

Antibiotic resistance genes in the aquaculture sector: global reports and research gaps

Justin O.G. Kemp, Jessica J. Taylor, Lisa A. Kelly, Renée Larocque, Armando Heriazon, Kevin H.D. Tiessen, and Steven J. Cooke

Abstract: Aquaculture has been one of the fastest-growing food production systems over the last decade and increased intensification of production has created conditions that favour disease outbreaks. Antibiotics are commonly applied in the food animal sector to fight against bacterial infections; however, their inappropriate use contributes to the emergence of antibiotic-resistant bacteria. Investment in research and capacity-strengthening, in parallel with enforcing existing regulations around antimicrobial use, are potentially powerful tools in tackling the threat of antimicrobial resistance (AMR) emanating from animal producing systems such as aquaculture. However, directing investment effectively is challenging due to the limited data available that hinder the identification of risk areas for current and future AMR emergence. Here, we aim to partially fill this gap by analyzing the current peer-reviewed literature reporting antibiotic resistance genes (ARGs) in aquaculture food production systems and combining the data in a systematic map. Systematic searches of three bibliographic databases, a search engine, and 120 reviews returned 10 699 articles that were screened at title and abstract and then by full text ($n = 1100$). Two hundred and eighteen articles, spanning 39 countries and six continents, met all inclusion criteria and were coded to retrieve bibliographic, methodology, and study outcome data. ARG detections were associated with 44 families of fish and crustaceans and 75 genera of bacteria, with most studies employing primer-based methods to detect ARGs. A narrative synthesis explores implications for future research and policy as well as limitations of the systematic mapping methodology.

Key words: aquaculture, antimicrobial resistance (AMR), genes, fish, crustacean, One Health.

Résumé : L'aquaculture a été l'un des systèmes de production alimentaire qui a connu la croissance la plus rapide au cours de la dernière décennie et l'intensification accrue de la production a créé des conditions qui favorisent l'éclosion de maladies. Les antibiotiques sont couramment utilisés dans le secteur de l'alimentation animale pour lutter contre les infections bactériennes, mais leur utilisation inappropriée contribue à l'émergence de bactéries résistantes aux antibiotiques. L'investissement dans la recherche et le renforcement des capacités, parallèlement à l'application des réglementations existantes concernant l'utilisation des antimicrobiens, sont des outils potentiellement puissants pour lutter contre la menace de la résistance aux antimicrobiens (RAM) émanant des systèmes de production animale tels que l'aquaculture. Toutefois, il est difficile d'orienter efficacement les investissements en raison du peu de données disponibles qui empêchent d'identifier les zones à risque pour l'émergence actuelle et future de RAM. Ici, les auteurs visent à combler partiellement cette lacune en analysant la littérature actuelle revue par les pairs, qui fait état de gènes de RAM dans les systèmes de production alimentaire en aquaculture et en combinant les données dans une carte systématique. Des recherches systématiques dans trois bases de données bibliographiques, un moteur de recherche et 120 synthèses ont permis de trouver 10 699 articles qui ont été passés au crible quant au titre et au résumé, puis au texte intégral ($n = 1100$). Deux cent dix-huit articles, couvrant 39 pays et six continents, ont répondu à tous les critères d'inclusion et ont été codés pour récupérer les données bibliographiques, la méthodologie et les résultats des études. Les détections de gènes de la RAM ont été associées à 44 familles de poissons et de crustacés et à 75 genres de bactéries, la plupart des études utilisant des méthodes basées sur des amorces pour détecter les gènes de résistance aux antibiotiques (GRA). Une synthèse narrative explore les implications pour la recherche et les politiques futures ainsi que les limites de la méthodologie de cartographie systématique. [Traduit par la Rédaction]

Mots-clés : aquaculture, résistance aux antimicrobiens (RAM), gènes, poissons, crustacés, Un monde, une santé.

Introduction

Aquaculture is currently responsible for producing close to half of all aquatic animals consumed globally (FAO 2019). Driven by dwindling stocks in wild capture fisheries and increased demand for fish and seafood products globally, aquaculture has been one of

the fastest growing food production sectors since the turn of the century, with an annual growth rate of 5.8% during the period 2001–2016 (FAO 2018; Lulijwa et al. 2020). This growth has been supported in part by the intensification of production methods, much of which has occurred in low and middle-income countries (LMIC), particularly

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J.O.G. Kemp, R. Larocque, A. Heriazon, and K.H.D. Tiessen. International Development Research Centre, 150 Kent Street, Ottawa, ON K1G 3H9, Canada.

J.J. Taylor, L.A. Kelly, and S.J. Cooke. Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON K1S 5B6, Canada; Canadian Centre for Evidence-Based Conservation, Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, ON K1S 5B6, Canada.

Corresponding author: Justin O.G. Kemp (email: jkemp@idrc.ca).

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in Asia (Brunton et al. 2019). Intensification increases the proximity of animals to each other and can negatively impact water quality, creating crowded and environmentally challenging conditions that lead to physiological stress and impaired immune function favouring disease emergence (Cabello et al. 2013; Santos and Ramos 2018; Lulijwa et al. 2020). Antibiotics are commonly applied to treat pathogen outbreaks and mitigate associated economic losses (Santos and Ramos 2018; Brunton et al. 2019). Although prophylactic use of antibiotics is prohibited in most countries, inappropriate antibiotic use (including for growth promotion), which is partly supported by limited regulations and controls, creates selective pressures that favour the emergence of antibiotic-resistant bacteria (Watts et al. 2017; Henriksson et al. 2018; Brunton et al. 2019; Reverter et al. 2020).

Aquaculture differs from other food production sectors in terms of its biodiversity and socio-economic context, presenting unique opportunities for antimicrobial resistance (AMR) emergence and distinct challenges to addressing this emergence. 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) (FAO 2018; Henriksson et al. 2018). 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 (Leung and Bates 2013; Reverter et al. 2020). As no antibiotics have been specifically developed for aquaculture, those designed for livestock and humans are used, some of which are extremely important in human medicine (e.g., kanamycin) (Henriksson et al. 2018). These are generally incorporated into feed and applied metaphylactically at the population level. Unfortunately, as fish do not efficiently metabolize 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% (Watts et al. 2017; Santos and Ramos 2018; Lulijwa et al. 2020). These antibiotics then interact with an aquatic microbiome that harbours a large variety of mobile genetic elements where significant genetic exchange and recombination can occur (Watts et al. 2017; Santos and Ramos 2018; Thornber et al. 2020). In addition, the regulatory framework governing the use of antibiotics in aquaculture varies greatly among countries, with limited capacity for monitoring and enforcement in many of the developing countries that are major aquaculture producers (Santos and Ramos 2018; Brunton et al. 2019).

Research and capacity-strengthening (both in the technical and institutional sense) are potentially powerful tools in tackling the threat of AMR emanating from aquaculture as they directly address many previously identified mechanisms for controlling antimicrobial use around biosecurity, diagnostics, education, vaccines, alternative treatments, and legislation (Henriksson et al. 2018). However, gaining maximum impact from programs addressing AMR requires ways of identifying areas of greatest risk for current and future AMR emergence to effectively direct resources. Accessing this information through current global AMR surveillance systems is difficult as they are generally disconnected and underdeveloped, with a strong focus on humans (IACG 2018). The World Health Organization Global Antimicrobial Surveillance System (GLASS) has only enrolled 71 countries, with fewer than 50 countries reporting AMR rates in the latest report (WHO 2018). In terms of the food and agriculture sector, surveillance systems are even less developed and coordinated. While some high-income regions and countries, particularly Europe, the United States, Canada, Japan, and Australia have established some form of veterinary surveillance program (Schrijver et al. 2018; Sharma et al. 2018), there has been less activity in LMIC around this issue. Current initiatives, such as the Food and Agriculture Organization Assessment Tool for Laboratories and AMR Surveillance Systems (ATLASS) (FAO 2020) are at the level of mapping AMR surveillance capacity in LMIC with the aim of strengthening

technical capacity, coordination, and harmonization among actors, both internally and regionally/globally.

