water environment into humans

Authors of studies included in this REA highlighted the following:

- Awareness about the risk of posttraumatic wound infection by bacteria from the sea, brackish and fresh water (Joseph et al., 1979; Gold & Salit, 1993).
- Advice for people to wash hands after cleaning and feeding fish and avoid using sinks to clean out aquaria (Kirk *et al.*, 2006; OzFoodNet Working Group, 2004).
- Awareness that people with pre-existing injuries/wounds or those that sustain them in aquatic environments and people who are immunocompromised or chronically ill are at greatest risk of antibiotic resistant infection (Fernandez & Pankey, 1975; Joseph et al., 1979 Gold & Salit., 1993; Haenen et al., 2014).
- Potential risks of water sports for transmission from the aquatic environment into humans, especially those that are immersive/increase likelihood of water ingestion (Leonard *et al.*, 2015; Leonard 2016 unpublished).
- Lack of mandatory notification systems for some bacteria/bacterial infections in some countries or states (e.g. *Vibrio* spp.) to understand the extent of the problem and guide appropriate prevention messages (Le Roux *et al.*, 2015; Yoder *et al.*, 2008), (these systems may not record antibiotic resistance but may help prevent infection).
- During heavy rainfall bacteria maybe involved in the dissemination of antibiotic resistance via the aquatic environment (Riberio *et al.*, 2014).

Implications for research

The following research gaps have been identified by this REA:

- Primary research to investigate how the natural environment spread ARB to the human microbiome.

- Primary research to understand the direction of transmission of ARB/ARG between humans and the environment.

- Primary research to investigate the health effects of exposure to ARB/ARG in aquatic environments, including transmission resistant bacteria to other (especially vulnerable) members of the community.

- Epidemiological and numerical modelling studies, to fully understand the role of aquatic exposure routes to assess real risk to human health.

- A review of the evidence to better understand to what extent does the transfer of genes play a role in the spread of resistance and what are the implications of this to human health.

Background

The emergence of antibiotic resistant bacteria (ARB) of clinical significance, poses a serious global threat to humans. The G8 science ministers meeting in 2013 (FCO, 2013) and the O'Neill Review (2016) have highlighted antibiotic resistance as one of the top threats facing humanity. The burden of antimicrobial resistance (AMR) cannot be tackled solely by focusing on antibiotic development and to overcome this global threat, a multidisciplinary approach is required (Kelly et al., 2016). Antimicrobial resistance is a phenomenon whereby microorganisms (viruses, fungi, bacteria, parasites) survive and grow in the presence of chemicals or drugs designed to kill them. Antibiotic resistant bacteria, as a subgroup of AMR, are specifically bacteria that are able to survive and grow in the presence of antibiotics (a type of antimicrobial). Genes in an organism that confer resistance to antibiotics are known as antibiotic resistance genes (ARGs). Natural environments are increasingly being recognised not only as reservoirs of clinically important ARB, AMR and ARGs but also as potentially significant pathways for human exposure to ARB. However, compared to other priority topic areas surrounding antibiotic resistance the environment has received less attention and research funding at national (UK) and European level (Kelly et al., 2016).

Aquatic environmental settings subject to anthropogenic pressure for example wastewater effluent, aquaculture and animal husbandry facilities have been identified as hotspots and pathways for AMR and ARGs into the environment (Berglund, 2015; O'Neill., 2015). These pathways introduce bacteria harbouring ARGs to the aquatic environment as well as resulting in a build-up of biologically active antimicrobial residues increasing the chances of clinically relevant pathogens acquiring genes conferring resistance to antimicrobial drugs. For example, studies have shown the dissemination of antibiotic resistance genes from manure to soil actinobacteria (Byrne-Bailey *et al.*, 2011) and from actinobacteria to pathogenic bacteria (Jiang *et al.*, 2017). Antibiotic resistance can evolve in the aquatic environment through genetic mixing of human and animal bacteria with environmental bacteria, and genetic mutation and recombination via direct selection pressures imposed by antibiotic residues or indirect selection by other bioactive compounds such as heavy metals and biocides (e.g. Gaze *et al.*, 2011). Evidence suggests that even very low concentration of antibiotics found in some aquatic environments can be effective to select and maintain resistance traits in bacteria (Gullberg *et al.*, 2011). The aquatic environment therefore acts not only as a reservoir of clinical resistance genes, but also as a medium for the spread and evolution of resistance genes (Young, 1993).

The routes by which humans can come into contact with these resistant bacteria and microbes via aquatic environments are numerous. Examples include consumption of crops grown with contaminated sludge used as fertiliser or irrigated using treated or untreated water (e.g. Kinney *et al.*, 2006); consumption of shellfish or fish (e.g. Heuer *et al.*, 2009); drinking of water drawn from contaminated ground or surface water (e.g. Coleman *et al.*, 2012), recreational activities in river or streams and marine water linked to contaminated surface water (e.g. Leonard *et al.*, 2015). These resistant bacteria and microbes have an opportunity to spread ARGs to the human microbiome making future treatment of clinically important infections with antimicrobial drugs increasingly difficult (Wellington *et al.*, 2013). A better understanding of the pathways that are most responsible for disseminating AMR, ARB and ARGs (hereafter referred to as 'resistance') from the aquatic environment into humans is needed so that relevant authorities are better placed to develop effective strategies for dealing with the risks.

Objectives of the REA

The REA compiled both academic and grey literature relevant to the primary question:

'What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans?'

The population, exposure, comparator and outcome (PECO) elements of the primary question are detailed in Table 1.

Table 1 PECO Elements of the REA

Population	Antimicrobial Resistance (AMR), Antibiotic Resistant Bacteria (ARB), Antibiotic Resistance Genes (ARGs)
Exposure	Pathways of exposure, e.g. drinking water, bathing waters, shellfish or fish consumption, irrigation
Comparator	Alternative pathways of exposure
Outcome	Pathways responsible for disseminating resistance, human health

Two secondary questions were also addressed using evidence gathered for the primary question, limited searches for evidence relevant to the secondary questions and expert opinion:

1. What are the factors affecting the transmission of AMR and ARGs from the water environment into humans? E.g. exposure times and frequency, physical conditions in the environment etc.
2. What evidence is there that exposure to the pathways is contributing to clinical infections that cannot be treated with existing antibiotics?

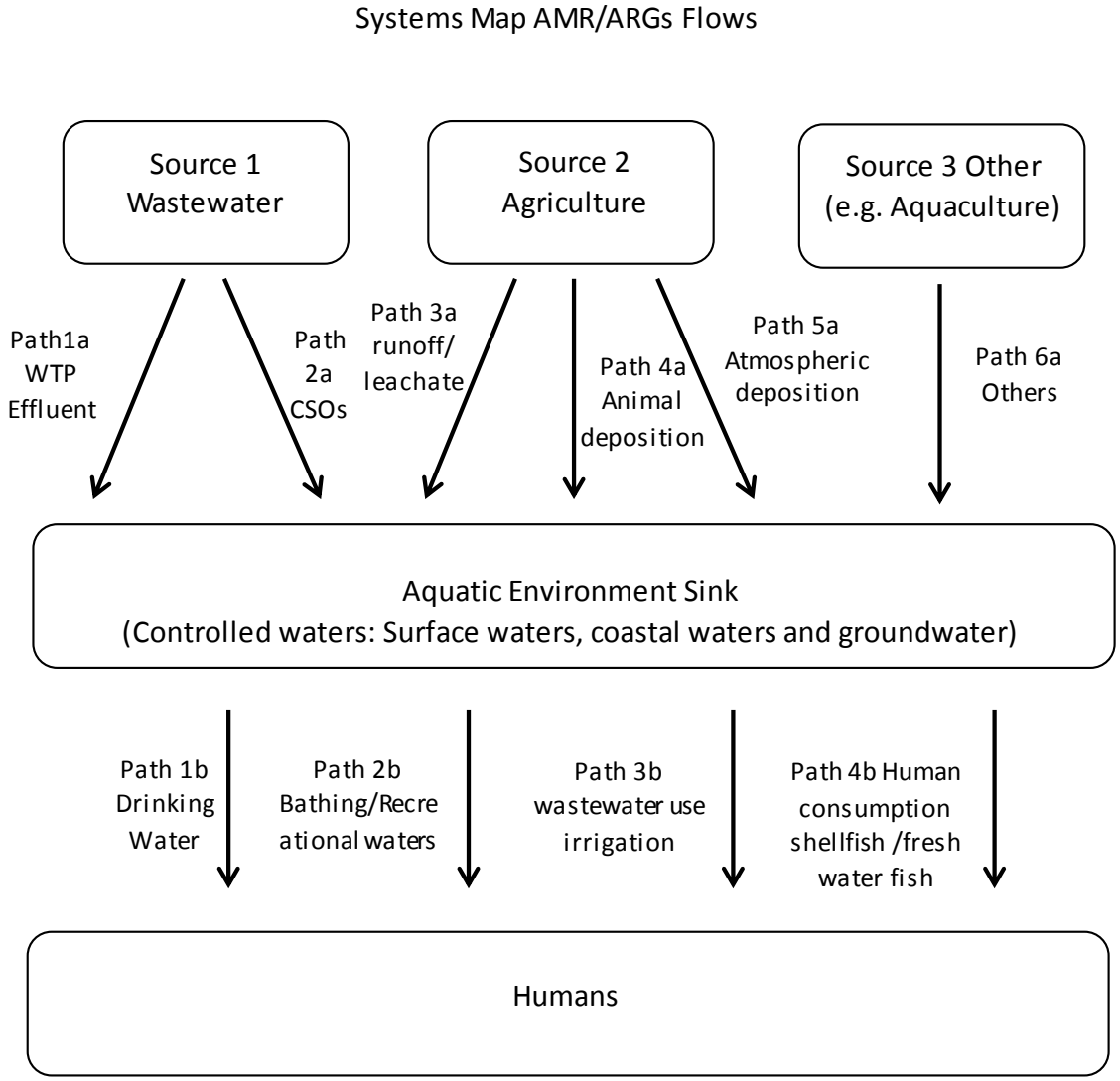
Evidence for horizontal gene transfer (HGT) in the context of environmental dissemination or environmental transmission to humans was also originally included as a secondary question,

but it was eventually decided to exclude this complex question from the REA, and instead just list in a separate file any potentially relevant articles that were found incidentally.

