# Where have the genes associated with antimicrobial resistance been reported in global aquaculture: A systematic map protocol.

Justin Kemp*, Renee Larocque & Armando Heriazon

*jkemp@idrc.ca, International Development Research Centre (IDRC), Ottawa, Canada.

## Abstract

### *Background*

The inappropriate use of antimicrobials in human medicine and animal husbandry creates selective pressures that favour the emergence of antimicrobial resistant organisms. Antibiotic consumption in livestock production likely exceeds human use and is projected to increase dramtically in the future. Information on antibiotic use in aqauculture is limited, however, the rapid growth and ongoing intensifcation of the industry, the geographic location of production and initial surveys would indicate inappropriate use of antimicrobials is occuring. Research and capacity development are potentially powerful tools in addressing the threat AMR as they directly address many mechanisms for controlling AM use around biosecurity, diagnostics, education, vaccines, alternative treatments and legislation. Generating maximum impact from research funding requires ways of identifying areas of greatest risk for ongoing and future AMR emergence. Unfortunately, an integrated global AMR surveillance system for animal production does not exit and their remains a strong need for baseline information to guide the allocation of research resources, design interventions to address AMR and ultimately monitor their effectiveness. This systematic review proposes to integrate the data generated by researchers investigating AMR in aquaculture food-production systems, in their respective local contexts, to provide baseline information regarding AMR prevalence in global aquaculture. This information is a useful first step in defining the scope and scale of the problem to inform current and future research programming.

### *Methods*

The systematic map will aim to capture all available peer-reviewed studies relevant to the question. The search will employ three databases focused on peer reviewed publications namely ISI Web of Science, Scopus and ProQuest. Reference sections of relevant review articles will also be examined to identify articles that were not found using the search strategy. All searches will

be conducted in English. Search results will be reviewed in two stages: (1) title and abstract; and (2) full text. All screening decisions will be included in the database. The systematic map will employ a narrative synthesis approach that will include the use of descriptive statistics, tables and figures, including a GIS map detailing the geographical distribution of genetic resistant determinants, categorized according to farming and culture animal type, in global aquaculture.

***Keywords***
Aquaculture, antimicrobial resistance, AMR, fish, crustaceans, antibiotic.

## Background

The availability and use of antimicrobials, and particularly antibiotics[1], has fundamentally transformed human and veterinary medicine[1]. The ability to treat once lethal infections has had major positive implications for global health and food security. However, the inappropriate use of antimicrobials in human medicine and animal husbandry creates selective pressures that favour the emergence of antimicrobial resistant organisms[2–5]. Antimicrobial resistance (AMR) can broadly be defined as the ability of microorganisms such as bacteria, viruses, fungi and parasites to develop resistance to previously effective antimicrobial substances. Within this broad grouping, the area of greatest concern remains the ability of bacteria to develop mechanisms to resist antibiotics, hereafter termed antibiotic resistance.

Antibiotic resistance is not a new phenomenon. Resistance to Penicillin was reported soon after its introduction in the early 1940s [6], and multidrug resistance as early as the 1950s [7]. Initially, loss of antibiotic efficacy was balanced by the rapid development, between the 1940s and 1980s, of multiple new antibiotic types. Unfortunately, only a handful of new antibiotics have been licensed over the last 40 y, despite the birth of bacterial genomics in the 1990s that rapidly revealed numerous novel antibacterial targets[7,8]. Genomics has however helped to elucidate the extent of the challenge that AMR poses. We now understand that the genetic mechanisms involved in resistance are diverse and widely distributed, even in geologically isolated environments[9]. This reservoir of antibiotic resistant genes (ARGs) suggests that resistance mechanisms may already exist for any new antibiotics that come though the development pipeline[9]. In addition, many ARGs are associated with mobile genetic elements of the bacterial genome and can be shared between bacterial species, and even between phyla, through a

---

[1] The term antibiotic refers to “naturally occurring, semi-synthetic or synthetic substances that exhibit antimicrobial activity to kill or inhibit the growth of bacteria, at concentrations attainable in vivo (biocide substances, such as disinfectants or antiseptics, are excluded)”, as per the definition adopted by the FAO, WHO, OIE and the G7 Chief Veterinary Officers in October 2017.

variety if mechanisms collectively referred to as horizontal gene transfer[10]. Resistance is therefore not confined to the bacterial population where it develops, with evidence to suggest the transfer of ARGs from environmental to pathogenic bacteria[11–13]. Furthermore, within common pathogenic bacterial species there has been the recent emergence of highly successful clades[2] that are resistant without any obvious impact on fitness, suggesting that even the removal of antibiotic-driven selection will not affect prevalence of this resistance[9]. Some of these clades appear to have evolved to utilise anthropogenic niches such as hospitals and often exhibit increased virulence[9]. AMR therefore poses a substantial global problem[14] and by all indications the global health and economic consequences of leaving AMR unchecked will be high. A recent review suggests that impacts as high as 10 million annual deaths (8.2 million currently caused by cancer) and a cumulative cost of USD 100 trillion are possible by 2050[15].

Recognising that the health of humans, animals and ecosystems are interconnected, the World Health Organisation (WHO) collaborated with the Food and Agriculture Organisation of the United Nations (FAO) and the World Organisation for Animal Health (OIE) to develop the Global Action Plan on AMR (GAPAMR)[16] using a One Health approach[17]. One of the key strategic objectives of the GAPAMR centres on optimising the use of antimicrobial medicines in human and animal health. Antibiotic use in animal production is considered a major driver of AMR, due in part to both the scale and nature of use[18]. A recent study by Van Boeckel *et al*. to model global antibiotic consumption by food animals, the first if its kind, estimated consumption at 63 151 tonnes in 2010[19]. Comparisons to human antibiotic consumption are hampered by a lack of reliable data, however given that estimates of the proportional use of antibiotics for livestock in major global consumers is high (84% in China[20] and 70% in the USA[21]), livestock consumption likely exceeds human use[18]. This figure is also projected to rise by 67% to over 105,000 tonnes by 2030, with 34% (46% in Asia) of that growth attributable to a shift towards more intense faming practices[19]. Furthermore, the predominant mode of antibiotic use in livestock husbandry involves the application of sub-therapeutic doses over long exposure periods, creating conditions conducive to the selection of AMR bacteria[18]. Interestingly, Van Boeckel *et al.* did not include aquaculture in their estimates of antibiotic use, possibly due to the complications of obtaining reliable data on a food production system defined a high diversity of species and culture systems, the often-unconsolidated nature of production and the unregulated use of antibiotics labelled for livestock in aquaculture[22].