Fundamentally, AMR surveillance systems track (either directly or indirectly) the genetic determinants of resistance. These are the genes that code for the protective mechanisms that microorganisms have developed, through Darwinian selection, to counter naturally occurring toxic substances produced by themselves or other microorganisms, including environmental fungi and saprophytic bacteria (Holmes et al. 2016). The majority of antimicrobial drugs are these naturally produced substances or synthetic derivatives thereof, with only a few fully synthetic types (Holmes et al. 2016). Culture-based AMR assessment methods, such as the disc-diffusion test, test for the phenotypic expression of resistance by exposing microorganisms to antimicrobials and observing susceptibility (Reller et al. 2009). More recently, advances in molecular biology have facilitated the direct identification of resistance genes in microorganisms, either through targeted primers or secondary analysis of whole genome sequences. Genes conferring resistance to antimicrobials can emerge in a microbial population either through mutation and dissemination via normal vertical inheritance or acquired from other strains or species through horizontal gene transfer mechanisms. These include conjugation by plasmids, transduction by bacteriophages, or natural transformation by extracellular DNA (Lerminiaux and Cameron 2019).

Despite the risks for AMR emergence and dissemination associated with the rapidly expanding aquaculture sector, there are limited data sources from which to extract information on the incidence and geographic distribution of AMR, and particularly the genetic determinants of resistance, in the context of global aquaculture. Recently, Reverter et al. (2020) conducted a meta-analysis to explore the impact of global warming and AMR on aquaculture, including using data from antimicrobial susceptibility studies to calculate a multi-antibiotic resistance index (MAR) of aquaculture-related bacteria for 40 countries. Data from research studies targeting resistance genes could provide complementary insight into the nature of AMR in aquaculture, with research microbiologists potentially functioning as a loose proxy for a global observation network. Here we set out to test this proposition. The objective of this synthesis was to identify, collate, and describe the peer-reviewed literature that has reported antibiotic-resistant genes (ARGs) in bacteria sampled from aquaculture food production systems. The goal was to provide preliminary insights into the distribution and nature of AMR in aquaculture in the absence of an integrated global AMR surveillance system in these food production systems. Specifically, we asked: What is the global incidence, composition, and geographic distribution of genetic determinants of antibiotic resistance in bacteria associated with aquaculture food production systems?

Approach

This systematic map followed the protocol published at the inception of this project on the Open Science Forum (<https://osf.io/ws5n/>) informed by the Collaboration for Environmental Evidence guidelines (CEE 2019) and complies with Reporting Standards for Systematic Evidence Syntheses (ROSES) (Haddaway et al. 2018). Our methods deviated from the protocol through the adjustment of the search string to fit requirements for the ProQuest database, the incorporation of additional terms in the coding sheet, and the method of data extraction, which was shifted from a Google form to an excel spreadsheet.

Searching for articles

The search strategy aimed to capture relevant studies in the peer-reviewed literature using three databases focused on peer-reviewed publications and a single web-based search engine. In addition, the reference sections of relevant review articles were searched to identify articles not previously found.

Definition of the question components

Population

Aquaculture food production systems are defined as those that involve cultivating an organism in an aquatic environment with direct human involvement in the form of seed addition, feed addition, habitat engineering, water quality manipulation, or a combination thereof. This synthesis aimed to target intensive aquaculture food producing systems where the application of antibiotics is likely. It therefore focused on the finfish and crustacean sectors of global production and excludes the extensively farmed plant and mollusk sectors.

Measure of antibiotic resistance

A genetic indicator of resistance was selected (i.e., the presence/absence of ARGs as defined by the Comprehensive Antimicrobial Resistance Database (CARD; <https://card.mcmaster.ca>) (Alcock et al. 2020). This methodology was adopted as it provides a standardized method for AMR detection that partially mitigates operational, reagent quality, and interpretational issues associated with culture-based methods and potentially provides information on non-culturable components of the microbiome.

Geographical scope

Global, no limits on geographical scope.

Search terms and language

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>) (FAO 2016). Lists of antibiotic names and ARGs were extracted from CARD, a curated collection of characterized, peer-reviewed resistance determinants, and associated antibiotics (Alcock et al. 2020). Initial attempts to develop search strings using specific gene names extracted from the CARD database were abandoned due to the nonspecificity of wildcards when using this approach.

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. The terms were broken into four components (aquaculture descriptors, cultured species/habitat descriptors, resistance descriptors, and resistance units) and combined using Boolean operators “AND” and (or) “OR” (see Supplementary Material A¹). The comprehensiveness of the search was assessed using a collection of benchmark papers ($n = 25$) to ensure that these articles identified as relevant were represented in search results. (see Supplementary Material A¹).

Searches

Three bibliographic databases (ISI Web of Science Core Collection, Scopus, and ProQuest Dissertations & Theses Global) were searched in July 2019 using the primary search string as described in Supplementary Material A¹. The search string for ProQuest was condensed by the removal of antibiotic names to meet the limitations of the search function of this database (Supplementary Material A¹). The Carleton University institutional subscription was used to conduct the searches (Supplementary Material A¹). A further search was also performed using a condensed search string (256-character limit for searches) on the web-based search engine Google Scholar. The top 200 most relevant results were exported. In addition, the reference sections of 120 review articles identified as potentially relevant (113 at title and abstract screening

and seven from full text screening) were screened manually for articles that were within the scope of this systematic map and not captured by the previous searches. No updates to the search were performed during the systematic mapping process.

Article screening and study eligibility criteria

Screening process

Results from the bibliographic database were exported as either an .RIS file (Scopus, ProQuest) or as a coded .txt files (ISI Web of Science, Google Scholar) and then imported into CADIMA (Kohl et al. 2018), an open access online tool for systematic review management, where duplicates were removed. Numeric outcomes of the search strategy are described in the ROSES report (see Supplementary Material B¹).

All articles were screened at two distinct stages. Initial screening at title and abstract was followed by a second round of screening at full text using a pre-established set of eligibility criteria (Table 1). Prior to each stage of screening, a consistency check was conducted between the reviewers using a subset of articles. At title and abstract, 1070/10 699 articles (10%) were screened by two reviewers (JK and LK) with a Kappa score of 0.61 (SE = 0.042, 95% confidence interval 0.528–0.693) indicating good agreement. All discrepancies were discussed between the two reviewers and reconciled before proceeding with screening. Any articles that were unclear were flagged for a second opinion and eligibility discussed between reviewers to reach a decision. At full text, 120/1150 articles (10%) were again screened by two reviewers with a Kappa score of 0.817 (SE = 0.058, 95% confidence interval 0.703–0.931) indicating very good agreement between reviewers.

Study validity assessment

We did not appraise the validity of individual studies.

Data extraction

Following screening, articles selected as eligible for data extraction were processed by one of two reviewers (JK and LK) using a standard template (Table 2). The template was established in an Excel spreadsheet and captured key information in the broad categories of (i) bibliographic information, (ii) culture system descriptors, and (iii) bacteria and resistance using a combination of pre-populated drop-down menus and open-ended input as required.