Conceptual model

Figure 1 illustrates how 'resistance' flows from different sources and pathways into the aquatic environment and subsequent pathways of exposure to humans. These pathways of exposure to humans such as drinking water, water based recreational activities, consumption of shellfish from aquaculture, irrigation water (e.g paths: 1b, 2b, 4b) and other pathways detailed in the scope section (e.g. animal transmission) were the focus of this REA.

Figure 1. Systems map of antimicrobial resistance and antibiotic resistance gene flows



Scope

All retrieved studies were assessed for relevance using inclusion criteria developed in collaboration with funders and subject experts as follows:

Relevant subjects: Studies that investigate pathways responsible for disseminating 'resistance' from the aquatic environment into humans, including drinking water, water based recreational activities (e.g. surfing, diving, kayaking etc), bathing waters, human consumption of crops irrigated with river water/treated waste water and human consumption of shellfish meat or fish from aquaculture (but not in fish/shellfish imports from excluded countries) – see exposure list below. Subjects for the secondary questions included factors affecting transmission into humans (e.g. exposure times and frequency, physical conditions in the environment etc.), studies showing that exposure to pathways is contributing to clinical infections that are resistant to antibiotics and evidence for HGT in the context of environmental dissemination or environmental transmission to humans.

Relevant types of study: Primary research and clinical case studies. Relevant reviews and modelling studies were collated and listed in a separate appendix as additional information. Reviews were screened for relevant studies to ensure they have been collated in the searches.

Population: Antimicrobial resistance, antibiotic resistance genes, antibiotic resistant bacteria, antibiotic resistance

Exposure: Drinking water, recreational activities such as swimming, surfing, diving and kayaking conducted in natural bathing waters, human consumption of crops irrigated with river water/treated waste water, human consumption of shellfish meat or fish from aquaculture, occupational pathways into general population (such as farmers and waste water treatment workers who have contact with water), waterborne and airborne (aerosol) transmission, animal and protist transmission.

Outcomes: Pathways responsible for disseminating resistance, human health, factors affecting the transmission of AMR and ARGs; exposure pathways contributing to clinical infections that are resistant to antibiotics

Geographical limits: Countries that are members of the Organisation for Economic Co-operation and Development (OECD): Australia, Austria, Belgium, Canada, Chile, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Latvia, Luxembourg, Mexico, Netherlands, New Zealand,

Norway, Poland, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom, United States.

Language: Studies published in the English language

Date of Publication: No date restrictions

Method

The REA was conducted following the Authority/NERC guidelines for the production of Quick Scoping Reviews and Rapid Evidence Assessments (Collins *et al.*, 2015).

Search keywords

Search terms were formulated by the review team and the Authority. A scoping search was performed to validate the methodology. Keywords were tested for specificity and sensitivity using the online database ISI Web of Knowledge (core collection). Wildcard (*) were used where accepted by the database/search engine to pick up multiple word endings. For example *water* picks up water, waters, freshwater etc. Table 2 shows the keywords and qualifiers used in the searches. A full list of keywords and qualifiers used for each online database searched is given in Appendix 1.

Table 2 shows keywords and qualifiers used in the literature search.

Population keyword	Exposure qualifier	Additional qualifier
"antimicrobial resistance"	river*	*health
“antibiotic resistan*”	aquatic	irrigate* crop*
	marine	aquaculture*
	sea	shellfish*
	lake*	sport*
	pond*	bathing
	water	drinking
	estuar*	farmer*
	stream	worker*
		transmission
		wild animal*
		colonis*
		microbiome
		carriage
		exposure
		recreation

Searches

A comprehensive search was undertaken using multiple information sources to capture an un-biased sample of literature. The search strategy was developed to identify both published and grey literature.

Online literature databases, search engines and websites of relevant organisations (identified by experts) were searched with keywords to identify relevant literature. Database and repository searches were conducted in the English language. The searches endeavored to be as thorough as possible within the limitations of the timescale of the REA. Recognised experts were asked for further recommendations and for provision of relevant unpublished material.

Online sources searched to identify relevant literature:

Academic databases:

Science Direct [http://www.sciencedirect.com]
Thomson Reuters Web of Science Core Collection [http://ipscience.thomsonreuters.com]
Wiley Online [http://onlinelibrary.wiley.com]
Pubmed [http://europepmc.org]
CAB abstracts [http://www.cabi.org/]
Greenfile [EBSCOhost www.greeninfoonline.com]
Ethos [http://ethos.bl.uk/Home.do]
DART-Europe E thesis [http://www.dart-europe.eu/basicsearch.php]

Organisational websites:

Defra online databases [https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs]
NERC Open Research Archive [https://nora.nerc.ac.uk/]
Centre for Disease Control and Prevention [https://www.cdc.gov/]
Environment & Natural Resources Canada
[ttps://www.canada.ca/en/services/environment.html]
European Centre for Disease Prevention and Control
[http://ecdc.europa.eu/en/Pages/home.aspx]
European Environment Agency [http://www.eea.europa.eu/]
Public Health England [https://www.gov.uk/government/organisations/public-health-england]
Medical Research Council [https://www.mrc.ac.uk/]
Umweltbundesamt [https://www.umweltbundesamt.de/en]
United Nations Environment Programme [http://web.unep.org/]
United Nations Environment Programme Mediterranean Action Plan
[http://web.unep.org/unepmap/]
United States Environment Protection Agency [https://www.epa.gov/]
World Health Organization [http://www.who.int/en/]

Search engines. The first 50 hits searched for relevant studies:

Google [http://www.google.co.uk]
Google Scholar [http:// scholar.google.com]

The results of each search term on each database was imported into a separate EndNote X7 library file. All the database libraries were incorporated into one library, recording the number of references captured. Using the automatic function in the EndNote X7 software

duplicates were removed. A record of each search was made to enable a re-run of the search if needed. The following data was be recorded: date the search was conducted; database name; search term; number of hits.

Study inclusion

Articles were screened for relevance by one reviewer, by applying study inclusion criteria (relevant subjects, geographic area and date of publication etc.) detailed in the scope section above. A list of key terms was also used to remove irrelevant literature and a record of these terms is available in Appendix 1. EPPI Reviewer 4 software was used to screen the articles.

The number of articles included and excluded at each stage was recorded. The resulting references were used to formulate a systematic map database.

Systematic map database

All primary research and clinical case studies were mapped in an Excel file. The systematic map database was used to describe the volume, nature and characteristics of the research/evidence relating to this REA.

Reviews and theses relating to the REA and evidence for HGT in the context of environmental dissemination or environmental transmission to humans were excluded from the Systematic map database, but listed separately.

Critical appraisal

No critical appraisal was conducted in this REA due to the type of evidence collated, its limited volume and highly heterogeneous nature.

Results

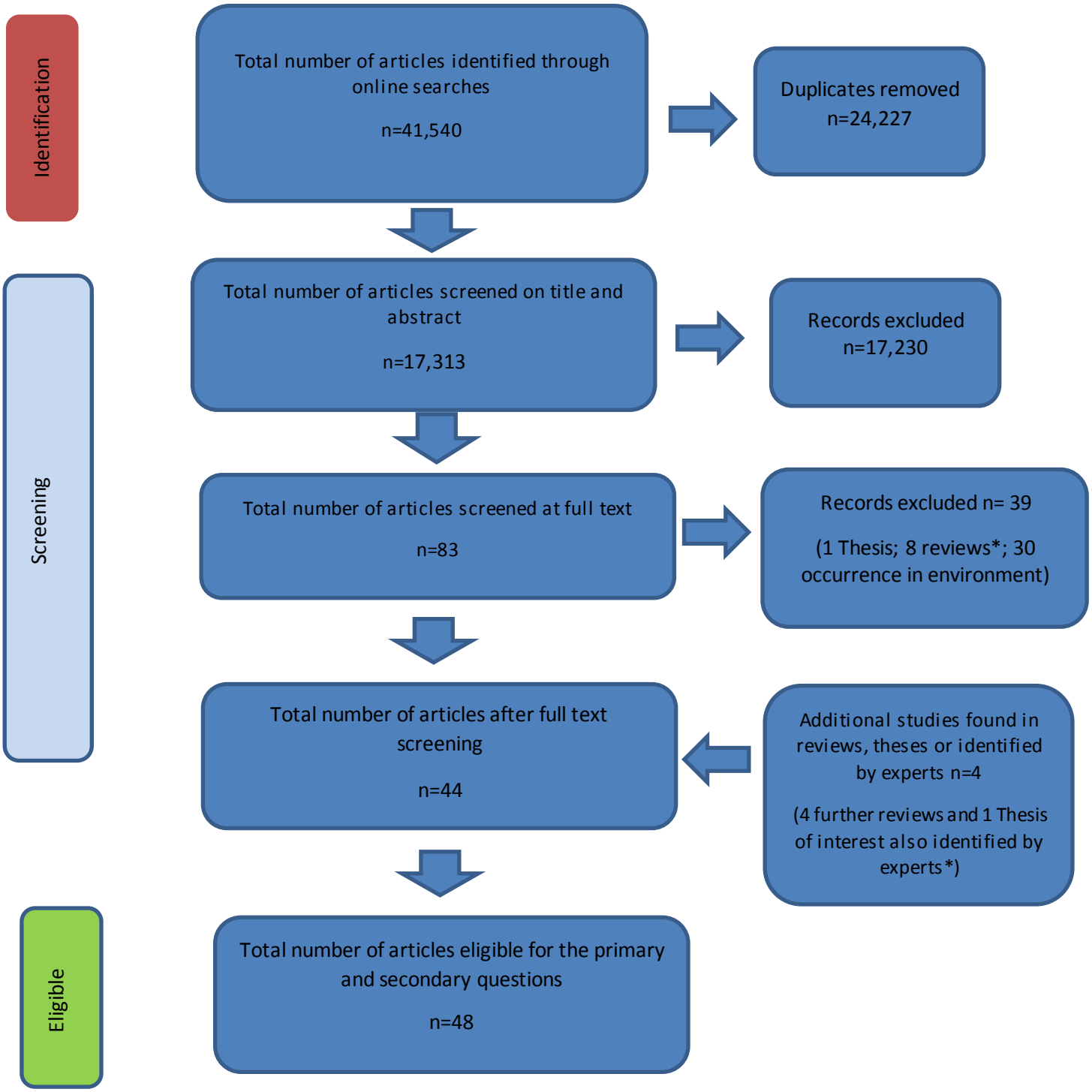
Review searching and screening

A total of 41,540 articles were identified through online searches for published and grey literature and a further 5 articles identified by experts or through review screening. Forty eight articles, comprising 49 studies were found to be eligible for inclusion in the REA. A record of the number of hits from each database searched is available in Appendix 1. Figure 2 shows the literature included and excluded at each stage of the REA process. All 48 articles included for mapping were found at full text and were summarised in an Excel database (Appendix 2).