The global aquaculture food production system exhibits a number of characteristics that mirror trends in livestock production. Despite global capture-fishery production having plateaued since the late 1980's, global per-capita fish consumption has continued to rise over the last 30 years. This increase was supported by the rapid growth in aquaculture over this period and

---

[2] A within species grouping that are genetically highly clonal and exhibit relatively limited core genetic variability.

aquaculture remains the fastest growing food production system globally[23]. In parallel with the growth in total aquaculture production, there has also been a shift in the modalities of culture. There is a growing trend of intensification in aquaculture with famers looking to improve yields by increasing the biological load on a given body of water, either by increasing fish density and/or adding fertiliser or feed[2,23]. These practices increase the proximity of animals to each other and can negatively impact water quality. Fish kept under crowded, physiologically stressful conditions are susceptible to disease emergence, commonly resulting in the application of antibiotics to mitigate pathogen outbreaks[3]. The inappropriate use of antibiotics is likely widespread in aquaculture, particularly in countries with limited regulation, and creates the same selective pressures that favour the emergence of antibiotic resistant bacteria in terrestrial systems[2–5].

However, the global aquaculture industry presents numerous unique and interconnected characteristics that together locate it as both an important area of concern for AMR emergence and a distinctly challenging industry to address. For example, aquaculture is an evolving food-production system cultivating close to 600 species in a variety of culture systems over a broad geographical area (194 producing countries)[22,23]. Furthermore, the majority of global aquaculture production is centred in sub-tropical and tropical regions which are prone to more rapid and severe disease outbreaks[24]. As no antibiotics have been specifically developed for aquaculture, those designed for livestock and humans are used, some of which are of critical importance to human medicine[22]. These are generally incorporated into feed and applied metaphylactically[3] at the population level. Unfortunately, as fish do not efficiently metabolise antibiotics and monitoring feed intake is difficult in the aquatic environment, a large proportion can be lost to the environment as uneaten feed, undigested feed and secreted antimicrobial metabolites, with some studies indicating retention as low as 20 - 30 %[2,3]. These antibiotics then interact with an aquatic microbiome that harbours a large variety of mobile genetic elements, in what have been referred to as “genetic reactors” or “AMR gene hotspots”, where significant genetic exchange and recombination[4] can occur[2,3]. In addition, the regulatory framework governing the use of AMs in aquaculture varies greatly between countries, with limited capacity and enforcement in many of the developing countries that are major aquaculture producers[3].

Antibiotic use in aquaculture, and the potential for AMR emergence, is the result of the interaction of multiple and often interacting biological, geographical, operational, social and regulatory characteristics and drivers[22]. Research and capacity development, both at the technical and institutional level, are potentially powerful tools in tackling the threat of AMR

---

[3] Mass application of medication to a group of animals composed of both diseased and healthy individuals to prevent or minimise an expected disease outbreak.

[4] the rearrangement of genetic material, especially by crossing over in chromosomes or by the artificial joining of segments of DNA from different organisms.

emanating from aquaculture as they directly address many previously identified mechanisms for controlling AM use around biosecurity, diagnostics, education, vaccines, alternative treatments and legislation[22]. However, generating maximum impact requires ways of identifying areas of greatest risk for current and future antibiotic use and AMR emergence to effectively direct research resources. Surveillance and monitoring have been identified a key component to the global AMR response and form one of the key pillars of the Global Action Plan on AMR (GAPAMR)[16]. Effective surveillance and monitoring allows one to not only detect AMR emergence and document prevalence, it also provides an empirical basis to asses the impact of interventions[25].

The majority of global effort on AMR surveillance has been centred on human health, coordinated under the WHOs Global Antimicrobial Resistance Surveillance System (GLASS)[26]. Data from this system is however still limited, with only 49 countries reporting AMR data in 2017 with the majority (>75%) being from high-income and upper middle-income countries[26]. No such global system exists for AMR surveillance in food producing animals. While some programmes, such as the EU Joint Interagency Antimicrobial Consumption and Resistance Analyses (JIACRA) and the US National Antimicrobial Resistance Monitoring System (NARMS), aim to integrate data from surveillance in humans, animals and food, there is limited integration at the global level[25]. Furthermore, while programmes such as the FAO Assessment Tool for Laboratories and Antimicrobial Resistance Surveillance Systems (FAO-ATLASS) are helping to build capacity, many countries lack the skills and infrastructure for effective AMR surveillance. The development of effective AMR surveillance systems locally, and their successful integration globally, will require considerable political will and sustained funding to realise. In the interim, there is a strong need for baseline information to inform the allocation of research resources, design interventions to address AMR and ultimately monitor their effectiveness. Where centralised, national data is limited or non-existent, it is proposed that the data generated by research scientists investigating AMR in aquaculture food-production systems, in their respective local contexts, can be integrated to provide baseline information regarding AMR prevalence in global aquaculture. The use of a genetic approach to identify AMR and a focus on peer-reviewed literature is intended to provide both a degree of comparability and quality control respectively. In addition, patterns of research effort can be further integrated with production data to identify potential knowledge and research gaps.

## Topic identification and stakeholder input

The second key strategic objective of the WHO GAPAMR is to “strengthen the knowledge and evidence base through surveillance and research”[16]. A recent survey by the Joint Programming initiative for AMR (JPIAMR) recorded a total allocated investment in AMR research of just over 2 billion USD in 2017, based on data from 22 of their member countries, the EU and the Wellcome trust[27]. Effectively directing this funding for maximum impact requires data on the nature and extent of the AMR problem. The InnoVet-AMR program, a partnership between the UK Department of Health and Social Care’s Global AMR Innovation Fund (GAMRIF) and Canada’s International Development Research Centre (IDRC), is representative of the type of funding initiative operating in the realm of AMR and food-producing animals (including aquaculture) and looking to employ an evidence-based approach to programming.

Accordingly, a review of the published literature on the prevalence in AMR in aquaculture has been proposed as a first step to defining the scope and scale of the problem to facilitate an informed approach to research programme design and implementation. Following initial search term development at the IDRC, aquaculture researchers and practitioners in the Asia Pacific region were engaged for comment on the search string during the World Aquaculture Society Asia Pacific Aquaculture Conference 2019, Chennai, India.

## Objective of the review

The objective of this systematic map is to identify, collate and describe the published literature (peer-reviewed) that has reported antimicrobial resistance (AMR) in bacteria sampled from aquaculture food productions systems. It is envisioned that these studies can be used as an interim proxy for an integrated global AMR surveillance system. Data extracted from these studies will be used to generate an interactive spatial map to provide a snap-shot view of the distribution of AMR in relation to global aquaculture.

## Primary question

What is global composition, prevalence and geographical distribution of antimicrobial resistance in bacteria associated with aquaculture food production systems?