Meta-data extraction was conducted down to the level of unique bacterial species or sample. Therefore, within each article, reports of the same gene in multiple strains/cultures of the same species were recorded as a single detection. However, reports of the same gene in multiple strains/cultures of the same species, but with differing aquaculture system, locality, or sample origin, were counted as separate detections.

Following extraction, each potential gene was referenced against the CARD database for a match to a gene and standardized to a single term based on the CARD database nomenclature if required (for example, tet(a), tet(A), tet-A etc. were standardized to tetA). Ancillary data relating to each matched gene, including the drug classes it is associated with, the resistance mechanism, and gene family were extracted as per the CARD ontology (Supplementary Material C¹).

Findings

Number and types of articles

A search of three bibliographic databases and Google Scholar returned 14 000 individual records. After duplicate removal, 10 699 articles were screened at abstract and title according to the eligibility criteria, of which 1150 records passed through to screening at full text. The majority of these articles ($n = 1100$)

¹Supplementary data are available with the article at <https://doi.org/10.1139/er-2020-0087>.

Table 1. Eligibility criteria.

Title and abstract
Population
1. Articles that report on a relevant food production system (i.e., aquaculture involving fish or crustacean species).
Study design/outcome
2. Articles that report the sampling of bacteria from the water, sediment, and other surfaces, infrastructure and resident biological organisms directly associated with an aquaculture farm, including the direct outflow.
Full text
Study design/outcome
2.1. Articles that extract DNA from bacterial or environmental samples and report the occurrence of genetic resistance determinants following PCR using suitable primers, or through secondary analysis of whole genomes.
Note: Articles that reported resistance to antimicrobials using culture-based methods were excluded, but flagged for future investigation.

Table 2. Data extraction template.

Category	Open input	Pre-populated categories
Bibliographic information		
Citation	x	
Journal	x	
Publication year		1900–2019
Publication title	x	
Primary author name	x	
Primary author country	x	
Corresponding author name	x	
Corresponding author contact	x	
Abstract	x	
Keywords	x	
Culture system descriptors		
Country of study		List – 246 countries
Region/province	x	
Latitude	x	
Longitude	x	
Year of study		1900–2019 or range
Water salinity	x	Freshwater brackish marine other no data
Cultured animal(s) common name	x	
Cultured animal(s) scientific name	x	
Cultured animal(s) family name	x	
Cultured animal(s) type		Fish crustacean combined no data
Culture system descriptor No. 1		Broodstock hatchery pond raceway tank cage RAS ornamental basket well fish/duck polyculture fish/chicken polyculture fish/goose polyculture fish/swine polyculture outflow no data
Culture system descriptor No. 2		as above
Culture system descriptor No. 3		as above
Culture system descriptor: other	x	
Bacteria and resistance		
Bacteria sample origin descriptors No. 1		Sediment aquaculture organism accessory organism feed waste soil
Bacteria sample origin descriptors No. 2		as above
Genetic analyses method		Primer genome
Genetic analyses method – other	x	
Bacterial species	x	
Genetic resistance determinant	x	

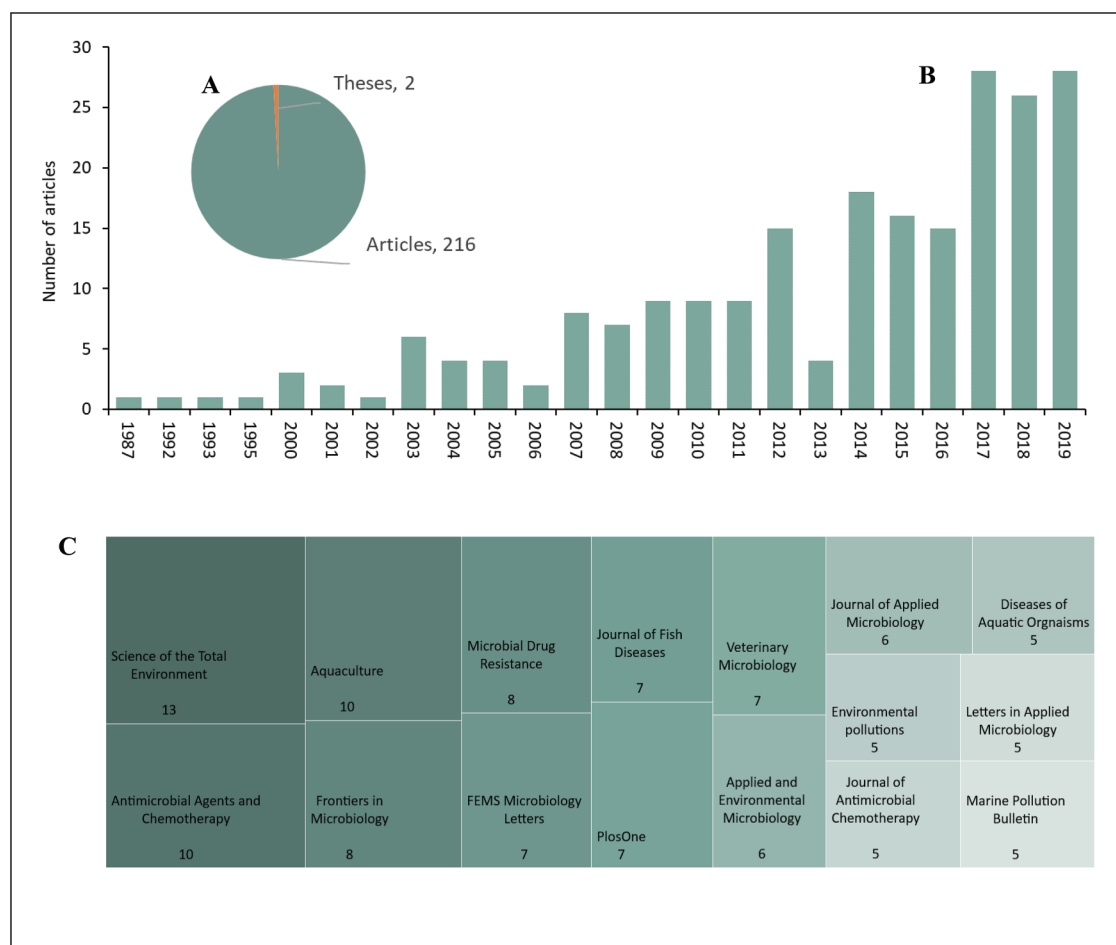
Note: RAS, recirculating aquaculture system.

were retrieved through Carleton University institutional subscriptions or inter-library loans, with 50 articles unobtainable given available resources (e.g., not accessible via inter-library loan system) or did not meet inclusion criteria (e.g., conference abstracts, non-English language publications). Following screening at full text, 890 articles were excluded for the following reasons: (i) study population ($n = 176$), (ii) study outcome ($n = 173$), (3) study methodology (culture-based; $n = 478$), duplicates ($n = 52$), article type (review article; $n = 7$), and article type (conference abstract; $n = 4$). A total of 210 articles were selected for inclusion in the systematic map. In addition, eight articles were included from searches of the bibliographic sections of relevant reviews.

Accordingly, 218 articles were included in the systematic map database and synthesis (see Supplementary Material B – ROSES form¹ and Supplementary Material D – Full-text screening outcomes¹).

The included articles varied across several metrics. There was a marked increase in the number of articles published annually since the first article in 1987 until 2019. Most articles (> 80%) were published in the last 10 years, with more than 50% being published in the last 5 years (Fig. 1A). All articles, barring two PhD theses, came from the commercially published literature (Fig. 1B). Articles came from 83 journals, with the top five contributors being *Science of the Total Environment* ($n = 13$), *Antimicrobial Agents and Chemotherapy* ($n = 10$), *Aquaculture* ($n = 10$), *Microbial Drug Resistance*

Fig. 1. Number of articles included in the systematic map by (A) publication type, (B) publication year, and (C) journal name for journals contributing five or more articles.