During title and abstract screening evidence for HGT in the context of environmental dissemination or environmental transmission to humans was collated but not included in the final REA, as it was decided this complex subject would be better address by a separate review. A total of 94 articles of potential relevance to this topic were collated and a list of these articles is presented in Appendix 2 as useful additional information for a future review.

Two theses and 12 reviews were identified and are listed in Appendix 2 as additional information.

Figure 2 Literature included and excluded at each stage of the REA process



*Theses and reviews are listed in Appendix 2

Review statistics

Forty eight articles, comprising 49 studies were included in the REA consisting of a mixture of observational cross-sectional (n=10), cross-sectional, controlled (n=3), case-controlled (n=2), survey (n=26) and clinical case studies (n=8). Twenty seven studies were relevant to the primary question, and 24 for secondary question 1. Two studies were relevant to both the primary and secondary questions. No evidence was found for secondary question 2 but studies included in the REA were used to discuss this question.

The literature gathered fell into 5 'pathways':

1. Drinking water (Primary question n= 5; Secondary question 1 n= 3)
2. Sea or fresh water contact and aquatic recreation (Primary question n=7; Secondary question 1. n=10)
3. Irrigation (Primary question n= 6; Secondary question 1. n= 1)
4. Seafood (Primary question n= 6; Secondary question 1. n= 6)
5. Companion and wild animals (primary question n=3; Secondary question 1 n=2)

No studies were found that showed direct evidence that any of the pathways disseminated 'resistance' from the aquatic environment into humans. In lieu of these studies, evidence that indicated a potential causal link between environmental exposure to ARB/ARGs and infection/colonisation in humans was included.

The earliest study dated from 1975 and the most recent from 2017. Most articles were from the US (n= 17) followed by Poland (n=4), Spain (n=4), UK (n=4), France (n=3), Australia (n=2), Canada (n=2), Chile (n=2), Norway (n=2), Mexico (n=2), Turkey (n=2), Portugal (n=1), Italy (n=1), Germany (n=1), Netherlands (n=1), Sweden (n=1).

With the exception of 2 studies, where the focus was tetracycline resistance and integrase genes, all other studies focused on ARB. Organisms studied included *Aeromonas* sp. (n=4) in particular *Aeromonas hydrophila*, *Campylobacter jejuni* (n=1), *Escherichia* coli, *Enterococcus* sp. and *Enterobacter* sp. (n=13), *Legionella pneumophila* (n=1), *Pseudomonas* sp. (n=2), *Salmonella* sp. (n=9) including *Salmonella enterica* and *Salmonella* Java, *Staphylococcus* sp. (n=6) including *Staphylococcus aureus*, *Vibrio* sp. (n= 9) including *Vibrio parahaemolyticus* and *Vibrio vulnificus*, *Yersinia ruckeri* (n=1), unknown (n=1).

Review synthesis primary question

Drinking water

Five studies were found linking colonisation/infection with consumption of drinking water.

Krovacek *et al.* (1989) describe the case of a young boy in Sweden (1.5 years old) who drank from a well and had long term diarrhoea. Ampicillin, penicillin and erythromycin resistant *A. hydrophila* was isolated from drinking water samples taken from the well. Once the boy ceased drinking the well water his diarrhoea stopped. However, no samples were taken from his stools to verify the well water was indeed the cause of his illness and other people and animals drinking from the well showed no signs of infection.

Bayram *et al.* (2011) reported on an outbreak of *S. enterica* serovar Typhi in Turkey. Clinical isolates were resistant to ampicillin, ampicillin/sulbactam, chloramphenicol, cefuroxime, amikacin, gentamicin and trimethoprim sulfamethoxazole. The authors hypothesised that the outbreak was probably caused by consumption of tap water contaminated with sewage.

An observational case-control study by The Campylobacter Sentinel Surveillance Scheme Collaborators (2002) investigated factors associated with acquisition of a ciprofloxacin-resistant *C. jejuni* infection in England and Wales. 55% of cases of Campylobacter infection acquired abroad were ciprofloxacin-resistant compared to those acquired in the UK (10%). Travel-associated ciprofloxacin-resistant infection cases were independently associated with travel to Spain, Portugal or Cyprus and the consumption of chicken or bottled water. On the other hand, in the UK this was associated with consumption of pre-cooked cold meats.

Coleman *et al.*, 2012 carried out an observational, cross-sectional study and found the risk of carriage of resistant *E. coli* was higher for users of water contaminated with resistant *E. coli*, with 41% of individuals carrying a resistant strain of E. coli and 28% carrying a multidrug-resistant strain. However, there were potential confounders in this study including households with children in nappies and frequent handling of raw meat.

Finally, an observational cross-sectional study by Rosenberg & Hernandez Duquino (1989) compared antibiotic resistant bacteria in mineral waters for purchase in Germany with clinical isolates. Nine species of *Pseudomonas* were found in in mineral waters, with *P. cepacia* and *P. maltophilia* showing the greatest antibiotic resistance. Similar resistance patterns were found between mineral water and clinical isolates. The authors concluded that immune compromised or chronically ill individuals should consider very carefully the consumption of mineral water given the presence of antibiotic resistant species associated with nosocomial infections.

Sea, brackish & fresh water contact & aquatic recreation pathways

Seven studies were found linking contact with sea, brackish or fresh water with colonisation/infection.

Three of the clinical case studies reported *Vibrio* sp. and *Aeromonas* sp. infection following wound exposure to sea, brackish or freshwater, with clinical isolates showing resistance to specific antibiotics.

Fernandez and Pankey (1975) described a case where a man with ant bites developed *Vibrio* sp. infection after paddling in seawater in the Gulf of Mexico. Although he was treated with tetracycline and penicillin, he later died. Culture specimens indicated the organism was similar to *V. parahaeolyticus* and was resistant to colistimethate *in vitro*. Joseph *et al.* (1979) described an *Aeromonas* spp. infection (*Aeromonas sobria* and *A. hydrophila*) in the leg wound of a scuba diver from US. The diver sustained a wound in brackish water and was admitted to hospital following a dive in sea water the following evening. Both isolates were resistant to ampicillin, carbenicillin, and cephalothin, but only *A. hydrophila* was resistant to tetracycline *in vitro*. However, despite these results the patient's infection responded to tetracycline therapy. *A. sobria* and *A. hydrophila* were also isolated from water samples where the infection took place and had the same biotype as those isolated from the infection wound and were resistant to tetracycline. Gold and Salit **(**1993) reported a case of cloxacillin-resistant *A. hydrophila* infection in a woman caused by micro-dermal abrasions after a

swimming incident in freshwater. The infection was subsequently treated successfully by surgically draining the wound.

Two case studies reported on ingestion of seawater leading to infection, with clinical and environmental isolates showing resistance to some antibiotics. Kelly *et al.* (1980) described a case study from US where a resuscitated drowning victim on an intensive care unit later developed pneumonia and septicaemia. Characteristics of lactose positive *Vibrio* sp. was isolated from seputum and blood samples and seawater samples from the drowning site. Environmental and clinical samples were similar and colistin-resistant. Goncalves *et al.* (1992) described the death of a previously healthy young man in Portugal who went swimming in the sea and subsequently died of *A. hydrophila* fulminant pneumonia. Bacterial isolates from the patient were ampicillin, carbenicillin and cephalothin resistant.

In a case control study in Norway Søraas *et al.* (2013) found that recreational swimming in freshwater is a risk factor for community-acquired urinary tract infections caused by extended spectrum beta-lactamases (ESBL) – producing *E. coli.*

Finally, a cross-sectional study conducted in the UK (Leonard 2016 unpublished) found that regular surfers were at an increased risk of being faecal carriers of cefotaxime-resistant *E. coli* (9.1%) compared to non-surfers (3.1%). Characterisation of these ARB revealed that the majority of cefotaxime-resistant *E. coli* were harbouring the mobile resistance gene, $bla_{\text{CTX-M}}$, and that many of these *E. coli* were the uropathogenic *E. coli*, ST131. Similar isolates were also found in coastal bathing waters in the UK. However, the study could not provide conclusive evidence that surfers were not colonised by CTX-M-producing bacteria before exposure.

Irrigation

Three studies reported that serotypes of *Salmonella* sp. found in irrigation water were the same as those found in outbreaks of infection.

Li *et al.* (2014) sampled irrigation pond water from vegetable produce farms between 2011 and 2013 in south eastern US. PulseNet patterns of some isolates were identical to strains associated with an outbreak of *Salmonella* Thompson in 2010, 2012, and 2013, *Salmonella* Enteritidis in 2011 and 2013, and *Salmonella* Javiana in 2012. *Salmonella* Newport isolates of the multidrug resistant-AmpC (MDR-AmpC) phenotype were found, exhibiting resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT), and to 1st, 2nd, and 3rd generations of cephalosporins (cephalothin, amoxicillin-clavulanic acid, and ceftriaxone). These isolates had a pulsed-field gel electrophoresis pattern indistinguishable from the patterns of the isolates from clinical settings. Clinically relevant *Salmonella* Virchow isolate (C243), was also found and demonstrated reduced susceptibility to kanamycin. *Salmonella* sp. prevalence varied with season and was significantly higher in summer months possibly influenced by temperature and precipitation.