## Components of the primary question

### *Aquaculture*

This study is relevant to aquaculture food-production systems, defined here as a food production system that involves cultivating an organism in an aquatic environment and that involves direct human involvement in the form of seed addition, feed addition, habitat engineering, water quality manipulation or a combination thereof.

### *Target organisms and systems*

This study aims to target aquaculture food-producing systems where the application of antibiotics is likely. Antibiotic use is commonly associated with increasing intensification of aquaculture production in enclosed systems. Consequently, this review will initially focus on the finfish and crustacean sectors of global production and exclude the extensively farmed plant and mollusk sectors.

### *Measure of resistance*

A genetic approach is favoured as it standardises the measure of resistance and mitigates against bias or poor data associated with variable methodologies or antibiotic quality used in culture-based methods. In addition, genetic results also potentially provide information on resistance beyond the scope of culture-based methods (i.e. the selection of antibiotics for testing) as single genetic traits can code for resistance to multiple antibiotics.

## Methods

The review will broadly follow CEE guidelines[28] and conform to ROSES reporting standards[29].

## Searching for articles

### *Search strategy*

The search will aim to capture all available studies relevant to the question found in the peer-reviewed literature. The search will leverage three databases focused on peer reviewed publications and a single web-based search engine (see below). In addition, reference sections of relevant review articles will be cross-checked to identify articles that were not found using the search strategy.

### ***Search String***

An initial set of English search terms relevant to the different components of the research question were compiled. A list of common names of cultured fish and crustacean species was extracted from the FAO Fishery Statistical Collection: Global Aquaculture Production accessed through the FAO FishStatJ software (http://www.fao.org/fishery/statistics/global-aquaculture-production/en). A list of antibiotic names was extracted from the Comprehensive Antibiotic Resistance Database (CARD), a curated collection of characterized, peer-reviewed resistance determinants and associated antibiotics[30]. Next, a set of search strings was developed and modified through a scoping exercise using Web of Science Core Collections and Scopus to evaluate the sensitivity associated with alternate terms and wildcards. Initial attempts to develop search strings using specific genetic resistant determinants extracted from the CARD database were abandoned due to the non-specificity of wildcards. The terms were broken into four components and were combined using Boolean operators "AND" and/or "OR" (Table 1). The comprehensiveness of the search was tested against a collection of benchmark papers (n = 25) to ensure articles identified as relevant were being captured when possible.

### ***Bibliographic databases***

The following online databases will be searched using subscriptions from Carleton University.

1. ISI Web of Science (WoS, Core Collection): multidisciplinary database of peer-reviewed literature, books, and conference proceedings.
2. Scopus: database of peer-reviewed literature including journals, books, and conference proceedings.
3. ProQuest Dissertations and Theses Global: database of graduate dissertations and theses from around the world.

### ***Web-based search engines***

In addition to the above bibliographic databases, internet searches will be performed using Google Scholar. Due to search string limitations, an adapted string (256 character limit, Table 2) will be used and the first 200 hits—sorted by relevance—will be added to the database for screening).

### ***Other literature searches***

In addition to the above searches, the reference sections of relevant reviews will be hand searched for articles that are within the scope of this systematic map and not captured by the searches.

Table 1: Proposed search string to execute the search strategy (Web of Science Core Collection and Scopus)

| Component | Search terms |
|---|---|
| Aquaculture | (aquaculture OR mariculture OR pisciculture OR culture OR cultivation OR aquafarm* OR farm* OR hatchery OR pond OR raceway OR tank* OR cage OR RAS OR ornamental) |
|  | AND |
| Target species / habitats | (crab* OR crawfish* OR crayfish* OR crustacean* OR decapod* OR lobster* OR marron OR prawn* OR shrimp* OR swimcrab* OR yabby OR aba OR arapaima OR asp OR astyanax OR atipa OR barb$ OR barbel$ OR bass OR bayad OR bluegill$ OR bonytongue$ OR bream$ OR buffalofish OR bullhead OR burbot$ OR cachama OR carp$ OR catfish* OR catla$ OR chub OR cichlid$ OR cod$ OR crappie OR dorada OR dorado OR drum OR eel$ OR fish* OR gobie$ OR goby OR goldfish OR gourami OR grunter OR guapote OR gudgeon OR jewelfish OR knifefish OR labeo OR loach OR mahseer OR mudfish* OR murrels OR nase OR ningu OR orfe OR pacu OR pangas OR pangasius OR parachanna OR perch OR pike OR pike-perch OR pirapatinga OR prochilod* OR rhinofish OR roach OR roho OR rohtee OR rohu OR rudd OR sampa OR siluroid* OR sleeper OR snakehead* OR sorubim* OR suckermouth OR tambacu OR tambatinga OR tench OR tilapia* OR trahira OR vimba OR wallago OR wuchang OR amberjack OR batfish OR bluefish OR bream$ OR brill OR cobia OR cod OR conger OR coralgrouper OR crevalle$ OR croaker OR dentex OR drum$ OR filefish OR finfish OR flounder$ OR gobie$ OR grouper$ OR haddock OR halibut OR jack OR jobfish* OR mackerel$ OR meagre OR mojarras OR mudskipper OR mullet$ OR pandora OR parrotfish OR percoid* OR pollack OR pompano OR porgie$ OR porgy OR pufferfish* OR rabbitfish* OR redfish* OR robalo$ OR rockfish OR scats* OR scorpionfish* OR seabass* OR seabream* OR seaperch OR silverside$ OR smelt OR snapper$ OR snook$ OR sole$ OR spadefish OR spinefoot OR tarpon OR threadfin OR trevally OR tuna OR turbot OR whiptail OR wolffish OR yellowtail OR barramundi$ OR bass OR beluga$ OR char$ OR eel$ OR grayling$ OR huchen$ OR icefish OR milkfish OR paddlefish OR salmon$ OR salmonid$ OR shad OR smelt OR stickleback OR sturgeon OR sweetfish OR trout$ OR whitefish OR fish$ OR freshwater OR seawater OR marine OR brackish) |
|  | AND |
| Resistance | ("anti-microbial resistan*" OR "drug-resistan*" OR "drugresistan*" OR "multi-drug resistan*" OR "multidrugresistan*" OR "multidrug resistan*" OR "resistan*" OR "ARG" OR "virginiamycin S2 resistan*" OR "virginiamycin resistan*" OR "vertilimicin resistan*" OR "vernamycin C resistan*" OR "vernamycin resistan*" OR "vernamycin B-gamma resistan*" OR "verdamicin resistan*" OR "vancomycin resistan*" OR "valnemulin resistan*" OR "unphenelfamycin resistan*" OR "UK-69,753 resistan*" OR "tylosin resistan*" OR "tunicamycin resistan*" OR "tuberactinomycin resistan*" OR "trovafloxacin resistan*" OR "trimethoprim resistan*" OR "triclosan resistan*" OR "tobramycin resistan*" OR "tinidazole resistan*" OR "ticarcillin resistan*" OR "tiamulin resistan*" OR "thusin resistan*" OR "thiostrepton resistan*" OR "thiamphenicol resistan*" OR "tetroxoprim resistan*" OR "tetracycline resistan*" OR "temocillin resistan*" OR "telithromycin resistan*" OR "telavancin resistan*" OR "teixobactin resistan*" OR "teicoplanin resistan*" OR "tedizolid resistan*" OR "tebipenem resistan*" OR "sulfisoxazole resistan*" OR "sulfasalazine resistan*" OR "sulfamethoxazole resistan*" OR "sulfamethizole resistan*" OR "sulfadoxine resistan*" OR "sulfadimidine resistan*" OR "sulfadiazine resistan*" OR "sulfacetamide resistan*" OR "streptothricin resistan*" OR "streptomycin resistan*" OR "spiramycin resistan*" OR "spectinomycin resistan*" OR "sparfloxacin resistan*" OR "solithromycin resistan*" OR "sitafloxacin resistan*" OR "sisomicin resistan*" OR "SB22484 resistan*" OR "roxithromycin resistan*" OR "rosaramicin resistan*" OR "rokitamycin resistan*" OR "ristocetin resistan*" OR "rifaximin resistan*" OR "rifapentine resistan*" OR "rifampin resistan*" OR "rifabutin resistan*" OR "ribostamycin resistan*" OR "rhodamine resistan*" OR "retapamulin resistan*" OR |