($n = 8$), and *Frontiers in Microbiology* ($n = 8$) (Fig. 1C). The residence of the primary authors aligned with the country of sampling or the location of the experiments in most articles (190 of 218 articles).

Systematic map

The systematic map is composed of two key components, namely (i) a database containing meta-data and coding for all studies selected for inclusion (see Supplementary Material E – Data¹) and (ii) a series of heatmaps to visualize patterns in the data extracted from these studies. Due to space limitations some heatmaps are truncated; however, the full datasets used to generate the heatmaps are provided (see Supplementary Material F – Heatmaps¹).

Geographic distribution of studies

The 218 articles included in the systematic map reported on 226 studies. A study was defined by the location of sample collection at the country level, as such, some articles reported on samples collected in more than one country. More than half of the studies were conducted in just five countries, namely China ($n = 47$), Japan ($n = 23$), Thailand ($n = 17$), Republic of Korea ($n = 17$), and the United States ($n = 14$). At a continental scale, Asia accounted for over half of the studies ($n = 129$), followed by Europe ($n = 47$), North America ($n = 20$), South America ($n = 17$), Africa ($n = 9$), and Australia ($n = 4$) (Fig. 2, Supplementary Material E – Data¹).

Study characteristics

To detect ARGs, 85% of studies employed primer-based polymerase chain reaction (PCR) techniques. A further 13% adopted

whole genome sequencing of bacteria (or plasmids) combined with gene databases to identify sequences that matched known ARGs, while 2% employed alternative methods such as DNA probes (Fig. 3A).

Primer-based studies had a higher mean number of samples (5.0 ± 6.5) per study compared to those using genome-based methods (1.8 ± 1.7) (Fig. 3B). By contrast, primer-based studies reported less ARGs per study (20.0 ± 28.4 versus 41.7 ± 79.5) and per sample (5.0 ± 7.1 versus 17.8 ± 24.6) compared to genome-based methods (Figs. 3C, 3D).

Sample characteristics

Fish aquaculture systems accounted for 90% of the 1023 separate detections extracted from the 226 studies. Within fish aquaculture systems, samples taken directly from aquaculture organisms accounted for 50% of the samples, followed by water samples, sediment samples, and feed samples, which accounted for a further 32%, 13%, and 3% of samples, respectively (Fig. 4). Data detailing the culture system where samples were taken from were not available in 39% of studies. Where such data were available, pond and cage culture were the most prevalent sources of samples, accounting for 61% of samples (Fig. 4). In terms of the bacterial genus associated with samples, *Aeromonas*, *Vibrio*, *Pseudomonas*, and *Enterococcus* were the most commonly reported, accounting for 20%, 9%, 6%, and 4% of samples, respectively. No bacterial genus was associated with 16% of samples, reflecting studies where the bacterial cultures were not identified or where DNA was

Fig. 2. Geographic distribution of studies selected for inclusion in the systematic map. Numbers correspond to the number of studies from each country or regions. Map created with Microsoft Excel using data available in Supplementary File E¹.

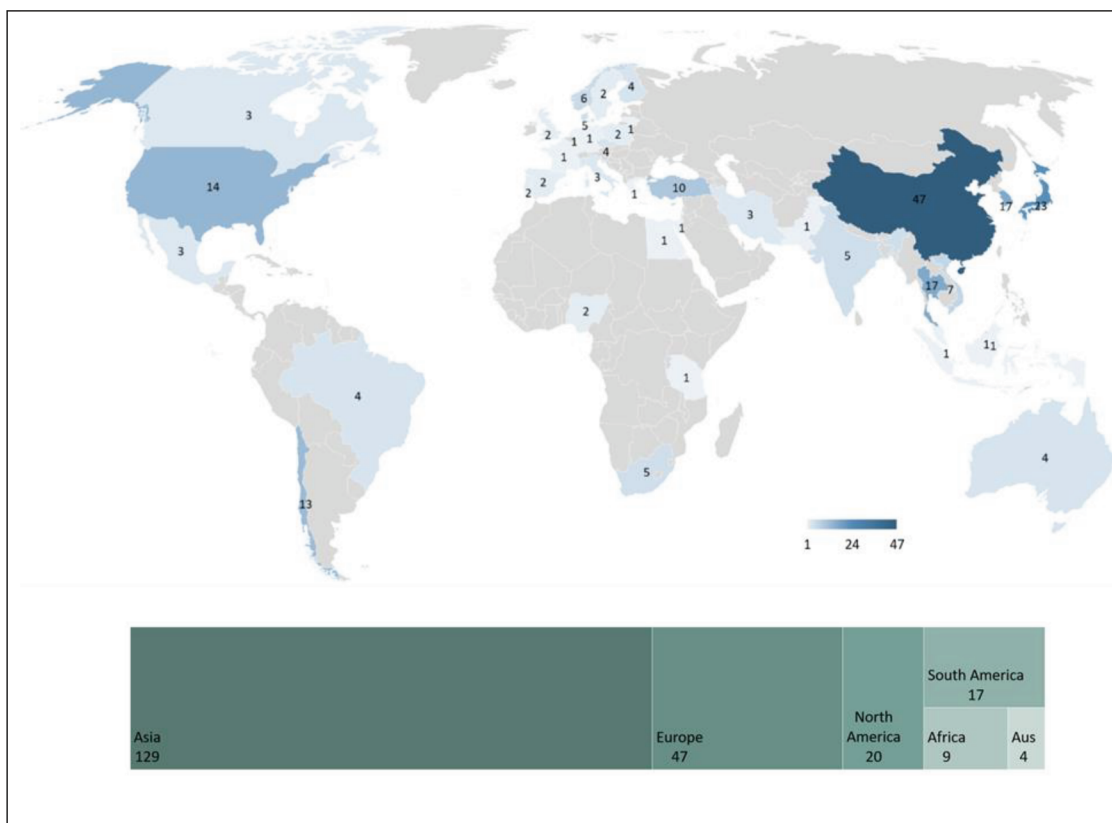
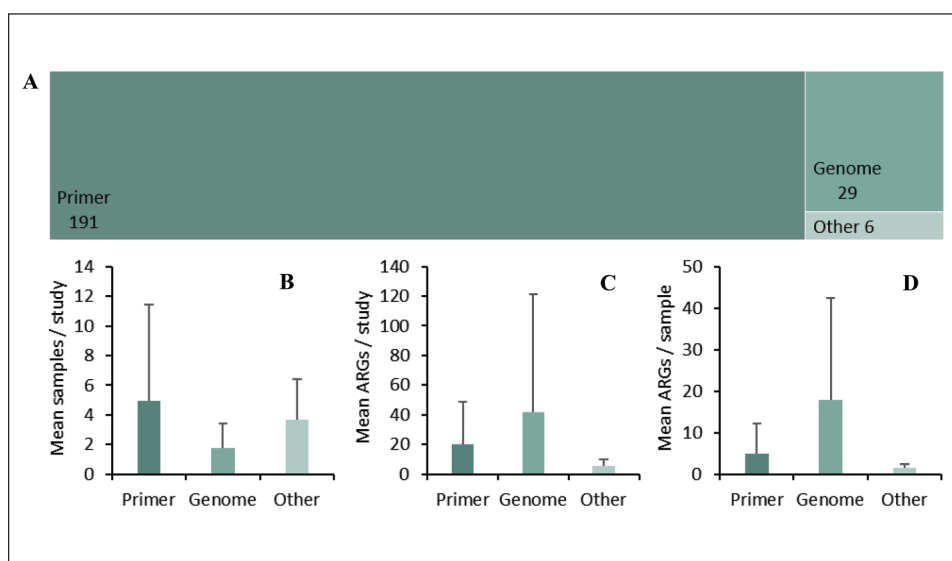


Fig. 3. (A) Methods employed to detect genetic resistance determinants (antibiotic resistance genes, ARGs), (B) the mean \pm SD number of unique samples analyzed per study by methodology, (C) the mean \pm SD number of ARGs detected per study by methodology, and (D) the mean \pm SD number of ARGs reported per sample by study.



sampled directly from the environment or aquaculture organisms (Fig. 5).