Martinez *et al.* (2017) characterised *Salmonella* sp. from rivers and irrigation channels in Chile. Pulsotypes from seven *Salmonella* isolates (serotypes Enteritidis, Typhimurium and Infantis) were identical to outbreak-associated clinical isolates and almost all isolates showed a multidrug resistance phenotype. Frequency of detection was greater in rural compared to peri-urban or urban areas.

Micallef *et al.* (2013) identified *Salmonella* Braenderup, *S.* Javiana and *S.* Newport from irrigation water and *S.* Typhimurium from irrigation ditch soil from tomato farms in the US. These serotypes have previously implicated in *Salmonella* outbreaks associated with tomato consumption. There was widespread resistance to sulfisoxazole and some resistance to ampicillin, cefoxitin, amoxicillin/clavulanic acid, and tetracycline. To the authors knowledge at the date of publication all tomato-associated *Salmonella* sp. outbreaks reported by the Centre for Disease Control have been caused by susceptible strains.

Two articles by the same author investigated occupational exposure to antibiotic resistant bacteria in irrigation workers compared to office workers.

Goldstein *et al.* (2014) compared odds of colonisation with methicillin-resistant *S. aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), vancomycin-resistant enterococci (VRE), and vancomycin-susceptible enterococci (VSE) between spray irrigation workers using reclaimed water and office worker controls in the US. No significant difference was found but number of study participants was low.

Goldstein *et al.* (2017) investigated antibiotic-resistant coagulase-negative staphylococcus (CoNS) carriage in US spray irrigators exposed to reclaimed water compared to controls (office workers). Spray irrigators were significantly more likely to be carriers of antibiotic-resistant CoNS and methicillin-resistant coagulase-negative staphylococci (MRCoNS) compared to controls. However, the authors could not confirm that antibiotic-resistant CoNS, and MRCoNS originated from reclaimed water and the small number of subjects was a limitation of the experiment. Due to the short duration of the study it was also not known whether CoNS carriage influenced the risk of CoNS infection among the study participants. The authors suggest that this relationship has not been fully explored in the literature and therefore public health implications are unclear.

Seafood

Six studies were found linking seafood with infection.

Haenen *et al.* (2014) reported the case of a Dutch eel farmer who suffered a severe *V. vulnificus* biotype 2 (ST 112) zoonosis from his infected eels. The biotype had resistance to Cefox and intermediate resistance to Cefa but was treatable with other antibiotics. It was hypothesised that skin injuries and change in the immune status of farmer led to the zoonosis. Strains from the eels showed resistance to cefoxitin, and one strain showed increasing resistance to quinolones, trimethoprim and with sulphonamide, and tetracycline. The authors stated that "risk assessment and prevention are needed to protect farmers and processors of eels as well as brackish-water and marine fish. Virulence testing of *V. vulnificus* strains is recommended. Medics should be aware of the potential zoonotic risk of *V. vulnificus* infections in our temperate geographical zone associated with indoor eel farming." The authors also highlighted the fact that at the time of publication human infections with *V. vulnificus* were not yet notifiable in the Netherlands but they are in the US.

Fernandez and Pankey (1975) described the case of a man in Mexico who spiked himself on a crab (shell) he was preparing to eat and the resulting *Vibrio* sp. infection was treated with penicillin. Cultures showed *Vibrio* sp. that was resistant to colistimethate.

Hernandez-Diaz *et al.* (2015) conducted an epidemiological surveillance of *V. parahaemolyticus* strains in both environmental (eg. sediment, seawater and shrimp) and clinical samples (from clinical gastroenteritis patients who had eaten seafood and sought health care during the period 2011–2013) in Mexico. The first Mexican outbreak of gastroenteritis caused by pandemic *V. parahaemolyticus* strain O3:K6 was in 2004 with further recurrent sporadic cases that have spread in that geographic area. Serovars O3:K6 and O6:K46 were identified in both environmental and clinical strains. 93.8% of strains were resistant to ampicillin but sensitive to chloramphenicol (98.8%). However, multidrug resistance significantly increased from 2004 to 2013. The authors concluded that the pandemic O3:K6 clone has endemically established in the Pacific Coast of Mexico.

Dauros *et al.* (2011) investigated the genetic relationship, genotype dominance and antibiotic resistance of *V. parahaemolyticus* in two outbreaks from clinical and environmental sources in Chile in 2005 and 2007. Antibiotic resistance was found to ampicillin, cephalothin, cefoxitin, cefpodoxime, amikacin, streptomycin and kanamycin. The authors concluded "These results suggest the persistence and dominance of a unique pulsed-field gel electrophoresis (PFGE) clone of *V. parahaemolyticus* during 2005 and 2007, and the absence of genetic elements that capture antibiotic resistance genes described in other species of *Vibrio*."

Grevskott *et al.* (2017) investigated antibiotic resistant rates in *E. coli* and other Enterobacteriaceae isolates from marine bivalves in Norway. 38% of isolates were resistant to at least one antibiotic and 5% were multi-drug antibiotic, with one isolate resistant to 15 drugs. The authors found that multiple-locus variable number tandem repeat analysis (MLVA) profiles were associated with community-acquired *E. coil* strains causing bacteraemia.

Martinez-Urtaza *et al.* (2006) investigated *Salmonella* serotype Paratyphi B isolates obtained from shellfish and human infections in Spain for antibiotic resistance. The authors concluded that there is a potential risk to the public of acquiring enteric fever linked to eating raw shellfish. However, only two clinical isolates showed resistance to streptomycin out of any of the antibiotics tested.

Companion and wild animals

Four articles investigated the risk that companion and wild animals in aquatic environments pose to the dissemination of resistance to humans, three of which (Kirk *et al.*, 2006; OzFoodNet Working Group. 2004; Weir *et al.*, 2012 - a systematic review) all reported the same outbreak of *S.* Java infections (2003/2004) in Australia. Twenty two cases were reported of which 82% had contact with fish aquariums during the incubation period. Seventy-two per cent of cases were infected with strains that were resistant to ampicillin, streptomycin, tetracycline, chloramphenicol, sulfonamides, spectinomycin. Households affected reported high risk behaviours such as cleaning aquaria in sinks and 61% of cases reported fish in aquarium had been sick or died in the week prior to their illness. *S.* Java was also isolated from the water or gravel of 5 cases. The authors of these articles recommended the public to wash hands after cleaning and feeding fish and avoid cleaning aquaria in sinks.

One study looked at transmission of methicillin-resistant *S. aureus* (MRSA) in between humans and pilot whales at a rehabilitation centre in the US. Hower *et al.* (2013) sampled

human volunteers, whales, seawater, and sand from multiple sites at a marine mammal rehabilitation centre, nearby recreational beaches, and a canal and analysed them for MSSA and MRSA. The study identified a potential link between human and cetacean carriage of *S. aureus* which may be shed into an environment by humans or pilot whales and subsequently colonize or infect exposed new hosts. The authors concluded that "Future studies are needed to include sampling prior to the animals direct exposure to potentially colonized humans to determine the sources of *S. aureus* and subsequent risks to animals and people involved in their care."

Review synthesis secondary questions

What are the factors affecting the transmission of AMR and ARGs from the water environment into humans? E.g. exposure times and frequency, physical conditions in the environment etc.

Drinking water

The impact of rainfall on the occurrence of ARB was reported in four articles. Three articles were related to drinking water and investigated ARB in karstic hydro systems (aquifer) in France (Laroche *et al.*, 2010; Flores Riberio *et al.*, 2012 & 2014). Heavy rainfall and increased turbidity resulted in elevated prevalence of ARB (*E.coli* & *Pseudomonas* sp.). During turbid events Flores Riberio *et al.* (2014) isolated antibiotic resistant *Pseudomonas* sp. from final drinking water and concluded that "*Pseudomonas* could be involved in the dissemination of antibiotic resistance via drinking water during critical periods." Rosenberg & Hernandez Duquino (1989) also pointed out that people who are immunocompromised or chronically ill are most at risk from infection by ARB.

Sea, brackish & fresh water contact & aquatic recreation pathways

Environmental factors that may affect ARB/ARG transmission from recreational fresh and seawater to humans have not been directly proven, but may include season (Shaw *et al.*, 2014), weather (Haack *et al.*, 2003), landscape (Haack *et al.*, 2003), water depth (Hamelin *et al.*, 2006; Vignaroli *et al.*, 2013), beach zone (Plano *et al.*, 2013; Skórczewski *et al.*, 2014; Mudryk *et al.*, 2013) and re-suspension of sediments induced for example by recreational activities, natural events or fishing (e.g. Vignaroli *et al.*, 2013; Marion *et al.*, 2015). These factors are likely however, to be location specific, for example beach orientation (e.g. Haack *et al.*, 2003), seasonal influence of local sewage plants (e.g. Hamelin *et al.*, 2006), and natural (e.g. tidal currents) or local activities that increase re-suspension of sediments (e.g. Vignaroli *et al.*, 2013) or large recreational events (Marion *et al.*, 2015).

Different types of water sport may also increase risk. Leonard *et al.* (2015) assessed the amount of water ingested during different water sports (surfers, sea swimmers, divers and kayakers) and combined this with water sampling data to estimate people's exposure to bacteria resistant to antibiotics. While only 0.12% of *E. coli* found in coastal waters and rivers running into beaches were resistant to third generation cephalosporins, the authors stated that this was enough to present a potential risk of exposure to water users. Those at highest risk of exposure were surfers and sea swimmers due to their tendency to swallow more water.

Furthermore, as discussed above factors such as having an open wound or being wounded in sea water or fresh waters can increase the likelihood of antibiotic resistant infection with *Vibrio* sp. or *Aeromonas* sp. (e.g. Fernandez and Pankey., 1975; Joseph *et al.*, 1979; Gold & Salit, 1993).

Irrigation

Luo *et al.* (2015) found ARB in irrigation ponds in the USA, however they concluded that the influence of rainfall is complicated as it may either dilute pathogen concentration or suspend bacteria from sediments or pond banks making it available in the irrigation pond water.