| | |
|---|---|
| | "quinupristin resistan*" OR "pyrazinamide resistan*" OR "pulvomycin resistan*" OR "prothionamide resistan*" OR "propicillin resistan*" OR "pristinamycin IIA resistan*" OR "pristinamycin IB resistan*" OR "pristinamycin resistan*" OR "polymyxin B resistan*" OR "pleuromutilin resistan*" OR "plazomicin resistan*" OR "pistinamycin IA resistan*" OR "piperacillin resistan*" OR "pikromycin resistan*" OR "phenoxymethylpenicillin resistan*" OR "phenelfamycin H resistan*" OR "phenelfamycin G resistan*" OR "phenelfamycin F resistan*" OR "phenelfamycin E resistan*" OR "phenelfamycin D resistan*" OR "phenelfamycin C resistan*" OR "phenelfamycin B resistan*" OR "phenelfamycin A resistan*" OR "phenelfamycin resistan*" OR "penicillin N resistan*" OR "penicillin resistan*" OR "pefloxacin resistan*" OR "patricin resistan*" OR "patricin resistan*" OR "paromomycin resistan*" OR "para-aminosalicylic acid resistan*" OR "panipenem resistan*" OR "palmitic acid resistan*" OR "pactamycin resistan*" OR "oxytetracycline resistan*" OR "oxacillin resistan*" OR "ostreogrycin B3 resistan*" OR "ostreogrycin resistan*" OR "oritavancin resistan*" OR "omadacycline resistan*" OR "oleic acid resistan*" OR "oleandomycin resistan*" OR "ofloxacin resistan*" OR "nybomycin resistan*" OR "novobiocin resistan*" OR "norfloxacin resistan*" OR "nitrofurantoin resistan*" OR "niddamycin resistan*" OR "netilmicin resistan*" OR "neomycin resistan*" OR "narbomycin resistan*" OR "nalidixic acid resistan*" OR "nafcillin resistan*" OR "mycinamicin resistan*" OR "mupirocin resistan*" OR "moxifloxacin resistan*" OR "moxalactam resistan*" OR "minocycline resistan*" OR "midecamycin resistan*" OR "microcin J25 resistan*" OR "microcin resistan*" OR "mezlocillin resistan*" OR "metronidazole resistan*" OR "methymycin resistan*" OR "methicillin resistan*" OR "meropenem resistan*" OR "megalomycin resistan*" OR "mecillinam resistan*" OR "magainin resistan*" OR "mafenide resistan*" OR "madumycin II resistan*" OR "madumycin resistan*" OR "lysocin resistan*" OR "lugdunin resistan*" OR "loracarbef resistan*" OR "lomefloxacin resistan*" OR "lividomycin resistan*" OR "lipopeptide resistan*" OR "linoleic acid resistan*" OR "linezolid resistan*" OR "lincomycin resistan*" OR "LFF571 resistan*" OR "levofloxacin resistan*" OR "lefamulin resistan*" OR "lassomycin resistan*" OR "L-681,217 resistan*" OR "kitasamycin resistan*" OR "kirrothricin resistan*" OR "kirromycin resistan*" OR "kasugamycin resistan*" OR "kanamycin A resistan*" OR "kanamycin resistan*" OR "josamycin resistan*" OR "isopenicillin N resistan*" OR "isopenicillin resistan*" OR "isoniazid resistan*" OR "isepamicin resistan*" OR "imipenem resistan*" OR "iclaprim resistan*" OR "hygromycin B resistan*" OR "hygromycin resistan*" OR "heneicomycin resistan*" OR "griseoviridin resistan*" OR "grepafloxacin resistan*" OR "gramicidin S resistan*" OR "gramicidin C resistan*" OR "gramicidin B resistan*" OR "gramicidin A resistan*" OR "gramicidin resistan*" OR "glycylcycline resistan*" OR "gentamicin C resistan*" OR "gentamicin B resistan*" OR "gentamicin A resistan*" OR "gentamicin resistan*" OR "GE37468 resistan*" OR "GE2270A resistan*" OR "gatifloxacin resistan*" OR "ganefromycin resistan*" OR "G418 resistan*" OR "fusidic acid resistan*" OR "furazolidone resistan*" OR "fosmidomycin resistan*" OR "fosfomycin resistan*" OR "formicin resistan*" OR "flucloxacillin resistan*" OR "florfenicol resistan*" OR "flomoxef resistan*" OR "fleroxacin resistan*" OR "fidaxomicin resistan*" OR "faropenem resistan*" OR "factumycin resistan*" OR "ethionamide resistan*" OR "ethambutol resistan*" OR "erythromycin resistan*" OR "ertapenem resistan*" OR "Eravacycline resistan*" OR "enoxacin resistan*" OR "enacyloxin IIa resistan*" OR "efrotomycin resistan*" OR "edeine F resistan*" OR "edeine D resistan*" OR "edeine B resistan*" OR "edeine A resistan*" OR "edeine resistan*" OR "doxycycline resistan*" OR "doripenem resistan*" OR "dirithromycin resistan*" OR "dihydromocimycin resistan*" OR "dicloxacillin resistan*" OR "dibekacin resistan*" OR "demeclocycline resistan*" OR "delafloxacin resistan*" OR "defensin resistan*" OR "dapsone resistan*" OR "dalfopristin resistan*" OR "dalbavancin resistan*" OR "cycloserine resistan*" OR "coumermycin A1 resistan*" OR "coumermycin resistan*" OR "colistin B resistan*" OR "colistin A resistan*" OR "colistin resistan*" OR "cloxacillin resistan*" OR "clorobiocin resistan*" OR "Clofazimine resistan*" OR "clindamycin resistan*" OR "clinafloxacin resistan*" OR "clarithromycin resistan*" OR "ciprofloxacin resistan*" OR "cinoxacin resistan*" OR "chlortetracycline resistan*" OR "chloroeremomycin resistan*" OR "chloramphenicol resistan*" OR "chalcomycin resistan*" OR "cephapirin resistan*" OR "cephaloridine resistan*" OR "celesticetin resistan*" OR "cefuroxime resistan*" OR "ceftriaxone resistan*" OR "ceftolozane resistan*" OR "ceftobiprole resistan*" OR "ceftizoxime resistan*" OR "ceftiofur resistan*" OR "ceftibuten resistan*" OR "ceftazidime resistan*" OR "ceftaroline resistan*" OR "cefradine resistan*" OR "cefprozil resistan*" OR "cefpodoxime resistan*" OR "cefoxitin resistan*" OR "cefotiam resistan*" OR "cefotetan resistan*" OR "cefotaxime resistan*" OR "cefoperazone resistan*" OR "cefonicid resistan*" OR "cefmetazole resistan*" OR "cefixime resistan*" OR "cefiderocol resistan*" OR "cefetamet resistan*" OR "cefepime resistan*" OR "cefditoren resistan*" OR "cefdinir resistan*" OR |

