

## **A systematic review on antimicrobial resistance in aquaculture** (Tinjauan sistematik mengenai kerintangan antimikrobia dalam akuakultur)

Mohd Tarmizi Haimid<sup>1,2</sup>, Syahrin Suhaimi<sup>1</sup>, Cantira Mannun<sup>1</sup>, Sarah Nadira Hurairah<sup>1</sup> and Mark Buda<sup>2\*</sup>

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### **Abstract**

Antimicrobial-resistant bacteria have been commonly introduced into fish farming systems through animal waste or overfeeding. About 80% of antimicrobials in aquaculture are used to pick the bacteria from changeable genetic components that contain various resistance causal factors transmittable to other bacteria. This causes a detrimental change in aquatic biodiversity and organisms. This paper aims to perform a systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method to study the occurrence of AMR in the aquaculture industry. The objective of this study is to facilitate further studies in the field, given this gap found in the literature. This systematic review was conducted and reported based on the PRISMA approach, and the study selection process was completed using Mendeley Desktop and Microsoft Excel. Tetracyclines, sulphonamide, penicillins and phenols are the primary antimicrobial agents, whereas shrimp has been identified to be among the top species affected by AMR. This systematic review study provides critical information related to AMR, a current issue in the aquaculture industry.

### **Introduction**

For decades, the aquaculture industry has contributed a growing share of nutrition for the global population. Aquaculture refers to farming of fishes, crustaceans and molluscs in a controlled environment. It involves using of artificial feed, medicines and other inputs to ensure optimum growth rate that will be profitable for the aquaculture farm. Aquaculture has contributed 85 million tonnes or 48% of the global fish, crustaceans, and molluscs supply. For instance, freshwater fish production in aquaculture has tripled in 20 years, from 15.8 million tonnes in 1998 to 46 million tonnes in 2018 (FAO 2019). Aquaculture is part of the puzzle to answer

the challenges of feeding a rapidly growing human population for the years to come and lowering the impact of captured fish on the environment. Nevertheless, fish farming still relies heavily on antibiotics to prevent diseases (Hinchliffe et al. 2018).

In aquaculture, antibiotics act as a preventive or remedial measure besides being a component in feed supplements. The formation of antibiotic-resistant bacteria (ARB) in the surroundings has been attributed to antibiotics used as feed supplements in aquaculture (Aly et al. 2014). Antimicrobials have commonly been found in feed supplements as an additive to accelerate growth, disease prevention or treatment (Miranda et al. 2001).

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<sup>1</sup>Socio-Economy, Market Intelligence and Agribusiness Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

<sup>2</sup>Department of Agribusiness and Bioresource Economics, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

E-mail: markbuda@upm.edu.my

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Antimicrobial-resistant bacteria have been widely introduced into fish farming systems through animal waste or overfeeding. About 80% of antimicrobials in aquaculture are used to select the bacteria from mutable genetic components that contain various causative resistance factors that are transmissible to other bacteria. This leads to an adverse change in aquatic biodiversity and organisms.

The use of antimicrobials such as antibiotics, antifungals and antivirals is increasing proportionally with the growth of the aquaculture industry. Overuse of antimicrobials lead to antimicrobial resistance (AMR), which is the ability of microorganisms to resist antimicrobials, due to the exposure of the organisms such as bacteria, fungi, viruses and parasites to the antimicrobial drugs, resulting in the ineffective use of medicines towards the hosts in fighting diseases and infections. Limited research focusing on the antimicrobial resistance problem, specifically in aquaculture seems to be a major problem in the industry. However, it is an essential topic as the threat posed by antimicrobial resistance to human and animal health is escalating.

Each country and aquafarm have varying antibiotic usage trends (Rodgers et al. 2009). Usage increment is noticeable in locations with growing antimicrobial utilisation and prosecution competency (Schar et al. 2018). It was predicted that the use of antimicrobials would rise as the industry grows, with a change in dietary inclination and the progression to total production. The reports for antimicrobial use needs to be better documented, including the high-production species. The current estimated world usage of aquaculture antimicrobials is 33% from the year 2017 until the year 2030. The trend is mainly affected by the growing aquaculture industry, China being one of the biggest country to expand in aquaculture. The worldwide aquaculture industry has observed the increasing trend of antibiotic

resistance or failed remedial treatments in multiple fish species (FAO et al. 2020).