Seafood

Very little evidence was found for the secondary question and seafood. Seasonal occurrence of ARB in fish and shellfish was mentioned in 7 papers (Depaola, Peeler & Rodrick, 1995; Martinez-Urtaza., 2004; Kayis *et al.*, 2009; Barkovskii *et al.*, 2010 & 2012; Lopatek *et al.*, 2015; Garrido-Maestu *et al.*, 2016). The limited evidence was conflicting, and may be influenced by differences in preferable conditions for particular bacterial species and the impact of local conditions and sources of antibiotic resistance.

Lopatek *et al.* (2015) and Kayis *et al.* (2009) found a greater occurrence of ARB in the spring and summer for shellfish in Poland (*V. parahaemolyticus*) and farmed rainbow fish in Turkey (ARB including *Y. ruckeri*) respectively. Garrido-Maestu *et al.* (2016) sampled cultivated mussels in Spain and found 78% of samples had a combination of tdh, trh and/or tdh/trh virulence factors and resistance to erythromycin. In 52% of samples *V. parahaemolyticus* possessed the ermB gene for resistance to erythromycin. No *Vibrio cholerae* (non-O1/non-O139) or *V. vulnificus* was found between June and October and the authors stated this reflected other researchers findings that there is low incidence of *V. vulnificus* and no detection of *V. cholera* in summer months. *V. parahaemolyticus* was found during October and September and again they stated that this was similar to other research that found higher densities of *V. parahaemolyticus* during warmer months. The authors also stated that *Vibrio* "abundance and distribution have been linked, to temperature and salinity, depending on the pathogen, its habitat, and the geographical location and that "dissolved oxygen, chlorophyll and plankton have also been found to be important in the ecology of vibrios."

Martinez-Urtaza *et al.* (2004) found isolates of antibiotic resistant *S. enterica* serovar Typhimurium varied in seasonal occurrence with some isolates exclusively detected in summer months, whist others were found exclusively in winter months. Barkovskii *et al.* (2012) investigated the occurrence of ARGs in pristine oyster reefs in the US and frequencies varied with season between two estuaries. One estuary was positively correlated to water turbidity, dissolved solids and potential water density and agricultural and municipal run off were the source of the ARG. No correlation was found in the other estuary but the authors suggested that there was ARG migration between the two estuaries possible due to physical conditions (currents, tides, wind), or animal movement (fish, oyster larvae or waterfowl).

Companion and wild animals

One study found that prevalence of antibiotic resistance in small mammals had a seasonal trend. Bennett (2004) investigated the role of wildlife as sources of resistance for domestic animals and man in the UK and found that the prevalence of antibiotic resistant *E. coli* in wood mice, peaked in late summer/early autumn, with a trough in late winter/early spring but no evidence for such seasonality was found for bank voles. Water samples taken from a lake bordering the mixed woodland where the study took place as well as puddles in the wood had similar prevalence of resistance to those within the woodland.

Another study found a possible relationship between prevalence of antibiotic resistant bacteria in marine mammals and algal blooms. Stewart *et al.* (2014) surveyed antibiotic-resistant bacteria isolated from bottlenose dolphins in the US. Antibiotic resistant bacteria varied temporally and an increase in one year may have been connected with a *Karenia brevis* red tide harmful algal bloom. The authors suggested proliferation of antibiotic-resistant bacteria may have occurred though increased quantities of decomposing fish in the water, or through exposure to brevetoxins and that the associations between antibiotic-resistant and harmful algal bloom events warrant further study. Some of the bacteria isolated were of clinical importance e.g. MRSA.

What evidence is there that exposure to the pathways is contributing to clinical infections that cannot be treated with existing antibiotics?

Clinical case studies (e.g. Fernandez & Pankey, 1975; Kelly *et al.*, 1980; Goncalves *et al.*, 1992; Gold & Sailt, 1993) have shown occurance of specific antibiotic resistance, but in every case, alternative antibiotics were available. However, environmental isolates have also shown resistance to a range of front line antibiotics (e.g. Joseph *et al.*, 1979; Micallef *et* al., 2012; Li *et al.*, 2014; Hernandez-Diaz *et al.*, 2015; Grevskott *et al.*, 2017). Shaw *et al.* (2014) studied antimicrobial susceptibility of *V. vulnificus* and *V. parahaemolyticus* from recreational and commercial areas of Chesapeake Bay and Maryland coastal bays. They found that, whilst antibiotics recommended to treat adult *Vibrio* sp. infections were susceptible, those recommended for paediatric treatment were not effective against some of the recovered isolates. Sikora *et al.* (2017) isolated azithromycin resistant *Legionella pneumophilia* from a sanatorium and concluded that therapeutic failures may be associated with bacterial resistance. Furthermore, a review included in this REA as additional information highlighted the risk of tap water to nosocomial transmission of antibiotic-resistant *Pseudomonas aeruginosa* (Muscarella, 2004).

Conclusions

Primary question: 'What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans?'

This REA found no studies showing direct causal evidence of a link between the pathways investigated and transmission of 'resistance' into humans, but did find observational and case studies that indicated a potential causal link between exposure to ARB/ARG through aquatic pathways and colonisation/infection in humans (e.g. Coleman *et al.*, 2012; Søraas *et al.*, 2013; Leonard 2016 unpublished; Goldstein *et al.*, 2017). The studies collated were highly heterogeneous and low in volume and therefore it is not possible at present, to identify which

pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans.

For example, Authors of studies that compared the similarity of ARB/ARG from aquatic environments to clinical isolates suggested that the similarities between environmental and clinical bacteria could be due to transmission of ARB/ARG from environment to humans (eg. Li *et al.*, 2014; Hernandez-Diaz *et* al., 2015; Grevskott *et* al., 2017; Martinez *et al.*, 2017). but, the observational design of the studies makes causal links difficult to prove. Also symptomatic bacterial infections associated with aquatic pathways are often sporadic in nature, and mediated by numerous factors such as extreme heatwaves (e.g. Huehn *et al.*, 2014), seawater contact with open wounds (e.g. Joseph *et al.*, 1979) or immune status (e.g. Haenen *et al.*, 2014) It is therefore perhaps not surprising that the volume of evidence is limited.

Other limitations of the research were study design, sample size, and existing confounders. For example, many studies were cross-sectional, making it uncertain whether people were colonised/infected prior to exposure (e.g. Kirk *et al.*, 2006; Coleman *et al.*, 2012; Hower *et al.*, 2013; Goldstein *et al.*, 2014; Leonard 2016 unpublished). The correlative nature of the studies suggested that there are transmission pathways but there was often insufficient evidence to conclude this was actually occurring (e.g. Krovacek *et al.*, 1989; Coleman *et al.*, 2012; Martinez *et al.*, 2017; Leonard 2016 unpublished; Goldstein *et al.*, 2017).

Whilst the 'best quality' evidence is likely to come from randomised controlled trials and prospective cohort studies, antibiotic resistant (symptomatic) infections in healthy people are likely to be rare, and so sample size would have to be impractically large. It may be possible to look for other markers of infection such as gut or skin colonisation by resistant bacteria but these conditions are probably asymptomatic and transient in the individual, although there exists the potential to pass on resistant bacteria to other, vulnerable members of the community. Studies to examine the effects of direct or indirect transmission to humans are therefore difficult and potentially very expensive (Leonard *et al.*, 2015) and possibly the reason why these types of study are uncommon in the literature.

There is a large body of related research that has not been considered in this REA, but that may also contribute to decision-making in this area.

Examples of research that does not provide evidence of dissemination into humans, but may inform decision-making include:

- Research investigating the occurrence of AMR, ARB and ARG in the aquatic environment, such as in fish, rivers, coastal waters, flood water, irrigation water and irrigated food .
- Evolution of clinically important AMR in the aquatic environment with increased prevalence, and potential risk of transmission downstream of waste water treatment plants (e.g. Amos 2013 unpublished; Amos *et al.*, 2014).
- The identification of small mammals as potentially useful environmental sentinels of AMR transmission from the aquatic environment into mammalian hosts. For example, Furness *et al.* (2017) found proximity to a polluted water body increased the likelihood of carriage of AMR *E. coli* in small mammals which excreted greater numbers of AMR *E. coli* and a greater diversity of *E. coli* phylotypes, including

human-associated pathogenic strains. Carriage of cefotaxime resistant *E. coli* was also only found in the small mammals associated with polluted water source.

- Studies that show that bathers are at a significantly higher risk of experiencing a variety of symptoms of ill health compared to non-bathers (e.g. Leonard unpublished 2016) and whilst resistance was not measured, experts have speculated that a subset or even majority of bacteria will be resistant in some way.

In conclusion, there is evidence that diverse AMR/ARB/ARG are present in aquatic environments/pathways, and the literature suggests that the human microbiome is linked with microbial communities in these environments. It is therefore possible for people coming into contact with these aquatic pathways containing ARB/ARG to become colonised and either subsequently develop a drug-resistant infection, or pass on ARB/ARG to other members of the community who are more vulnerable to developing drug-resistant infections (e.g. immunocompromised individuals). If colonisation or infection by ARB/ARG is dependent on the dose people are exposed to, then any factor that increases the abundance of ARB/ARG in water bodies/seafood/irrigated food/companion animals/drinking water or factors that increase the volume of water ingested (e.g. particular water sports such as swimming), would increase the likelihood of developing colonisation/infection status. Likewise, the health and immune status of the exposed individual might interact with these factors to mediate infection. People with pre-existing injuries/wounds or those that sustain them in aquatic environments and people who are immunocompromised or chronically ill are at greatest risk of antibiotic resistant infection (e.g. Fernandez & Pankey, 1975; Joseph *et al.*, 1979 Gold & Salit., 1993; Haenen *et al.*, 2014). Healthy people can usually fight off infection and do not need antibiotics, but the elderly and young children may also be more susceptible. Many authors highlighted that medical practitioners need to be aware of the bacterial infections associated with the pathways in this REA not only in terms of diagnosis and treatment but also in making recommendations to reduce risk to immunocompromised and chronically ill patients.

This REA identifies a research gap that needs to be addressed to better understand the role of these pathways in the spread of resistant bacteria to people and their impacts on human health so that relevant authorities are better placed to develop effective strategies for dealing with the risks.