| | |
|---|---|
| | "cefazolin resistan*" OR "cefamandole resistan*" OR "cefalotin resistan*" OR "cefalexin resistan*" OR "cefadroxil resistan*" OR "cefaclor resistan*" OR "CBP C1-aminomethylene vancomycin resistan*" OR "carbomycin resistan*" OR "carbenicillin resistan*" OR "capreomycin resistan*" OR "butirosin resistan*" OR "brodimoprim resistan*" OR "BPI resistan*" OR "bleomycinic acid resistan*" OR "bleomycin B2 resistan*" OR "bleomycin A2 resistan*" OR "bleomycin resistan*" OR "bicyclomycin resistan*" OR "benzylpenicillin resistan*" OR "benzalkonium chloride resistan*" OR "balofloxacin resistan*" OR "balhimycin resistan*" OR "BAL30072 resistan*" OR "bacitracin F resistan*" OR "bacitracin B resistan*" OR "bacitracin A resistan*" OR "bacitracin resistan*" OR "aztreonam resistan*" OR "azlocillin resistan*" OR "azithromycin resistan*" OR "azidamfenicol resistan*" OR "azdimycin resistan*" OR "azamulin resistan*" OR "avoparcin resistan*" OR "aurodox resistan*" OR "astromicin resistan*" OR "arsphenamine resistan*" OR "arbekacin resistan*" OR "apramycin resistan*" OR "Antibiotics resistan*" OR "antibiotic A47934 resistan*" OR "antibiotic A40926 resistan*" OR "amythiamicin A resistan*" OR "ampicillin resistan*" OR "amoxicillin resistan*" OR "aminonucleoside resistan*" OR "amikacin resistan*" OR "actinomycin resistan*" OR "acriflavin resistan*" OR "acridine dye resistan*" OR "aminocoumarin resistan*" OR "aminoglycoside resistan*" OR "antibacterial free fatty acids resistan*" OR "beta-lactam resistan*" OR "diaminopyrimidine resistan*" OR "elfamycin resistan*" OR "fluoroquinolone resistan*" OR "glycopeptide resistan*" OR "lincosamide resistan*" OR "macrocyclic resistan*" OR "macrolide resistan*" OR "nitrofuran resistan*" OR "nitroimidazole resistan*" OR "nucleoside resistan*" OR "organoarsenic resistan*" OR "oxazolidinone resistan*" OR "peptide antibiotic resistan*" OR "phenicol resistan*" OR "pleuromutilin resistan*" OR "polyamine resistan*" OR "rifamycin resistan*" OR "streptogramin resistan*" OR "sulfonamide resistan*" OR "sulfone resistan*" OR "tetracycline resistan*") |
| | AND |
| Bacteria | (gene* OR sequenc* OR bacteri* OR plasmid OR allele OR integron) |

The asterisk (*) is a wildcard and represents any characters (e.g., fish* includes fish, fishes, fishing, fisher) while the dollar sign ($) includes zero or one character (e.g., gobie$ includes gobie and gobies). All searches will be conducted in English.

Table 2: Proposed condensed search string to execute the search strategy (Google Scholar – 256-character limit)

| Component | Search terms |
|---|---|
| Aquaculture | (aquaculture OR mariculture OR pisciculture OR aquafarm OR hatchery OR pond OR raceway OR tank OR cage OR ornamental) |
| | AND |
| Resistance | (antimicrobial resistant OR drug resistant OR multidrug resistant OR resistant OR ARG) |
| | AND |
| Bacteria | (gene OR sequence OR bacteria OR plasmid) |

### *Estimating the comprehensiveness of the search*

The comprehensiveness of the entire search (all sources) was tested using a collection of benchmark papers identified as relevant (n = 25). The search strategy was tested using the search string in Web of Science Core Collection (WoS) and Scopus. Articles yielded by the search string were cross-checked with the list of key references to see which articles were found and which were not. During the scoping exercise (conducted on 13 May 2019), the above search string, limited to 1999 – 2009, generated 5729 records on WoS and 5830 records on Scopus. A single article (Wu *et al*, 2019) was not found using the WoS search and further searches revealed that it was not present in the WoS database. However, this article was found in the Scopus search. Any alterations to the search string and search strategy as the systematic map is conducted will be recorded and all amendments will be reported in the final published document.

## Search record database

Search results from the online literature review will be exported into a single CADIMA database. Duplicate results will be identified and merged. The final search record database will serve as an archive and remain untouched.