Antibiotic resistance genes (ARGs)

Cross-referencing all extracted potential ARGs against the CARD database resulted in 201 studies with a match, resulting in a total of

4467 potential gene detections. Of these, 375 were discarded as the match related to a gene family, enzyme, bacteria, integron, or plasmid rather than a specific gene. Ultimately 4092 individual gene detections were considered for further analysis (Fig. 6).

ARGs associated with resistance to a single antibiotic class accounted for 75% of all detections. Within this group of ARGs

Fig. 4. Heatmap depicting the number of unique samples extracted from 226 studies for each organism type, focused by the origin of the sample and the primary aquaculture system descriptor. RAS, recirculating aquaculture system.

	fish						crustacean					combined				no data			Grand Total
	Aquaculture organism	Feed	no data	Sediment	Waste	Water	Aquaculture organism	Sediment	soil	Waste	Water	Aquaculture organism	Feed	Sediment	Water	no data	Sediment	Water	
no data	261	1	10	36		63	11	3			4					5	1	2	397
Pond	63	26	4	29		53	10	14	1	1	25	1	1	3	11				242
Cage	48	1		42		48													139
Ornamental	34					22													56
Tank	28	3		5		11									1				48
Fish / Swine polyculture						40													40
IMTA						27													27
Outflow				5		10		1			1								17
RAS	13				1	2													16
Fish / Duck polyculture	1			2		7													10
Hatchery	8					1													9
Fish / Chicken polyculture	6			1	1														8
Other						4					1							1	6
Inflow						3													3
Well	1																		1
Broodstock	1																		1
Basket	1																		1
Fish / Goose polyculture						1													1
Raceway				1															1
Grand Total	465	31	14	121	2	292	21	6	1	1	31	1	1	3	12	5	1	3	1023

associated with a single antibiotic class, five antibiotic classes accounted for over 85% of the detections. The classes were tetracycline antibiotics (39%), sulfonamide antibiotics (22%), aminoglycoside antibiotics (13%), phenicol antibiotics (6%), and diaminopyrimidine antibiotics (6%) (Fig. 7). In terms of organism type, 76% of detected ARGs were associated with finfish aquaculture, 22% with crustacean aquaculture, and the remainder either combined fish/crustacean aquaculture or no data were provided. Within fish aquaculture, data relating to specifics of the culture system were not available in 38% of detections. Where data were available, many detections were associated with freshwater pond aquaculture (17%), marine cage culture (11%), and ornamental culture (6%). Within crustacean aquaculture, pond culture was dominant, associated with 64% of detections (Fig. 7, Fig. 8).

In terms of specific ARGs, 418 unique genes were reported, with just 60 of these responsible for over 75% of all reported detections. Within this frequently reported group, those associated with resistance to tetracycline antibiotics accounted for 46% of the detections, followed by ARGs associated with resistance to sulphonamides (21%), aminoglycosides (10%), and multiple antibiotics (8%). The 10 most commonly detected ARGs were *su1* (*n* = 339), *tetA* (*n* = 248), *su2* (*n* = 252), *tetM* (*n* = 219), *tetB* (*n* = 184), *flrR* (*n* = 105), *tetE* (*n* = 103), *tetD* (*n* = 103), *tetC* (*n* = 79), and *tetW* (*n* = 70) (Fig. 9, Supplementary Material F – Heatmaps¹).

The Salmonidae were the family most commonly associated with reported ARGs, accounting for 22% of all detections. Other prominent families included the Cyprinidae (7%) and the Cichlidae (6%). The Penaeidae accounted for 15% of detections. A family name could not be assigned to 18% of the detections (Fig. 9).

It was not possible to associate ARGs with a bacterial genus in just under half the detections (46%). Where data on the bacterial genus of ARGs were reported, ARGs were most commonly associated with the genus *Aeromonas* (27%), *Vibrio* (10%), *Escherichia* (8%), *Pseudomonas* (7%), and *Enterococcus* (5%).

Limitations of the map

Limitations related to the search strategy

The search strategy was wide ranging given the use of a broad search string that included both generic terminology and specific aquaculture organism and antibiotic names. However, scientific names were not included in the search-string component related to the targeted aquaculture organisms, and this may have influenced the number of results obtained. Furthermore, the finite time and resources available for this synthesis meant that the search was confined to the commercially published peer-reviewed literature. It is possible that valuable complementary information can be found in the grey literature, particularly databases and reports emanating from country and regional surveillance programs and networks; however, searching these sources was beyond the resources of this synthesis.

The use of English as the search language could have biased the search results. While the search engines used were locating non-English language articles that provided English abstracts, we acknowledge that a section of the relevant literature published entirely in non-English languages was excluded. The inclusion of non-English language literature sources and the exploration of the grey literature, particularly as it relates to government and producer commissioned studies, should be considered to improve the robustness of future syntheses on this subject.

Fig. 5. A heatmap depicting the number of unique samples extracted from 226 studies by bacterial genus and the origin of the sample (categorized by organism type).

	fish						crustacean					combined					no data		Grand Total
	Aquaculture organism	Water	Sediment	Feed	no data	Waste	Water	Aquaculture organism	Sediment	soil	Waste	Water	Sediment	Aquaculture organism	Feed	no data	Water	Sediment	
Aeromonas	150	32	10		6		1	6	4										209
no data	15	71	44	2		1	7	3	8	1		4	3	1	1				161
Vibrio	42	23	4				6	8	0			3				3	2	1	92
Pseudomonas	37	20	4						0										61
Enterococcus	15	13	14	3					0										45
Escherichia	9	22	3				2	2	1		1								40
Photobacterium	15	8	1						0							2	1		27
Citrobacter	18	5	2						1										26
Shewanella	13	8	1				1	1	0										24
Acinetobacter	5	6	3	2	1	1	5		1										24
Enterobacter	12	9	2						0										23
Edwardsiella	20	2	0						0										22
Pseudoalteromonas	4	5	1				3		0			4							17
Plesiomonas	8	2	2		4				0										16
Streptococcus	16		0						0										16
Bacillus		3	8	2			2		0										15
Lactococcus	9	2	0		2			1	0										14
Klebsiella	3	6	3	1					1										14
Yersinia	14		0						0										14
Staphylococcus	1	5	0	2					0										8
Serratia	5	2	1						0										8
Exiguobacterium		5	1						0										6
Psychrobacter	3		0	2					0										5
Salmonella	1	4	0						0										5
Hafnia	4	1	0						0										5
Stenotrophomonas	3	1	1						0										5
Nocardia	4		0						0										4
Flavobacterium	3		1						0										4
Carnobacterium	2		0	2					0										4
Corynebacterium			0	3					1										4
Kluyvera	3		1						0										4
Burkholderia	4		0						0										4
Proteus		3	0						0										3
Sphingobacterium		2	1						0										3
Lactobacillus			1	2					0										3
Rahnella	3		0						0										3
Kurthia		1	0	2					0										3
Arthrobacter		2	1						0										3
Chryseobacterium	2		1						0										3
Vagococcus		1	0	2					0										3
Shigella	1	2	0						0										3
Pantoea	2	1	0						0										3
Other	19	25	10	6	1		4		1			1							67
Grand Total	465	292	121	31	14	2	31	21	18	1	1	12	3	1	1	5	3	1	1023

Fig. 6. Flowchart of outcomes resulting from the cross-referencing of potential genetic resistance determinants, extracted from 226 studies, against the Comprehensive Antibiotic Resistance Database (CARD).