This paper aims to perform a systematic review using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method, as introduced by Moher et al. (2009), to study the occurrence of AMR in the aquaculture industry. PRISMA is a writing guideline used primarily in studies of the health sectors to create valuable reports of systematic reviews. This study aims to understand the AMR in the aquaculture industry and thus facilitate further studies in the field, given this gap found in the literature.

### **Methodology**

This systematic review was conducted and reported based on the PRISMA approach, as described in Section 1. According to the PRISMA checklist, several points must be addressed in a systematic review report.

### **Eligibility criteria**

Data on AMR in aquaculture was collected. The eligibility criteria for the selected studies are as follows where the examination must be: (1) a full-text journal article or conference proceedings; and (2) written in English.

### **Information source**

Three well-known electronic abstract and citation databases: (1) Emerald Insight, (2) Science Direct, and (3) Scopus, were used to collect existing studies.

### **Search strategy**

Finding relevant search terms, the following search terms were used in the three electronic abstract and citation databases: 'Antimicrobial Resistance' and 'Aquaculture'.

### **Study selection**

The study selection process was conducted using Mendeley Desktop and Microsoft Excel. Mendeley Desktop

was used for checking the duplications of papers. Microsoft Excel was used for easier management and sorting of study information. PRISMA flow chart was used to present the complete study selection process.

**Data collection process and data items**

In the process of data collection, the following items was extracted from the full-text paper: (1) the name of the author and title of the paper; and (2) research highlights. The data collected was managed in an Excel sheet and presented in a table.

**Results**

**Study selection**

The study selection process is shown in the PRISMA flow chart above (Figure 1). It entails four stages: identification, screening, eligibility and inclusion. During the first identification round, 411 studies were found in Scopus, Science Direct and Emerald Insight. Duplicated papers were excluded using the Check for Duplicates tool in Mendeley Desktop. Only journal articles and conference proceedings were included. At the beginning of the screening phase itself, 81 papers were excluded as it consisted