## Article screening and study eligibility

### *Screening process*

Search results will be screened at two stages: (1) title and abstract, and (2) full text. Articles that meet the inclusion criteria at the title and abstract stage will be reviewed at the full text stage. Any articles that do not have abstracts will automatically be screened at the full text level. In cases where a reviewer is uncertain about inclusion, it will be flagged for screening by a second reviewer. If there is still uncertainty, the article will be discussed by the research team until consensus has been reached regarding inclusion/exclusion. All screening decisions will be included in the database. A list of articles excluded at the full text assessment will be provided as an additional file in the systematic map with details regarding the reasons for exclusion. When English title and abstracts are found, with article content in other languages, the articles will be screened as part of the title and abstract screening process. If the article is included to full text screening, a translated version of the article will be searched for online (using Google Scholar) and, when translated copies are not found, the first author of the relevant article will be contacted to see if a translated copy exists and is available.

### *Consistency checking*

Prior to the title and abstract screening, a random subset of articles (5% of the total) will be used for a consistency check to ensure consistent and repeatable decisions are made regarding inclusion/exclusion. The consistency check will involve a double-blind method at the title and abstract stage and be done with two reviewers to ensure the accurate interpretation of the screening criteria. Inter-rater reliability will be calculated using a Kappa test (score of ≥ 0.6 required) and inconsistencies will be discussed and reconciled prior to moving forward with screening. The same process will be repeated prior to screening articles at the full text stage. Articles or datasets found by means other than database or search engine searches (i.e., specialist website or other literature searches) will be entered at the second stage of this screening process (i.e., full text) but will not be included in consistency checks. If the decision to include or exclude a specific article is unclear, that article will be retained and will go on to the next level of screening. A list of all articles excluded at the full text level will be provided as an additional file to the systematic map and will include the reason for exclusion.

### *Eligibility criteria*

The following eligibility criteria will be used to screen articles at title/abstract and full text screening.

#### *Relevant food production system*

The term "aquaculture" refers to any aquatic food production system that involves human intervention in the form of seed addition, feed addition, habitat engineering or water quality manipulation.

#### *Relevant target species*

Only articles that relate to aquaculture involving fish or crustacean species will be included. Articles relating to aquaculture involving mollusks, aquatic plants or amphibians will excluded.

#### *Relevant study type*

Studies which sample bacteria from the water, sediment and other surfaces, infrastructure and resident biological organisms directly associated with an aquaculture farm, including the direct outflow, will be included. Furthermore, only studies that extract DNA from said bacteria and report the incidence of genetic resistance determinants following PCR using suitable primers and sequencing, or through secondary analysis of whole genomes will be included. Studies that use culture-based methods such as disk diffusion to assess antimicrobial resistance of bacterial cultures will be excluded, however these studies will be flagged and listed for later study

*Relevant genetic resistance determinant*

Any genetic resistant determinant (GRD) reported in the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/) will be included. Studies reporting novel GRDs not yet listed in the CARD database will be considered for inclusion on a case-by-case basis.

*Geographic scope*

The scope of this study will be global, as such there will be no geographic exclusion boundaries for studies.

### ***Demonstrating procedural independence***

Reviewers who have authored articles to be considered within the review will be prevented from influencing inclusion decisions through the appropriate delegation of tasks.

## Study validity assessment

In this systematic map we do not intend to appraise the validity of the studies.

## Data coding strategy

Following the full-text screening, the remaining studies to be included will be coded using a standard questionnaire (Appendix 2). The questionnaire is designed to capture key descriptive information about the studies regarding five general categories.

A Google Form—which automatically compiles the results—will be developed to facilitate the coding and metadata extraction. Prior to metadata extraction, a subset of articles (5% of included articles) will be used for a consistency check to ensure consistent and repeatable decisions are made regarding the meta-data coding.

The following general categories of variables will be extracted from the articles: (1) bibliographic information; (2) study location; (3) aquaculture system characteristics including water salinity, culture water body, culture species and history; (4) Bacterial sample characteristics including taxonomic classification and source; (5) Genetic resistance determinants. Missing or unclear bibliographic information will be cross-referenced using the Google search engine or through contacting the authors when necessary.

## Study mapping and presentation

All included studies and their meta-data will be recorded in an MS-Excel database that will be made available with the published systematic map article, as additional supporting files. The final narrative report will describe the review process and the volume and key characteristics of the evidence base, detailing methodology, results and trends. The GRD prevalence data will be categorized according to farming and culture animal type and mapped against geographic distribution using QGIS.

# References

1. OIE & World Oragnization for Animal Health. *The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. World Oragnization for Animal Health* (2016).
2. Watts, J. *et al.* The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions. *Mar. Drugs* **15**, 158 (2017).
3. Santos, L. & Ramos, F. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *Int. J. Antimicrob. Agents* **52**, 135–143 (2018).
4. Done, H. Y., Venkatesan, A. K. & Halden, R. U. Does the Recent Growth of Aquaculture Create Antibiotic Resistance Threats Different from those Associated with Land Animal Production in Agriculture? *AAPS J.* **18**, 1583–1583 (2016).
5. Cabello, F. C. *et al.* Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* **15**, 1917–1942 (2013).
6. Rammelkamp, C. H. & Maxon, T. Resistance of Staphylococcus aureus to the Action of Penicillin. *Exp. Biol. Med.* **51**, 386–389 (1942).
7. Bloom, D. E., Black, S., Salisbury, D. & Rappuoli, R. Antimicrobial resistance and the role of vaccines. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 12868–12871 (2018).
8. Baker, S. J., Payne, D. J., Rappuoli, R. & De Gregorio, E. Technologies to address antimicrobial resistance. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 12887–12895 (2018).
9. Klemm, E. J., Wong, V. K. & Dougan, G. Emergence of dominant multidrug-resistant bacterial clades: Lessons from history and whole-genome sequencing. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 12872–12877 (2018).
10. Holmes, A. H. *et al.* Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* **387**, 176–187 (2016).
11. POST. *Reservoirs of Antimicrobial Resistance*. (2019).
12. von Wintersdorff, C. J. H. *et al.* Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Front. Microbiol.* **7**, 173 (2016).
13. Allen, H. K. *et al.* Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* **8**, 251–259 (2010).
14. Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S. & Laxminarayan, R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet. Heal.* **2**, e398–e405 (2018).
15. O'Neill, J. *Tackling drug-resistant infection gloablly: Final report and recommendations. The Review on Antimicrobial Resistance.* (2016).

16. WHO. *Global action plan on antimicrobial resistance.* (2015).

17. AVMA. *One Health: A new professional imperative. One Health Inititiative Task Force Final Report.* (2008).

18. Robinson, T. P. *et al.* Antibiotic resistance is the quintessential One Health issue. *Trans. R. Soc. Trop. Med. Hyg.* **110**, 377–380 (2016).