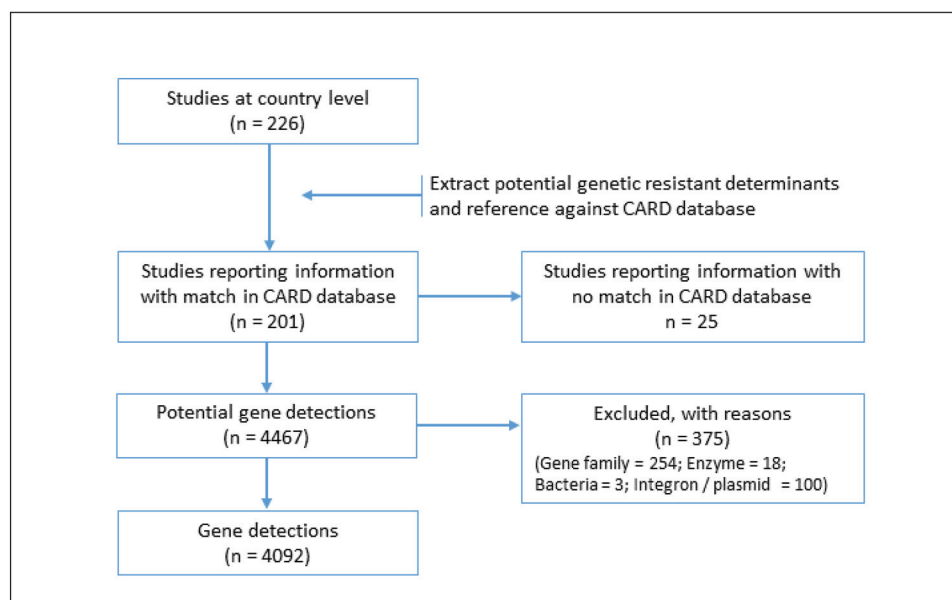


Fig. 7. Heatmap depicting the number of gene detections for each organism type, focused by culture salinity, primary aquaculture system descriptor, and the antibiotic class associated with the ARG (as per CARD). RAS, recirculating aquaculture system.

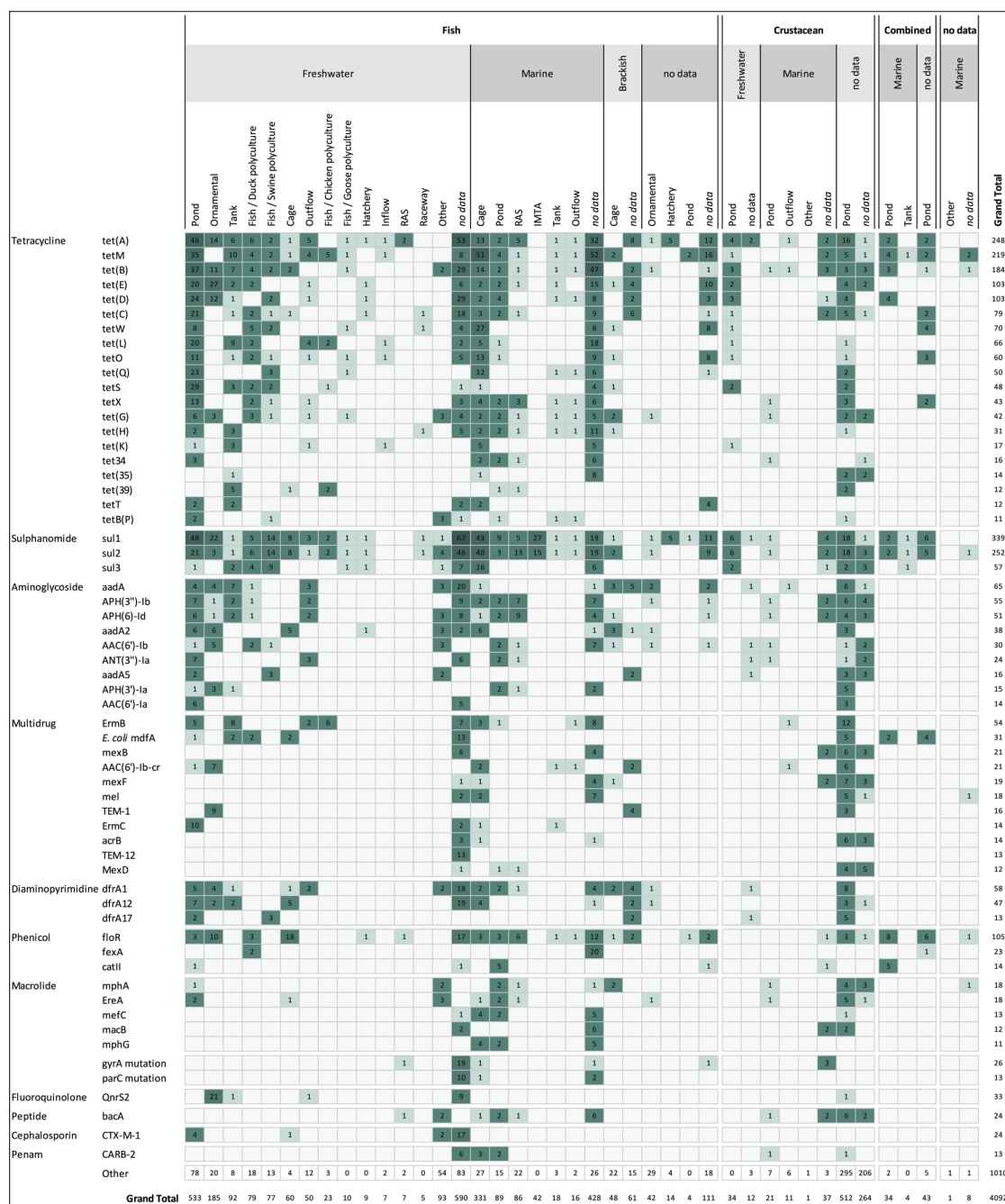
	Fish																											Crustacean							Combined			no data		Grand Total			
	Freshwater													Marine										Brackish	no data			Freshwater	Marine			no data	Marine	no data	Marine								
	Pond	Ornamental	Tank	Fish / Duck polyculture	Fish / Swine polyculture	Cage	Outflow	Fish / Chicken polyculture	Fish / Goose polyculture	Hatchery	Inflow	RAS	Raceway	Other	no data	Cage	Pond	RAS	IMTA	Tank	Outflow	no data	Cage		no data	Ornamental	Hatchery		Pond	no data													
Tetracycline antibiotic	252	53	48	28	17	5	13	10	6	3	4			3	8	115	144	26	11		9	8	214	9	14	2		2	52	16		4	1		8	39	13	11	1	12		3	1164
Sulfonamide antibiotic	70	25	4	15	37	17	4	4	3	3			2	6	120	99	12	18	42	2	2	44	3	2	5	1	20	14	1	2	7	38	7	4	3	11		1	648				
Aminoglycoside antibiotic	43	25	13	7	12	6	12	1		1				16	52	10	14	23			25	8	10	12		6			4	5	1		5	47	35				393				
Phenicol antibiotic	14	11		5		18	2			1			1	2	20	4	10	7		1	1	33	2	5	3	1	4			1	2	11	6		13		7	1	186				
Diaminopyrimidine antibiotic	18	6	3		5	8	2							2	44	9	5	5			9	2	11	10	4				3	2		26	4					178					
Fluoroquinolone antibiotic		22	4	1	1		5				1	2		5	18	3	1	1			1	1		1			1			1		21	4					94					
Macrolide antibiotic	3					1								5	4	10	9	3			18	3		1					2		2	18	5				1	85					
Peptide antibiotic		1		2							1			2	1	2	2	1			6								1			2	23	11				55					
Cephalosporin	7					2								5	17						1	1																33					
Glycopeptide antibiotic														12			1	1			3	3										5	1				26						
Rifamycin antibiotic	4	2												4				2						1					1				5	5				24					
Aminocoumarin antibiotic														2	2						2												13	3			22						
Penam antibiotic		2												6	4	2				1		1									2	2	2				24						
Fosfomycin antibiotic																1											1						6	3			11						
Carbapenem antibiotic		1																					1			6								2			10						
Streptogramin antibiotic		1												3							2											2	1				9						
Pleuromutilin antibiotic														1																			3				4						
Lincosamide antibiotic																																	3	1				4					
Nitroimidazole antibiotic														1																			1	1				3					
Mupirocin antibiotic																																	1	1				2					
acridine dye																																	1					1					
Multiple antibiotics ¹	122	36	20	21	5	3	12	8	1	1	2	2	0	23	156	37	7	14	0	5	5	66	12	21	10	5	0	20	4	3	2	9	1	6	241	150		6	0	13	1	1	1051
no data												1		2	29	8						3	3				1					3	9	6						65			
Grand Total	533	185	92	79	77	60	50	23	10	9	7	7	5	93	590	331	89	86	42	18	16	428	48	61	42	14	111	34	12	21	11	1	37	512	264		34	4	43	1	8	4092	