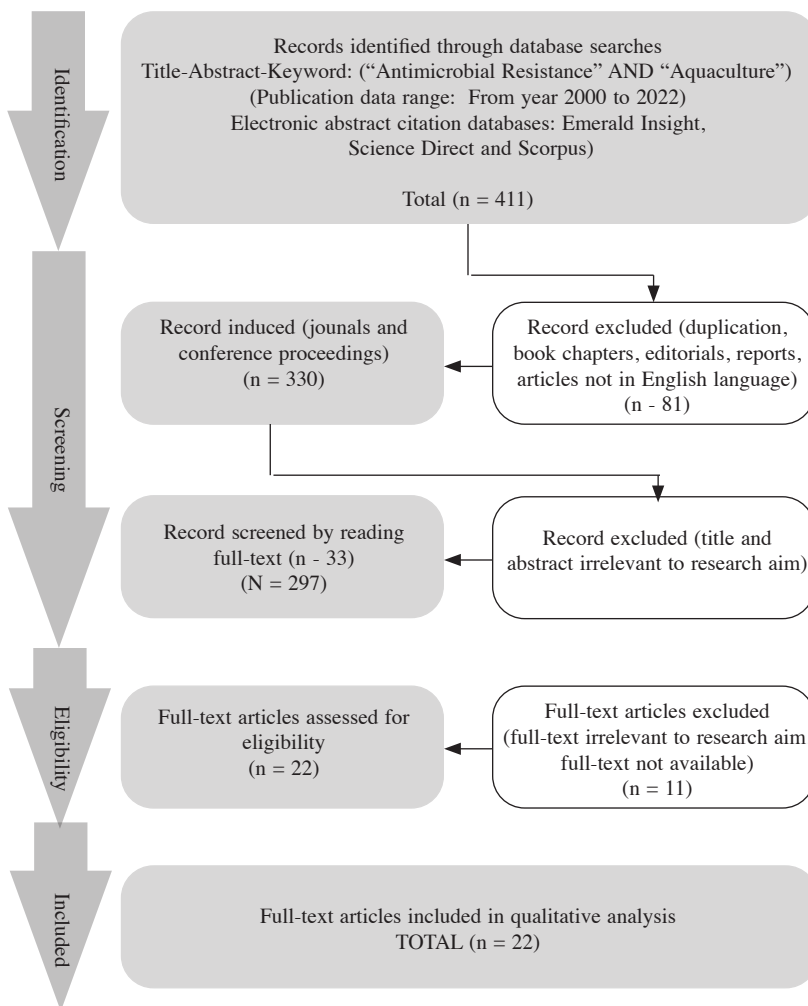


Figure 1. Study selection process

duplicated papers, book chapters, editorials, reports and those not written in English. The title and abstract of 330 articles were then screened to exclude 297 forms that didn't meet this research's aim. In the eligibility stage, 33 full-text papers were downloaded and read, 11 of it excluded, where primarily, the documents of AMR were not specified in aquaculture. Lastly, 22 articles were included in the review for qualitative analysis.

## Discussion

### *Antimicrobial use and resistance in aquaculture*

According to a survey by Tusevljak et al. (2013), the most often reported antimicrobial agents used across all aquaculture species worldwide are tetracyclines, followed by sulphonamide, penicillins and phenols. Tetracyclines are widely used in aquaculture as it is one of the few approved cost-efficient antimicrobials in most countries

### Highlights of the study

Paper	Author	Highlights
Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia	(Akinbowale et al. 2006)	Assessed AMR in bacteria in Australian aquaculture. Varied resistance has been identified in ampicillin, Amoxycillin, cephalixin and erythromycin.
Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms	(Gordon et al. 2007)	We determined the number of antimicrobial resistances in <i>Aeromonas</i> spp.
Antimicrobial use and resistance in aquaculture: Findings of a globally administered survey of aquaculture-allied professionals	(Tuševljak et al. 2013)	Observed the opinions of aquaculture professionals regarding AMR in 25 countries. Tetracyclines are mainly used as antimicrobials, and the resistance in 5 species is not higher than 52%. The most high-risk species to AMR is shrimp.
Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and animal and human health.	(Cabello et al. 2013)	Examined the use of antimicrobials and the effects on AMR and the health of animals and humans.
Water metagenomic analysis reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam.	(Nakayama et al. 2017)	Identified the role of polluted water in disseminating AMR by analysing the antimicrobial residues and AMR genes in 12 aquaculture freshwater in Vietnam. Backyard-based aquacultures have a wide range of water microbiota depending on aquaculture management practices.
A pharmaco-epidemiological study of antibacterial treatments and bacterial diseases in Norwegian aquaculture from 2011 – 2016	(Lillehaug et al. 2018)	Performed analyses on antibacterial sales and the prescription of Norwegian fish farming through examination, fish category and the production stage. The industry has used a small volume of antibacterial agents.

Paper	Author	Highlights
Antimicrobial drug resistance in fish pathogens	(Miller and Harbottle 2018)	Conducted antimicrobial susceptibility testing of aquatic bacterial pathogens.
Antimicrobial resistance and the environment: Assessment of advances, gaps and recommendations for agriculture, aquaculture and pharmaceutical manufacturing	(Topp et al. 2018)	Presented the primary issues regarding the environmental effects of antibiotic usage in agriculture and aquaculture and the discharge released by antibiotic manufacturing.
Composition and antimicrobial resistance profile of Gram-negative microbiota prevalent in aquacultured fish	(Ruzauskas et al. 2018)	We identified and analysed antimicrobial-resistant Gram-negative bacteria in pond fish aquaculture. <i>Pseudomonas spp.</i> has the highest multiresistant isolates and the most common resistance to beta-lactams.
The AMR problem: demanding economies, biological margins, and co-producing alternative strategies	(Hincliffe et al. 2018)	The author demonstrated the problems of AMR in Bangladesh's aquaculture sector. The ways to overcome the difficulties are lowering disease incidence and transmission rates, regulating antimicrobial uses and educating drug users.
Raising awareness of antimicrobial resistance in rural aquaculture practice in Bangladesh through digital communications: a pilot study.	(Thornber et al. 2019)	Investigated the effectiveness of delivering AMR knowledge and awareness to rural aquaculture farmers in Bangladesh.
An understated danger: Antimicrobial resistance in aquaculture and pet fish in Switzerland, a retrospective study from 2000 to 2017	(Delalay et al. 2020)	Conducted an AMR test for 14 antimicrobials from farmed and ornamental fish in aquaculture. The lowest resistant samples are amoxicillin, gentamycin and norfloxacin.
Aquaculture at the crossroads of global warming and antimicrobial resistance	(Reverter et al. 2020)	Conducted meta-analyses to explore the impact of AMR on aquaculture. Findings show that countries more vulnerable to climate change have a higher risk of AMR.
Aquaculture component of a national action plan on antimicrobial resistance in Malaysia	(Noordin et al. 2020)	Presented the outcomes of activities by the Department of Fisheries related to AMR in Malaysia. The AMR results suggest that <i>Escherichia coli</i> isolates are most resistant to erythromycin.