Secondary questions

What are the factors affecting the transmission of AMR and ARGs from the water environment into humans? E.g. exposure times and frequency, physical conditions in the environment etc.

The variables that lead to increased environmental exposure are to some extent independent of AMR and so this literature will not have been searched for and collated in this review. AMR exposure is a function of total microbial exposure and AMR prevalence in that population.

Evidence not included in this REA has shown that high flow events increase ARGs and faecal indicator organisms (FIOs) loads in streams, rivers, estuaries and coastal waters (e.g. Wilkinson *et al.*, 2006; Kay *et al.*, 2008; Stapleton *et al.*, 2008; Garner *et al.*, 2017) consequently affecting drinking, recreational and shellfish water protection. Mechanisms that increase loads under these conditions are varied, including surface run off and its source (e.g. Edwards *et al.*, 2008) and the re-suspension of faecal indicator organisms (FIOs) stored in sediments entrained into flowing water (e.g. Wilkinson *et al.*, 2006).

As mentioned above, colonisation or infection by ARB/ARG is dependent on the dose to which people are exposed. Factors that increase the occurrence of ARB/ARG and the volume of water ingested, increase likelihood of developing colonisation/infection. It is therefore prudent to ensure immersion and ingestion of water (and consumption of shellfish) is minimised particularly during changes in flow conditions (e.g. Amos 2013 unpublished; Wilkinson *et al.*, 2006; Leonard *et al.*, 2015). This is particularly relevant to some water sports such as swimming and surfing where larger amounts of water are likely to be ingested (Leonard *et al.*, 2015). It should be noted that high flow events can also be artificially induced for recreational sports, such as canoeing and white water rafting and the same recommendations apply.

Other activities that increase the re-suspension of sediments such as dredging for shellfish (e.g. Vignaroli *et al.*, 2013) or large recreational events (Marion *et al.*, 2015) have also been shown to increase loads of ARB.

Local catchment conditions and mitigation measures in place to improve microbiological standards are also likely to impact ARB and ARG transmission as are other local factors mentioned previously such as beach orientation (e.g. Haack *et al.*, 2003), seasonal influence of local sewage plants (e.g. Hamelin *et al.*, 2006) and tidal currents. It is difficult therefore to provide blanket recommendation as advice would have to be tailored for specific locations.

Whilst evidence for water temperature increasing likelihood of transmission of ARB/ARG to humans was lacking, there is evidence that some bacterial infections occur more frequently in warm weather. For example, Gold & Salit, 1993 found *Aeromonas* sp. infections occurred more frequently in warm weather; Huehn *et al.* (2014) reported that in Germany the incidence of *Vibrio* sp. infections (usually related contact of seawater with wounds) increases after extreme heatwaves and *Vibrio* sp. infection in the US shows a clear seasonal peak in the summer (Yoder *et al.*, 2008). Le Roux *et al.* (2015) in a recent workshop to investigate the emergence of *Vibrio* sp. pathogens in Europe reported higher prevalence of human infections followed regional climatic trends with outbreaks occurring during episodes of unusually warm weather. Sea surface temperatures have increased in Europe and have been linked to outbreaks of *Vibrio*-associated human illness caused by *V. cholerae* non O1-non-O139, *V. parahaemolyticus*, and *V. vulnificus* in several European countries (Le Roux *et al.*, 2015). Lack of mandatory notification systems in Europe for *Vibrio* sp. associated illnesses, however, prevents accurate estimates of the number of *Vibrio* sp. infections occurring (Le Roux *et al.*, 2015) and it is not known to what extent antibiotic resistance is an issue. Warm saline environments with temperatures greater than 15^{o}C favour the growth of *Vibrio* spp. and it is speculated that warming of marine saline inland waters in Europe due to climate change is likely to increase populations of *Vibrio* spp. and the risk of infection (Le Roux *et al.*,

2015). Furthermore, elevated water temperatures may also facilitate successful invasion of pathogenic variants via food trade or wildlife (e.g. migrating birds) (Le Roux *et al.*, 2015).

As mentioned above, factors such as having an open wound or being wounded in sea water or fresh waters can increase the likelihood of antibiotic resistant infection with *Vibrios* sp. or *Aeromonas* sp. (e.g. Fernandez and Pankey., 1975; Joseph *et al*., 1979; Gold & Salit, 1993) and authors have highlighted that those most at risk from ARB are people who have a compromised immune system or are chronically ill (e.g. Rosenberg & Hernandez Duquino, 1989).

What evidence is there that exposure to the pathways is contributing to clinical infections that cannot be treated with existing antibiotics?

No evidence was found for this secondary question. This reflects current expert opinion that the number of infections that cannot be treated with existing antibiotics resulting from any type of exposure pathway, not just aquatic, is still rare (e.g. programmatically incurable tuberculosis (Dheda *et al*., 2017)). However, clinical antibiotic resistance is an increasing problem, there is evidence for the occurrence of antibiotic resistance in the aquatic environment and some evidence suggesting evolution of clinically important AMR. Moreover, experts speculate that that exposure outcomes will lead to increased gut resistome in humans which is of major concern. This combined with albeit more limited evidence for transmission of 'resistance' to humans suggests that the precautionary principle be followed and that action to mitigate AMR/ARB/ARG contamination of the environment and transmission pathways is needed now to prevent future problems arising.

Implications

Observational and case studies included in this REA indicate a potential causal relationship between exposure to ARB/ARG through aquatic pathways and colonisation/infection in humans. Factors that increase ingestion of water such as swimming and surfing, and the health, and immune status of people are also likely to influence the risk of colonisation/infection.

Overall, the evidence collated for the primary and secondary questions was highly heterogeneous and low in volume making it impossible to identify which pathways are most responsible for transmission into humans and difficult to make blanket recommendations to reduce environmental exposure due to factors which are often location specific (e.g. tidal currents). The lack of direct causal evidence does not mean that transmission is not occurring but rather that this type of evidence is difficult to obtain as the types of studies that generate this data are often too complex to undertake, cost prohibitive or would be unethical to perform.

The biology of AMR/ARB/ARG in the aquatic environment including the potential for transmission through aquatic pathways to humans is a highly complex subject. The evidence presented in this REA should be considered in combination with other evidence/components of the topic (e.g. occurrence, evolution, and increasing clinical antibiotic resistance) when considering implications for policy and practice. For example, a large amount of literature was excluded from the REA that showed the presence of diverse AMR, ARB and ARGs in

aquatic pathways. The literature suggested that the human microbiome is linked with microbial communities in these environments. It is therefore possible for people coming into contact with these aquatic pathways to become colonised and either subsequently develop a drug-resistant infection, or pass on ARB/ARGs to other members of the community who are more vulnerable to developing drug-resistant infections (e.g. immunocompromised individuals). When all the evidence is considered it suggests a precautionary principle be followed and that action is needed to mitigate AMR/ARB/ARG contamination of the aquatic environment and potential transmission pathways.

Implications for policy and practice

There is a gap in the knowledge base to fully understand:

- What pathways are most responsible for disseminating 'resistance' from the aquatic environment into humans
- Which factors affect the transmission of AMR and ARGs from the water environment into humans

Authors highlighted the following:

- Awareness about the risk of posttraumatic wound infection by bacteria from the sea, brackish and fresh water (Joseph et al., 1979; Gold & Salit, 1993).
- Advice for people to wash hands after cleaning and feeding fish and avoid using sinks to clean out aquaria (Kirk *et al*., 2006; OzFoodNet Working Group, 2004).
- Awareness that people with pre-existing injuries/wounds or those that sustain them in aquatic environments and people who are immunocompromised or chronically ill are at greatest risk of antibiotic resistant infection (Fernandez & Pankey, 1975; Joseph et al., 1979 Gold & Salit., 1993; Haenen et al., 2014).
- Potential risks of water sports for transmission from the aquatic environment into humans, especially those that are immersive/increase likelihood of water ingestion (Leonard *et al*., 2015; Leonard 2016 unpublished).
- Lack of mandatory notification systems for some bacteria/bacterial infections in some countries or states (e.g. *Vibrio* sp.) to understand the extent of the problem and guide appropriate prevention messages (Le Roux *et al*., 2015; Yoder *et al*., 2008), (these systems may not record antibiotic resistance but may help prevent infection).
- During heavy rainfall bacteria maybe involved in the dissemination of antibiotic resistance via the aquatic environment (Riberio *et al*., 2014).

Implications for research

The following research gaps have been identified by this REA:

- Primary research to investigate how the natural environment spread ARB to the human microbiome.

- Primary research to understand the direction of transmission of ARB/ARG between humans and the environment.

- Primary research to investigate the health effects of exposure to ARB/ARG in aquatic environments, including transmission resistant bacteria to other (especially vulnerable) members of the community.

- Epidemiological and numerical modelling studies, to fully understand the role of aquatic exposure routes to assess real risk to human health.

- A review of the evidence to better understand to what extent does the transfer of genes play a role in the spread of resistance and what are the implications of this to human health.

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Competing interests

Financial competing interests –The authors have been commissioned and funded by the Department of Environment Food and Rural Affairs to carry out this research.

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References

Amos G C A, Zhang L, Hawkey P M, Gaze W H, Wellington E M. (2014). Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes. Veterinary Microbiology 171 (3–4): 441-447.

Barkovskii A L, Green C, Hurley D. (2010). The occurrence, spatial and temporal distribution, and environmental routes of tetracycline resistance and integrase genes in Crassostrea virginica beds. Marine Pollution Bulletin 60(12):2215-2224.

Barkovskii A L, Thomas M, Hurley D, Teems C. (2012). Environmental factors responsible for the incidence of antibiotic resistance genes in pristine Crassostrea virginica reefs. Marine Pollution Bulletin 64(12):2692-2698.

Bayram Y, Guducuoglu H, Otlu B, Aypak C, Gursoy N C, Uluc H, Berktas M. (2011). Epidemiological characteristics and molecular typing of Salmonella enterica serovar Typhi during a waterborne outbreak in Eastern Anatolia. Annals of Tropical Medicine and Parasitology 105(5):359-365.