Limitations in coding and synthesis

Interpretation of the information presented in this systematic map should consider the following caveats regarding the data extraction, synthesis, and presentation process. First, no critical appraisal of the quality of the studies included in the systematic

map was conducted. This is likely less of an issue given the use of a present/absent genetic indicator of resistance, compared to cultured-based methods (e.g., diffusion disks) where both study design parameters and the interpretation of results are more variable.

Fig. 8. Heatmap depicting the number of gene detections of the most commonly reported ARGs (75% of total detections), focused by culture organism, water salinity, and primary culture system. RAS, recirculating aquaculture system.



Second, interpretation of heatmaps that include the variables of either “culture system descriptor” or “bacterial sample origin descriptor” should be undertaken with the knowledge that in some cases multiple values were assigned to these parameters. For example, multiple samples collected from an aquaculture organism, water, and sediment were pooled before analysis. However, only the first of these was used for the heatmaps. Two or more bacterial sample descriptors or two or more culture systems were present in 16% and 3% of total samples, respectively (see Supplementary Material E – Data¹).

Third, while the CARD database served as a useful reference to identify and categorize potential ARGs, it is likely that some potential ARGs excluded using CARD were in fact valid and could

be identified using other means. These data points ($n = 597$) have been retained and are available (see Supplementary Material F – Heatmaps¹, sheet “DATA_Expanded”, column “AA”, value = “2”) for future analysis.

Limitations of the evidence base

This systematic map specifically selected studies that used a genetic indication of antibiotic resistance. This approach is advantageous in that it standardized to some degree the method for AMR detection and partially mitigated some of the limitations associated with culture-based methods. However, it also potentially introduces its own set of biases. First, the presence of

an ARG does not necessarily imply expression of the gene and associated antibiotic resistance in the phenotype. Simultaneous application of standardized culture-based, antibiotic-exposure tests and genetic sequencing would be required to confirm an association.

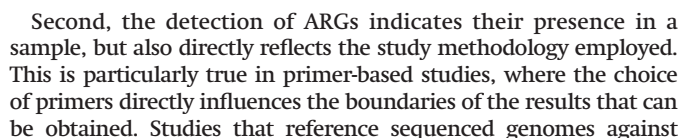
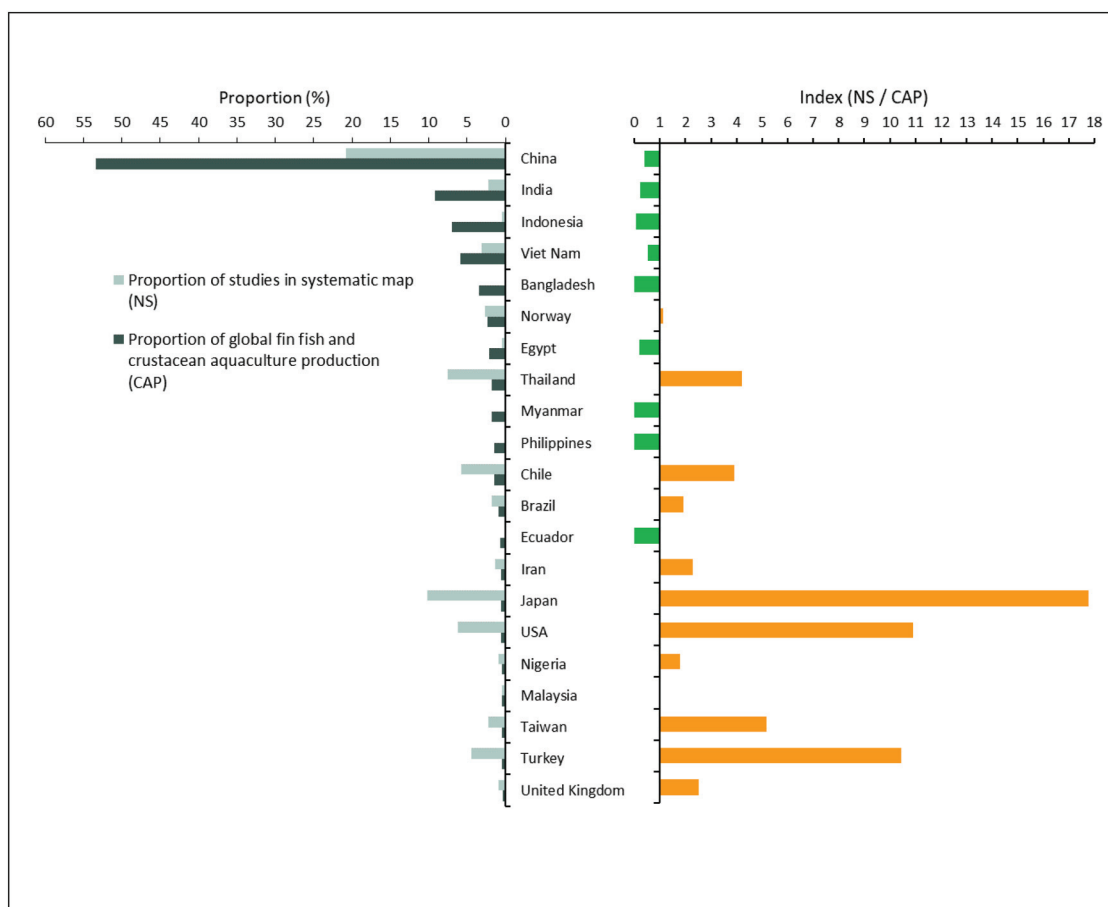


Fig. 10. Comparison of country aquaculture production (CAP, proportion of total global finfish and crustacean production) against the number of studies (NS, as a proportion of all studies) using an index (NS/CAP) from each of these 21 countries that cumulatively account for 95% of total finfish and crustacean aquaculture production.



gene databases are less prescriptive; however, selection criteria, such as the percentage similarity to confirm a match, can influence outcomes.