Paper	Author	Highlights
Complexities involved in source attribution of antimicrobial resistance genes found in aquaculture products	(Karunasagar et al. 2020)	Presented the origin, evolution and spread of AMR in aquaculture. Antibiotic resistance exists in bacteria even from environments without antibiotics exposure. However, overuse of antibiotics has led to the selection and spread of multidrug-resistant bacteria.
Investigation of the correlation between the use of antibiotics in aquaculture systems and their detection in aquatic environments: A case study of the Nera River aquafarms in Italy	(Sargenti et al. 2020)	Identified that a correlation exists between antibiotic concentrations detected in surface waters and the utilisation of flumequine and florfenicol.
Sources, behaviour and health risks of antimicrobial resistance genes in wastewaters: A hotspot reservoir	(Gwenzi et al. 2020)	Presented the hotspot sources, behaviour and fate in wastewater as well as health risks and risk factors of Antimicrobial Resistance Genes (ARGs).
Unravelling the menace: detection of antimicrobial resistance in aquaculture	(Preena et al. 2020)	Presented a few detection methods of AMR in aquacultures, such as antimicrobial susceptibility methods, molecular detection and rapid detection tool.
A case study on the distribution of the environmental resistance in Korean shrimp farms	(Seong et al. 2021)	Conducted analyses to investigate the distribution of AMR and Antibiotic-resistant bacteria (ARB) at multiple stages in shrimp farming. AMR and ARB distributions are different across the farming stages. Anthropogenic activity may result in the formation of environmental resistors.
Combined administration routes of marine yeasts enhanced immune-related genes and protection of white shrimp ( <i>Penaeus vannamei</i> ) against <i>Vibrio parahaemolyticus</i>	(Licona-Jain et al. 2022)	Identified that shrimp has been minimally protected from bacterial infection for seven days through combined yeast and route methods.
The intervention of antimicrobial peptide usage on antimicrobial resistance in aquaculture	(Lu et al. 2022)	Discussed the intervention of antimicrobial peptide usage on AMR in aquaculture. The study shows that cecropin treatment could have lessened the multi drug resistant bacteria.
Typology of interventions for antimicrobial use and antimicrobial resistance in aquaculture systems in low and middle income countries	(Garza et al. 2022)	Presented a typology to classify the interventions to reduce antimicrobial usage and lessen AMR in aquaculture.

Paper	Author	Highlights
Biodegradable peptide polymers as alternatives to antibiotics used in aquaculture	(Ma et al. 2022)	Conducted analyses on peptide polymers which exhibit effects on pathogenic bacteria in aquaculture and prevent the formation of bacteria which lead to antibiotic resistance.
A potential alternative to traditional antibiotics in aquaculture: Yeast glycoprotein exhibits antimicrobial effect in vivo and in vitro on <i>Aeromonas caviae</i> isolated from <i>Carassius auratus gibelio</i>	(Wu et al. 2020)	Performed the pathogenicity analysis to determine the antimicrobial activity of yeast glycoprotein (YG) against <i>Aeromonas caviae</i> for alternative antibiotic usage in aquaculture.

such as the United States, Canada, India, Indonesia and Japan. Their findings also highlighted that the species most often to be reported for AMR is shrimp, while the least is written for clams, crawfish, lobster, mussels, and oyster species. Cabello et al. (2013) highlighted that using prebiotics, probiotics and vaccines under the right circumstances could substitute the overuse of antimicrobials in aquaculture. Delay et al. (2020) found that antimicrobials resistance was higher in ornamental fish than in farm fish and recirculation systems. In summary, the shrimp industry may have the most significant use of antibiotics, although other, less written evidence that other species do not use the antibiotic is not presented. Therefore, more research needs to be directed towards aquaculture as a whole.