Bennett M. (2004). Wild rodents as reservoirs of antibiotic resistance for farm animals and man - current status, origins and mechanisms of persistance. Final project report for Defra. Defra project code OD2009.

Berglund B. (2015). Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infection, Ecology and Epidemiology 2015; 5: 10.3402/iee.v5.28564. Published online 2015 Sep 8. doi:10.3402/iee.v5.28564.

Byrne-Bailey K G, Gaze W H, Zhang L, Kay, P, Boxall A P, Hawkey P M, Wellington E M H. (2011) Integron Prevalence and Diversity in Manured Soil. Applied Environmental Microbiology 77(2): 684–687.

Campylobacter Sentinel Surveillance Scheme Collaborators (2002). Ciprofloxacin resistance in Campylobacter jejuni: case-case analysis as a tool for elucidating risks at home and abroad. Journal of Antimicrobial Chemotherapy 50(4):561-568.

Coleman B L, Salvadori M I, McGeer A J, Sibley K A, Neumann N F, Bondy S J, Gutmanis I A, McEwen S A, Lavoie M, Strong D, Johnson I, Jamieson F B, Louie M. (2012). The role of drinking water in the transmission of antimicrobial-resistant E. coli. Epidemiology and Infection, 140(4):633-642.

Coleman B L, Salvadori M I, McGeer A J, Sibley K A, Neumann N F, Bondy, S J, Gutmanis I A, McEwen S A, Lavoie M, Strong D, Johnson I, Jamieson F B, Louie M. (2012) The role of drinking water in the transmission of antimicrobial-resistant E. coli. Epidemiological Infections. 2012 Apr;140(4):633-42. doi: 10.1017/S0950268811001038. Epub 2011 Jun 23.

Collins A M, Coughlin D, Miller J, Kirk S. (2015). The Production of Quick Scoping Reviews and Rapid Evidence Assessments: A How to Guide. Available at: https://connect.innovateuk.org/documents/3058188/3918930/JWEG%20HtG%20Dec2015v2 [Accessed 30.01.17]

Dauros P, Bello H, Domínguez M, Hormazábal J C, González G. (2011). Characterization of Vibrio parahaemolyticus strains isolated in Chile in 2005 and in 2007. Journal of Infection in Developing Countries 5(7):502-510.

Depaola A, Peeler J T, Rodrick G E. (1995). Effect of oxytetracycline-medicated feed on antibiotic-resistance of gram-negative bacteria in catfish ponds. Applied and Environmental Microbiology, 61(6):2335-2340.

Dheda K, Gumbo T, Maartens G, Dooley K E, McNerney R, Murray M, Furin J, Nardell E A, London L, Lessem E, Theron G, van Helden P, Niemann S, Merker M, Dowdy D, Van Rie A, Siu GK, Pasipanodya J G, Rodrigues C, Clark T G, Sirgel F A, Esmail A, Lin H H, Atre S R, Schaaf H S, Chang K C, Lange C, Nahid P, Udwadia Z F, Horsburgh C R Jr, Churchyard G J, Menzies D, Hesseling A C, Nuermberger E, McIlleron H, Fennelly K P, Goemaere E, Jaramillo E, Low M, Jara C M, Padayatchi N, Warren R M. (2017) The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med. 2017 Mar 15. pii: S2213-2600(17)30079-6. doi: 10.1016/S2213-2600(17)30079-6. [Epub ahead of print].

Edwards A C, Kay D, McDonald A T, Francis C, Watkins J, Wilkinson JR, Wyer M D. (2008). Farmyards, an overlooked source for highly contaminated runoff. J Environ Manage. 87(4):551-9.

FCO. (2013). G8 Science Ministers Statement. Available at: https://www.gov.uk/government/news/g8-science-ministers-statement [Accessed 30.01.17]

Fernandez C R, Pankey G A. (1975). Tissue invasion by unnamed marine vibrios. Journal of the American Medical Association 233(11):1173-1176.

Flores Ribeiro A, Bodilis J, Alonso L, Buquet S, Feuilloley M, Dupont J P, Pawlak B (2014). Occurrence of multi-antibiotic resistant Pseudomonas spp. in drinking water produced from karstic hydrosystems. Science of The Total Environment 490:370-378.

Flores Ribeiro A, Laroche E, Hanin G, Fournier M, Quillet L, Dupont J P, Pawlak B. (2012). Antibiotic-resistant Escherichia coli in karstic systems: a biological indicator of the origin of fecal contamination? FEMS Microbiology Ecology 81(1):267-280.

Furness L E, Campbell A, Zhang L, Gaze W H, McDonald R A. (2017). Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. Environmental Research 154:28-34.

Garner E, Benitez R, von Wagoner E, Sawyer R, Schaberg E, Hession W C, Krometis L H, Badgley B D, Pruden A. (2017). Stormwater loadings of antibiotic resistance genes in an urban stream. Water Res.123:144-152.

Garrido-Maestu A, Lozano-León A, Rodríguez-Souto R R, Vieites-Maneiro R, Chapela M J, Cabado A G. (2016). Presence of pathogenic Vibrio species in fresh mussels harvested in the southern Rias of Galicia (NW Spain). Food Control 59:759-765.

Gaze W H, Zhang L, Abdouslam N A, Hawkey P M, Calvo-Bado L, Royle J, Brown H, Davis S, Kay P, Boxall A B, Wellington E M. (2011). Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. ISME J. 2011 Aug ;5(8):1253-61. doi: 10.1038/ismej.2011.15. Epub 2011 Mar 3.

Gold W L, Salit I E (1993).Aeromonas hydrophila Infections of Skin and Soft Tissue: Report of 11 Cases and Review. Clinical Infectious Diseases 16(1):69-74.

Goldstein R E. R, Micallef S A, Gibbs S G, He X, George A, Sapkota A, Joseph S W, Sapkota A R. (2014). Occupational Exposure to Staphylococcus aureus and Enterococcus spp. among Spray Irrigation Workers Using Reclaimed Water. International Journal of Environmental Research and Public Health 11(4):4340-4355.

Goldstein R R, Kleinfelter L, He X, Micallef S A, George A, Gibbs S G, Sapkota A R. (2017). Higher prevalence of coagulase-negative staphylococci carriage among reclaimed water spray irrigators. Science of the Total Environment 595: 35-40.

Goncalves J R, Brum G, Fernandes A, Biscaia I, Correia M J, Bastardo J. (1992). Aeromonas hydrophila fulminant pneumonia in a fit young man. Thorax 47(6):482–483.

Grevskott D H, Svanevik C S, Sunde M, Wester A L, Lunestad B T. (2017). Marine Bivalve Mollusks As Possible Indicators of Multidrug-Resistant Escherichia coli and Other Species of the Enterobacteriaceae Family. Frontiers in Microbiology 8:24.

Gullberg E, Cao S, Berg O G, Ilbäck C, Sandegren L, Hughes D., Andersson D I. (2011). Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7:e1002158. doi:10.1371/journal.ppat.1002158

Haack S K, Fogarty L R, Wright C. (2003). Escherichia coli and enterococci at beaches in the Grand Traverse Bay, Lake Michigan: Sources, characteristics, and environmental pathways. Environmental Science and Technology 37(15): 3275-3282.

Haenen O L. M, van Zanten , E , Jansen R, Roozenburg I, Engelsma M Y, Dijkstra A, Boers S A, Voorbergen-Laarman M, Moller A V. M. (2014). Vibrio vulnificus outbreaks in Dutch eel farms since 1996: strain diversity and impact. Diseases of Aquatic Organisms 108(3):201-209.

Hamelin K, Bruant G, El-Shaarawi A, Hill S, Edge T A, Bekal S, Fairbrother J M, Harel J, Maynard C, Masson L, Brousseau R. (2006). A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in Escherichia coli isolates from Great Lakes recreational waters. Applied and Environmental Microbiology 72(6):4200-4206.

Hernandez-Diaz L D, Leon-Sicairos N, Velazquez-Roman J, Flores-Villasenor H, Guadron-Llanos A M, Martinez-Garcia J J, Vidal J E, Canizalez-Roman A. (2015). A pandemic Vibrio parahaemolyticus O3:K6 clone causing most associated diarrhea cases in the Pacific Northwest coast of Mexico. Frontiers in Microbiology 6:221.

Heuer O E, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo F J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis. 2009 Oct 15;49(8):1248-53. doi: 10.1086/605667.

Hower S, Phillips M C, Brodsky M, Dameron A, Tamargo M A, Salazar N C, Jackson C R, Barrett J B, Davidson M, Davis J, Mukherjee S, Ewing R Y, Gidley M L, Sinigalliano C D, Johns L, Johnson F E, 3rd , Adebanjo O, Plano L R. (2013). Clonally related methicillin-resistant Staphylococcus aureus isolated from short-finned pilot whales (Globicephala macrorhynchus), human volunteers, and a bayfront cetacean rehabilitation facility. Microbial Ecology 65(4):1024-1038.

Huehn S, Eichhorn C, Urmersbach S, Breidenbach J, Bechlars S, Bier N, *et al*. (2014). Pathogenic vibrios in environmental seafood and clinical sources in Germany. Int.J.Med.Microbiol. 304,843–850.doi:10.1016/j.ijmm.2014. 07.010.

Jiang X, Hashim Ellabaan M M, Charusanti P, Munck C, Blin K, Tong Y, Weber T, Sommer M O A, Yup Lee S Y. (2017) Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nature Communications 8:15784 doi: 10.1038/ncomms15784.

Joseph S W, Daily O P, Hunt W S, Seidler R J, Allen D A, Colwell R R. (1979). Aeromonas primary wound infection of a diver in polluted waters. Journal of Clinical Microbiology 10(1):46-49.

Kay D, Kershaw S, Lee R, Wyer M D, Watkins J, Francis C. (2008). Results of field investigations into the impact of intermittent sewage discharges on the microbiological quality of wild mussels (Mytilus edulis) in a tidal estuary. Water Res. 42(12):3033-46.