19. Van Boeckel, T. P.*et al.* Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci.* **112**, 5649–5654 (2015).

20. Zhang, Q.-Q., Ying, G.-G., Pan, C.-G., Liu, Y.-S. & Zhao, J.-L. Comprehensive Evaluation of Antibiotics Emission and Fate in the River Basins of China: Source Analysis, Multimedia Modeling, and Linkage to Bacterial Resistance. *Environ. Sci. Technol.* **49**, 6772–6782 (2015).

21. FDA. *Antimicrobials Sold or Distributed for Use in Food-Producing Animals. Summary Report by the Food and Drug Administration Department of Health and Human Services.* (2014).

22. Henriksson, P. J. G. *et al.* Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: a review from a systems perspective. *Sustain. Sci.* **13**, 1105–1120 (2018).

23. FAO. *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. AqTHE STATE OF THE WORLD series of the Food and Agriculture Organization of the United Nations.uaculture* **35**, (2018).

24. Leung, T. L. F. & Bates, A. E. More rapid and severe disease outbreaks for aquaculture at the tropics: Implications for food security. *J. Appl. Ecol.* **50**, 215–222 (2013).

25. WHO. Surveillance and monitoring for antimicrobial use and resistance. Interagency Coordination group on Antimicrobial Resistance Discussion Paper, June 2018.

26. WHO. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018. (2018)

27. JPIAMR. *Mapping of AMR Research Funding: a comprehensive overview of the 2017 antimicrobial resistance research landscape in the JPIAMR member countries.* (2017).

28. Collaboration for Environmental Evidence. Guidelines and standards for evidence synthesis in environmental management. Version 5.0. In: Pullin A, Frampton G, Livoreil B, Petrokofsky G. http://www.environmentalevidence.org/information-for-authors. Accessed: 17 Aug 2018 (2018)

29. Haddaway NR, Macura B, Whaley P, Pullin AS. ROSES Reporting standards for systematic evidence syntheses: pro forma, flow-diagram and descriptive summary of the plan and conduct of environmental systematic reviews and systematic maps. Environ Evid. **7**:7 (2018).

30. Jia, B. *et al.* CARD 2017: expansion and model-centric curation of the comprehensive

antibiotic resistance database. *Nucleic Acids Res.* **45**, D566–D573 (2017).

# Appendix 1: Coding Sheet

The following form will be used to extract relevant meta-data from the final set of published studies included in the following: "Where have the genes associated with antimicrobial resistance been reported in global aquaculture: A systematic map." The following general categories of variables will be extracted from the articles: (1) bibliographic information; (2) study location; (3) aquaculture system characteristics including water salinity, culture water body, culture species and history; (4) Bacterial sample characteristics including taxonomic classification and source; (5) Genetic resistance determinants

SEPARATE MULTIPLE RESPONSES WITH COMMAS.

Note that if there are multiple case studies in different countries in a single study, please complete the questionnaire for each and include a parenthetical at the end of the corresponding author with (A, B, C, etc) to reflect each case study.

### *Article reference code*

☐

### *1. Coder Name*

(select one)

Coder 1
Coder 2
Coder 3

### *2. Screening*

(select one)

Include on Full Text
Include for second opinion

Exclude - Relevant Review
Exclude - Not Relevant
Exclude – Not genetic study (i.e. culture-based methodology)
Exclude – Aquaculture organism (not fish / crustacean)
Exclude – No aquaculture sample (only nearby site)
Exclude - Document type
Exclude – Other (describe)

# Bibliographic

***3. Year Published***

***4. Title***

(Title of Journal, book, report, thesis, etc.)

***5. Title of Article / Chapter***

(Full title of the article/chapter (NA = Not Applicable))

***6. Type of publication***

(Classification of Article Type | select one)

Peer-Reviewed Article
PhD Dissertation
Master's Thesis
Conference Paper
Other:

***7. Primary author country of affiliation and gender***

(list all, separate with commas)

Male
Female
Unknown

***8. Corresponding author name***

***9. Corresponding author email address***

***10. Abstract***

(copy verbatim)

***11. Publication keywords***

(copy verbatim, separate with commas, NA – none provided)

***12. Publication DOI***

(copy verbatim, NA = not applicable)

# Study location

***13. Country of study***

***14. Region / Province in Country***

(NR = nor reported)

# Aquaculture System

### ***15. Water salinity***

(Select one)

Freshwater
Brackish
Saltwater / Marine

### ***16.Cultured animal – common name***

(Common name, separate multiple species by comma)

### ***16.Cultured animal – scientific name***

(Scientific name, separate multiple species by comma)

### ***17. Culture system descriptors***

(Select one or more)

Broodstock
Hatchery
Grow-out
Pond
Raceway
Tank
Cage
RAS
Ornamental
Other:

# Bacterial Sample

### ***18. Sample site history***

Current aquaculture site
Historical aquaculture site
Time series samples

### ***19. Comparative samples***

Aquaculture site only
Aquaculture site vs. nearby unfarmed site

### ***20. Sample origin***

Water sample
Sediment sample
Aquaculture organism sample
Accessory organism

Broodstock
Hatchery
Pond
Tank
Cage
Outflow

# Genetic resistance determinants

### ***21. Genetic analysis method***

(Select one)

Primer | PCR | Sequencing
Whole genome

### *22. Reported GRD’s*

(separate multiple GRD’s with a comma)

### *23. Reported antibiotic resistance*

(separate multiple antibiotics with a comma)