Third, the current synthesis did not consider the temperature of aquaculture systems when extracting reports of ARGs. Recent research indicates that antimicrobial use is accompanied by a parallel factor, in the form of higher temperature, in driving the selection and emergence of AMR (MacFadden et al. 2018; Reverter et al. 2020). As such, the presence of ARGs reflects complexity beyond the outcomes of a simple linear process resulting from antimicrobial use.

Fourth, studies selected for inclusion in this systematic map did not necessarily form part of a systematic surveillance program and were in some cases conducted in response to disease outbreaks in aquaculture facilities. Reference to disease, either in terms of the health of individual culture organisms sampled or general outbreak conditions, was associated with 32% of the included articles (see Supplementary Material E¹). The remaining 68% either explicitly mentioned healthy culture organisms or no disease-specific information was provided. As such, both the location of the studies and particularly the species of bacteria associated with ARGs would be biased by the interest of the investigators and common pathogens, respectively. The situation prevailing the studies cannot be assumed to be similar amongst all studies.

Given the previous four points, any attempt to interpret the heatmaps presented as directly indicative of the distribution and prevalence of ARGs in global aquaculture should be undertaken

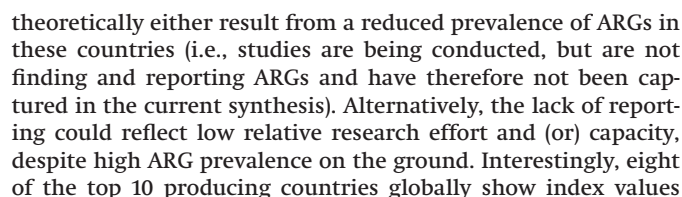
with caution. Clearly, the use of literature derived ARG distribution and prevalence is insufficient to provide a clear picture of the nature of AMR in global aquaculture. While this synthesis provides some insights into research gaps made apparent by the characteristics of the scientific literature on the subject, more robust data are needed to direct effective measures to address AMR in the sector. This could at least partly be achieved by combining ARG data with other measures of AMR, such as those derived from culture-dependent techniques.

Discussion and conclusions

The systematic map presented here provides a comprehensive synthesis of available information related to the distribution and composition of genetic resistance determinants in fish and crustacean aquaculture food production systems. This synthesis identified a total of 218 articles (226 unique sampling studies at country level) reporting potential ARGs in bacteria sampled from aquaculture systems, spanning 39 countries across six continents. These ARG detections were associated with 44 families of fish and crustaceans and 75 genera of bacteria, with most studies (85%) employing primer-based methods to isolate and amplify specific sequences associated with known ARGs. This map not only depicts general patterns in the available evidence, but also highlights knowledge gaps and biases in the existing evidence base, particularly imbalances between research output and total aquaculture production at the country level.

Approximately 95% of the global production of finfish and crustacean aquaculture can be attributed to 21 countries (FAO 2016).

Country aquaculture production (CAP, as a proportion of total global finfish and crustacean aquaculture production) can be compared to the number of studies from each of these 21 countries (NS, as a proportion of all studies included ($n = 226$)) in an index (NS/CAP) (Fig. 10). Values below 1 indicate a proportionally lower research output reporting ARGs relative to total aquaculture production in a country. This reduced reporting could



below 1, highlighting potential knowledge gaps in the prevalence and composition of ARGs in aquaculture systems in these countries. A recent review of culture-based studies reporting AMR in aquaculture (Reverter et al. 2020) found that the levels of AMR, calculated using a MAR index, were reasonably high (> 0.3) in many of these countries, suggesting that reduced prevalence of AMR is unlikely to be the cause of the lack of representation in the literature.

Implications for policy/management

One of the key strategic objectives of the Global Action Plan on AMR (GAPAMR) (WHO 2016) is to strengthen the knowledge and evidence base through surveillance and research. This strategy envisions both (i) generating knowledge on the incidence, prevalence, pathogen range, and geographical patterns of AMR and (ii) developing an understanding of how resistance develops and spreads, including how resistance circulates within and between humans, animals, and the environment. While large scale susceptibility testing would go a long way in addressing the first point, genetic approaches would offer considerable insight into the second. The outputs of this systematic map (i.e., the map database and heatmaps) provide a current collection of the existing peer-reviewed evidence regarding the incidence and global distribution of ARGs in aquaculture food production systems.

Furthermore, where data were available, the association between reported ARGs and bacterial genus offers mixed insights. The two most commonly reported genera, namely *Aeromonas* spp. and *Vibrio* spp., are considered major bacterial pathogens in aquaculture (Fig. 11) (Reverter et al. 2020). However, other major pathogens such as *Edwardsiella* spp., *Yersinia* spp., *Lactococcus* spp., and *Streptococcus* spp. were less commonly associated with ARGs. Moreover, the wide diversity of bacterial genera with ARGs reported from aquaculture settings would support the indication that these systems, and the larger aquatic environments they exist in, are active reservoirs of AMR (Marti et al. 2014). It is likely that AMR is already influencing production by limiting antimicrobial treatment options for at least some of the major bacterial disease-causing agents, with potential consequences for antimicrobial use as farmers seek out alternative antimicrobials or adjust dosage in response.

From an international policy perspective, this systematic map potentially highlights regions where support, either in the form of direct research funding or capacity-strengthening, can be directed to develop locally generated data on the genetic determinants of AMR in local aquaculture systems (Fig. 10). In addition, high costs associated with establishing genetic analyses capacity could be partially circumvented through the establishment of regional or international partnerships to facilitate knowledge and capacity sharing. Further to this, patterns emerging from this systematic map may allow targeting of research effort to aquaculture systems (i.e., marine fish cage, freshwater fish pond, freshwater ornamental fish, crustacean pond, and some polyculture systems) that show high incidences of ARGs (Fig. 7). However, this approach should be undertaken with the consideration that increased reporting of ARGs in these systems may reflect increased research effort rather than increased prevalence.

Implications for research

Several opportunities and considerations for future evidence synthesis or primary research are highlighted by the current systematic map.

1. The reported incidence of ARGs in ornamental fish would benefit from further investigation given the AMR dissemination risks associated with the high mobility of live animals on a global scale.
2. Gaps in geographic coverage from many of the large producers of aquaculture products, particularly in Asia. It is

possible that this is an artefact of language bias in the systematic map methodology used here.

3. Further synthesis to explore the incidence in aquaculture of ARGs considered important to human medicine. The World Health Organization list of Critically Important Antimicrobials for Human Consumption (WHO 2017) provides a useful reference in this regard.
4. While relatively few studies employed whole-genome approaches to detecting ARGs (Fig. 3), those studies that did generally reported a higher diversity of ARGs, likely an outcome of bypassing primer selection issues and (or) their ability to capture non-culturable or accessory components (i.e., the phageome) of the microbiome. Given the ability of ARGs to move between components of the microbiome, complementing targeted investigations of specific pathogens with ecosystem-level environmental sampling of microbiome DNA would provide a more nuanced understanding of ARG incidence and potential risk.

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