Kayis S, Capkin E, Balta F, Altinok I. (2009). Bacteria in Rainbow Trout (Oncorhynchus mykiss) in the Southern Black Sea Region of Turkey - A Survey. Israeli Journal of Aquaculture-Bamidgeh 61(4):339-344.

Kelly M T, Avery D M. (1980). Lactose-positive Vibrio in seawater: a cause of pneumonia and septicemia in a drowning victim. Journal of Clinical Microbiology 11(3):278-280.

Kelly R, Zoubiane G, Walsh D, Ward R, Goossens H. (2016) Public funding for research on antibacterial resistance in the JPIAMR countries, the European Commission, and related European Union agencies: a systematic observational analysis. Lancet Infectious Diseases 16: 431–40.

Kinney C A, Furlong E T, Zaugg S D, Burkhardt M R, Werner S L, Cahill J D, Jorgensen G R. (2006). Survey of organic wastewater contaminants in biosolids destined for land application. Environmental Sciences and Technology 40: 7207-7215.

Kirk M, Combs B G, Musto J, Mwanri L, Lightfoot D (2006). Multi-drug resistant Salmonella Java infections acquired from tropical fish aquariums, Australia, 2003-04. Communicable Diseases Intelligence Quarterly Report 30(2):222-227.

Krovacek K, Peterz M, Faris A, Mansson I. (1989). Enterotoxigenicity and drug sensitivity of Aeromonas hydrophila isolated from well water in Sweden: a case study. International Journal of Food Microbiology 8(2):149-154.

Laroche E, Petit F, Fournier M, Pawlak B. (2010). Transport of antibiotic-resistant Escherichia coli in a public rural karst water supply. Journal of Hydrology, 392(1-2):12-21.

Le Roux F K, Wegner M, Baker-Austin C, Vezzulli L, Osorio C R, Amaro C, Ritchie J M, Defoirdt T, Destoumieux-Garzón D, Blokesch M, Mazel D, Jacq A, Cava F, Gram L, Wendling C C, Strauch E, Kirschner A, Huehn S. The emergence of Vibrio pathogens in Europe: ecology, evolution, and pathogenesis (Paris, 11–12th March 2015). PERSPECTIVE ARTICLE. Front. Microbiol., 13 August 2015 https://doi.org/10.3389/fmicb.2015.00830 [Accessed 20.07.17].

Leonard A F C (2016). The beach bum survey. In: Are bacteria in the coastal zone a threat to human health? Exeter University, Unpublished PhD Thesis.

Leonard A F C, Zhang L, Balfour A J, Garside R, Gaze W H. (2015). Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environment International 82:92-100.

Li B G, Vellidis G, Liu H L, Jay-Russell M, Zhao S H, Hu Z L, Wright A, Elkins C A. (2014). Diversity and Antimicrobial Resistance of Salmonella enterica Isolates from Surface Water in Southeastern United States. Applied and Environmental Microbiology 80(20): 6355-6365.

Lopatek M, Weczorek K, Osek J. (2015). Prevalence and Antimicrobial Resistance of Vibrio parahaemolyticus Isolated from Raw Shellfish in Poland. Journal of Food Protection 78(5):1029-1033.

Luo Z, Gu G, Ginn A, Giurcanu M C, Adams P, Vellidis G, van Bruggen , A H, Danyluk M D, Wright A C. (2015). Distribution and Characterization of Salmonella enterica Isolates from Irrigation Ponds in the South eastern United States. Applied and Environmental Microbiology 81(13):4376-4387.

Marion J W, Burrowes V, Lee C S, and Lee J. (2015). Changes in microbial water quality associated with an extreme recreational water event in Ohio, United States. Water Quality, Exposure and Health 7(4):491-501.

Martinez M C, Retamal P, Rojas-Aedo J F, Fernandez J, Fernandez A, Lapierre L. (2017). Multidrug-Resistant Outbreak-Associated Salmonella Strains in Irrigation Water from the Metropolitan Region, Chile. Zoonoses Public Health 64 (4): 299–304.

Martinez-Urtaza J, Echeita A, Liebana E. (2006). Phenotypic and genotypic characterization of Salmonella enterica serotype paratyphi B isolates from environmental and human sources in Galicia, Spain. Journal of Food Protection 69(6):1280-1285.

Martinez-Urtaza J, Liebana E, Garcia-Migura L, Perez-Pineiro P, Saco M. (2004). Characterization of Salmonella enterica serovar Typhimurium from marine environments in coastal waters of Galicia (Spain). Applied and Environmental Microbiology 70(7):4030-4040.

Micallef S A, Goldstein R E, George A, Ewing L, Tall B D, Boyer M S, Joseph S W, Sapkota A R. (2013). Diversity, distribution and antibiotic resistance of Enterococcus spp. recovered from tomatoes, leaves, water and soil on U.S. Mid-Atlantic farms. Food Microbiology 36(2):465-474.

Mudryk Z J, Kosiorek A, Perliński P. (2013). In vitro antibiotic resistance of Vibrio-like organisms isolated from seawater and sand of marine recreation beach in the southern Baltic Sea. Hydrobiologia 702:141-150.

Muscarella L.F. (2004) Contribution of tap water and environmental surfaces to nosocomial transmission of antibiotic-resistant Pseudomonas aeruginosa.Infection Control and Hospital Epidemiology 25(4):342-345.

O'Neill J. (2015). The Review on Antimicrobial Resistance 2015. Antimicrobials in Agriculture and the Environment: Reducing unnecessary Use and Waste. Available at: https://amr-review.org/sites/default/files/Antimicrobials%20in%20agriculture%20and%20the%20environment%20-%20Reducing%20unnecessary%20use%20and%20waste.pdf [Accessed 30.01.17]

O'Neill J. (2016). The Review on Antimicrobial Resistance 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Available at: https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf [Accessed 30.01.17]

OzFoodNet Working Group (2004) Foodborne disease investigation across Australia: annual report of the OzFoodNet network, 2003. Communicable Diseases Intelligence Quarterly Report 28(3): 359-389

Plano L R, Shibata T, Garza A C, Kish J, Fleisher J M, Sinigalliano C D, Gidley M L, Withum K, Elmir S M, Hower S, Jackson C R, Barrett J B, Cleary T, Davidson M, Davis J, Mukherjee S, Fleming L E, Solo-Gabriele H M. (2013). Human-associated methicillin-resistant Staphylococcus aureus from a subtropical recreational marine beach. Microbial Ecology 65(4):1039-1051.

Rosenberg F A, Hernandez Duquino H. (1989). Antibiotic resistance of Pseudomonas from German mineral waters. Toxicity Assessment, 4(3):281-294.

Shaw K S, Goldstein R E R, He X, Jacobs J M, Crump B C, Sapkota A R. (2014). Antimicrobial Susceptibility of Vibrio vulnificus and Vibrio parahaemolyticus Recovered from Recreational and Commercial Areas of Chesapeake Bay and Maryland Coastal Bays. Plos One, 9(2):11.

Sikora A, Gladysz I, Koziol-Montewka M, Wojtowicz-Bobin M, Stanczak T, Matuszewska R, Krogulska B. (2017). Assessment of antibiotic susceptibility of Legionella pneumophila isolated from water systems in Poland. Annals of Agricultural and Environmental Medicine 24(1):66-69.

Skórczewski P, Mudryk Z, Miranowicz J, Perliński P, Zdanowicz M. (2014). Antibiotic resistance of Staphylococcus-like organisms isolated from a recreational sea beach on the southern coast of the Baltic Sea as one of the consequences of anthropogenic pressure. Oceanological and Hydrobiological Studies 43(1): 41-48.

Søraas A, Sundsfjord A, Sandven I, Brunborg C, Jenum P A (2013). Risk Factors for Community-Acquired Urinary Tract Infections Caused by ESBL-Producing Enterobacteriaceae –A Case–Control Study in a Low Prevalence Country. PLoS One 8(7): e69581.

Stapleton C M, Wyer M D, Crowther J, McDonald A T, Kay D, Greaves J, Wither A, Watkins J, Francis C, Humphrey N, Bradford M. (2008). Quantitative catchment profiling to apportion faecal indicator organism budgets for the Ribble system, the UK's sentinel drainage basin for Water Framework Directive research. J Environ Manage. 87(4):535-50.

Stewart J R, Townsend F I, Lane S M, Dyar E, Hohn A A, Rowles T K, Staggs L A, Wells R S, Balmer B C, Schwacke L H. (2014). Survey of antibiotic-resistant bacteria isolated from bottlenose dolphins Tursiops truncatus in the southeastern USA. Diseases of Aquatic Organisms 108(2):91-102.

Vignaroli C, Luna G M, Pasquaroli S, Cesare A di, Petruzzella R, Paroncini P, Biavasco F. (2013). Epidemic Escherichia coli ST131 and Enterococcus faecium ST17 in coastal marine sediments from an Italian beach. Environmental Science and Technology 47(23):13772-13780.

Weir M, Rajic´ A, Dutil L, Cernicchiaro N, Uhland F C, Mercier B, Tusevljak N. (2012). Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: a

systematic review of the existing research and survey of aquaculture-allied professionals. Epidemiology and Infection 140(2):192-206.

Wellington E M, Boxall A B, Cross P, Feil E J, Gaze W H, Hawkey P M, Johnson-Rollings A S, Jones D L, Lee N M, Otten W, Thomas C M, Williams A P. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. Lancet Infect Dis. 2013 Feb;13(2):155-65. doi: 10.1016/S1473-3099(12)70317-1.

Wilkinson J, Kay D, Wyer M, Jenkins A. (2006). Processes driving the episodic flux of faecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. Water Res. 40(1):153-61.

Yoder J S, Hlavsa M C, Craun G F, Hill V, Roberts V, Yu P A, Hicks L A, Alexander N T, Calderon R L, Roy S L, Beach M J (2008). Surveillance for Waterborne Disease and Outbreaks Associated with Recreational Water Use and Other Aquatic Facility-Associated Health Events --- United States, 2005—2006. Mortality and Morbidity Weekly Report. Surveillance Summaries. September 12 2008/57(ss09) 57(9):1-29

Young H K. (1993). Antimicrobial resistance spread in aquatic environments. J Antimicrob Chemother. 1993 May; 31(5):627-35.