# Appendix 2: Benchmark Studies

| | |
|---|---|
| Higuera-Llantén et al. 2018 | Higuera-Llantén, S., Vásquez-Ponce, F., Barrientos-Espinoza, B., Mardones, F. O., Marshall, S. H., & Olivares-Pacheco, J. (2018). Extended antibiotic treatment in salmon farms select multiresistant gut bacteria with a high prevalence of antibiotic resistance genes. *PloS one*, *13*(9), e0203641. |
| Hoa et al. 2008 | Hoa, P. T. P., Nonaka, L., Viet, P. H., & Suzuki, S. (2008). Detection of the sul1, sul2, and sul3 genes in sulfonamide-resistant bacteria from wastewater and shrimp ponds of north Vietnam. *Science of the Total Environment*, *405*(1-3), 377-384. |
| Nonaka et al. 2007 | Nonaka, L., Ikeno, K., & Suzuki, S. (2007). Distribution of tetracycline resistance gene, tet (M), in gram-positive and gram-negative bacteria isolated from sediment and seawater at a coastal aquaculture site in japan. *Microbes and Environments*, *22*(4), 355-364. |
| Muziasari et al. 2016 | Muziasari, W. I., Pärnänen, K., Johnson, T. A., Lyra, C., Karkman, A., Stedtfeld, R. D., ... & Virta, M. (2016). Aquaculture changes the profile of antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments. *FEMS microbiology ecology*, *92*(4), fiw052. |
| Nawaz et al. 2006 | Nawaz, M., Sung, K., Khan, S. A., Khan, A. A., & Steele, R. (2006). Biochemical and molecular characterization of tetracycline-resistant Aeromonas veronii isolates from catfish. *Appl. Environ. Microbiol.*, *72*(10), 6461-6466. |
| Xiong et al. 2015 | Xiong, W., Sun, Y., Zhang, T., Ding, X., Li, Y., Wang, M., & Zeng, Z. (2015). Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. *Microbial ecology*, *70*(2), 425-432. |
| Gao et al. 2012 | Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., & Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water research*, *46*(7), 2355-2364. |
| Song et al. 2016 | Song, C., Zhang, C., Fan, L., Qiu, L., Wu, W., Meng, S., ... & Chen, J. (2016). Occurrence of antibiotics and their impacts to primary productivity in fishponds around Tai Lake, China. *Chemosphere*, *161*, 127-135. |
| Zhao et al. 2018 | Zhao, S., Ma, L., Wang, Y., Fu, G., Zhou, J., Li, X., & Fang, W. (2018). Antimicrobial resistance and pulsed-field gel electrophoresis typing of Vibrio parahaemolyticus isolated from shrimp mariculture environment along the east coast of China. *Marine pollution bulletin*, *136*, 164-170. |

| | |
|---|---|
| Akinbowale et al. 2007 | Akinbowale, O. L., Peng, H., & Barton, M. D. (2007). Diversity of tetracycline resistance genes in bacteria from aquaculture sources in Australia. *Journal of applied microbiology*, *103*(5), 2016-2025. |
| Tamminen et al. 2010 | Tamminen, M., Karkman, A., Lõhmus, A., Muziasari, W. I., Takasu, H., Wada, S., ... & Virta, M. (2010). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environmental science & technology*, *45*(2), 386-391. |
| Lo et al. 2014 | Lo, D. Y., Lee, Y. J., Wang, J. H., & Kuo, H. C. (2014). Antimicrobial susceptibility and genetic characterisation of oxytetracycline-resistant Edwardsiella tarda isolated from diseased eels. *Veterinary Record*, *175*(8), 203-203. |
| Hossain et al. 2018 | Hossain, S., De Silva, B. C. J., Wimalasena, S. H. M. P., Pathirana, H. N. K. S., Dahanayake, P. S., & Heo, G. J. (2018). Distribution of antimicrobial resistance genes and class 1 integron gene cassette arrays in motile Aeromonas spp. isolated from goldfish (Carassius auratus). *Microbial Drug Resistance*, *24*(8), 1217-1225. |
| Wu et al. 2019 | Wu, J., Mao, C., Deng, Y., Guo, Z., Liu, G., Xu, L., ... & Feng, J. (2019). Diversity and abundance of antibiotic resistance of bacteria during the seedling period in marine fish cage-culture areas of Hainan, China. *Marine Pollution Bulletin*, *141*, 343-349. |
| Jacbos & Chenia 2007 | Jacobs, L., & Chenia, H. Y. (2007). Characterization of integrons and tetracycline resistance determinants in Aeromonas spp. isolated from South African aquaculture systems. *International journal of food microbiology*, *114*(3), 295-306. |
| Verner-Jeffreys et al. 2009 | Verner-Jeffreys, D. W., Welch, T. J., Schwarz, T., Pond, M. J., Woodward, M. J., Haig, S. J., ... & Baker-Austin, C. (2009). High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. *PloS one*, *4*(12), e8388. |
| Nguyen et al. 2014 | Nguyen, H. N. K., Van, T. T. H., Nguyen, H. T., Smooker, P. M., Shimeta, J., & Coloe, P. J. (2014). Molecular characterization of antibiotic resistance in Pseudomonas and Aeromonas isolates from catfish of the Mekong Delta, Vietnam. *Veterinary microbiology*, *171*(3-4), 397-405. |
| Zhang et al. 2013 | Zhang, R. Q., Ying, G. G., Su, H. C., Zhou, L. J., & Liu, Y. S. (2013). Antibiotic resistance and genetic diversity of Escherichia coli isolates from traditional and integrated aquaculture in South China. *Journal of Environmental Science and Health, Part B*, *48*(11), 999-1013. |

| | |
|---|---|
| Kim et al. 2018 | Kim, A., Lim, Y., Kim, N., Luan Nguyen, T., Roh, H. J., Park, C. I., ... & Smith, P. (2018). A comparison of genotypic and phenotypic methods for analyzing the susceptibility to sulfamethoxazole and trimethoprim in Edwardsiella piscicida. *Microbial Drug Resistance*, *24*(8), 1226-1235. |
| Su et al. 2017 | Su, H., Liu, S., Hu, X., Xu, X., Xu, W., Xu, Y., ... & Cao, Y. (2017). Occurrence and temporal variation of antibiotic resistance genes (ARGs) in shrimp aquaculture: ARGs dissemination from farming source to reared organisms. *Science of the Total Environment*, *607*, 357-366. |
| Huang et al. 2017 | Huang, L., Xu, Y. B., Xu, J. X., Ling, J. Y., Chen, J. L., Zhou, J. L., ... & Du, Q. P. (2017). Antibiotic resistance genes (ARGs) in duck and fish production ponds with integrated or non-integrated mode. *Chemosphere*, *168*, 1107-1114. |
| Muziasari et al. 2014 | Muziasari, W. I., Managaki, S., Pärnänen, K., Karkman, A., Lyra, C., Tamminen, M., ... & Virta, M. (2014). Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. *PLoS One*, *9*(3), e92702. |
| Nonaka et al. 2015 | Nonaka, L., Maruyama, F., Suzuki, S., & Masuda, M. (2015). Novel macrolide-resistance genes, mef (C) and mph (G), carried by plasmids from V ibrio and P hotobacterium isolated from sediment and seawater of a coastal aquaculture site. *Letters in applied microbiology*, *61*(1), 1-6. |
| Shah et al. 2014 | Shah, S. Q., Cabello, F. C., L'Abée-Lund, T. M., Tomova, A., Godfrey, H. P., Buschmann, A. H., & Sørum, H. (2014). Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. *Environmental microbiology*, *16*(5), 1310-1320. |
| Shah et al. 2012 | Shah, S. Q., Colquhoun, D. J., Nikuli, H. L., & Sørum, H. (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environmental science & technology*, *46*(16), 8672-8679. |