#### ***Causes of antimicrobial resistance in aquaculture***

Antimicrobial agents are often administered in aquaculture farms using broadcasted medicated feeds (Miller and Harbottle 2018). According to Delay et al. (2020), bacterial diseases, the leading cause of morbidity in aquaculture species, must be treated and often by antimicrobial use, such as antibiotics. The overuse of antibiotics contributes to the increase of AMR problems in aquaculture. Therefore, a sustainable solution to minimise the use of antibiotics is needed.

#### ***Input alternatives to replace the use of antibiotics in aquaculture***

The peptide polymers have been proven to be an excellent substitute for aquaculture antibiotics (Ma et al. 2022). The peptide polymer shows dominant actions against common pathogenic bacteria in aquaculture and produces minor cytotoxicity effects in vitro and a powerful remedial perspective in vivo. Furthermore, after multiple treatments, *S. aureus* and *E. coli* have not cultivated resistance against the peptide polymers. In addition, the treated *S. aureus* and *E. coli* also have not generated cross-resistance against typical antibiotics used in aquaculture. Enzymatic degradation in peptide polymers enabled biodegradation, transforming the peptide polymers into single amino acids and dipeptides. These amino acids and dipeptides possess no antibacterial activity; hence, no antimicrobial resistance will be produced. Besides that, *Aeromonas caviae* has been significantly inhibited by yeast glycoprotein through in vivo and in vitro methods which can be a promising alternative to antibiotics (Wu et al. 2020). This method should be used as a remedial step for *A. caviae* disease in aquaculture as it is safer and more ecological-friendly.

#### **Conclusion**

This systematic review study provides critical information related to AMR, a current issue in the aquaculture industry. Tetracyclines, sulphonamide, penicillins

and phenols are the primary antimicrobial agents, whereas shrimp has been identified to be among the top species affected by AMR. The extensive use of antibiotics has led to the occurrence of AMR. Previous studies have suggested the utilisation of peptide polymer and yeast glycoprotein to substitute antibiotics and thus reduce AMR in aquaculture. The findings have inclusively gathered knowledge from various literature studies, which can be utilised to understand the issue and improve the aquaculture industry's development.

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## **Abstrak**

Bakteria rintang terhadap antimikrob wujud dalam sistem penternakan ikan atau akuakultur melalui sisa najis haiwan dan pemberian makanan yang berlebihan. Kira-kira 80% daripada antimikrob yang digunakan dalam akuakultur memilih bakteria daripada komponen genetik boleh ubah yang mengandungi pelbagai faktor penyebab rintangan yang boleh dipindahkan kepada bakteria lain. Ini menyebabkan perubahan yang memberikan kemudahan dalam biodiversiti akuatik dan organisma. Artikel ini bertujuan untuk melakukan tinjauan sistematik menggunakan kaedah *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) untuk mengkaji kejadian AMR dalam industri akuakultur. Objektif kajian ini adalah untuk memudahkan kajian lanjutan dalam bidang tersebut, memandangkan terdapat jurang pengetahuan dalam literatur. Tinjauan sistematik ini telah dijalankan dan dilaporkan berdasarkan pendekatan PRISMA, dan proses pemilihan kajian telah dijalankan menggunakan Mendeley Desktop dan Microsoft Excel. Hasil tinjauan sistematik ini mendapati tetrasiklin, sulfonamida, penisilin dan fenol adalah agen antimikrob utama, manakala udang telah dikenal pasti sebagai antara spesies teratas yang terjejas oleh AMR. Kajian tinjauan sistematik ini menyediakan maklumat kritikal berkaitan AMR, yang merupakan isu semasa dalam industri akuakultur.