INPLASY

Incidence and antimicrobial resistance of Aeromonas hydrophila in seafood: a systematic review and meta-analysis

Corresponding author:
Saharuetai Jeamsripong

saharuetaij@gmail.com

Author Affiliation:
Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University.

Odoi, JO; Anuntawiru, S; Jeamsripong, S.

ADMINISTRATIVE INFORMATION

Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202490043

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 10 September 2024 and was last updated on 10 September 2024.

INTRODUCTION

Review question / Objective 1) What is the prevalence of Aeromonas hydrophila in seafood species, including fish, shrimp, and shellfish? (2) What are the phenotypic and genotypic characteristics of antimicrobial resistance in A. hydrophila strains isolated from seafood? (3) What virulence factors are commonly associated with A. hydrophila strains in seafood? (4) How do AMR and virulence factors in A. hydrophila impact public health and the sustainability of aquaculture?

Rationale The rationale for this study stems from the growing global concern over antimicrobial resistance (AMR) and its implications for both public health and food security. Aeromonas hydrophila, a pathogenic bacterium commonly found in aquatic environments, is frequently associated with seafood, including fish, shrimp, and shellfish. This bacterium poses a significant risk due to its ability to cause a range of infections in humans, from gastroenteritis to wound infections, and its capacity to develop resistance to commonly used antibiotics.

As aquaculture continues to expand to meet the increasing global demand for seafood, the overuse of antimicrobials in farming practices has intensified the emergence of AMR. This situation is further exacerbated by environmental contamination from terrestrial sources, such as agricultural run-off and sewage discharge, which introduces additional resistant bacteria and resistance genes into aquaculture systems. Consequently, seafood has become a key vector for the transmission of resistant bacteria, including A. hydrophila, to humans.

Despite the known risks, there is limited comprehensive data on the global prevalence of A. hydrophila in seafood, its AMR patterns, and the specific virulence factors that contribute to its pathogenicity. Understanding these factors is critical for developing strategies to mitigate the spread of AMR in aquaculture and protect public health. Therefore, this study aims to systematically

review and analyze existing research to provide updated insights into the prevalence, AMR characteristics, and virulence factors of A. hydrophila in seafood, ultimately guiding policy development and future research efforts.

Condition being studied The condition being studied in this systematic review and meta-analysis is the prevalence of Aeromonas hydrophila in seafood species, with a focus on its antimicrobial resistance (AMR) patterns and associated virulence factors. The review aims to assess the public health risks posed by A. hydrophila, particularly its role in foodborne illnesses and its potential to spread AMR, which has implications for both human health and the sustainability of aquaculture practices.

METHODS

Search strategy Comprehensive systematic review searches will be conducted in Google Scholar (https://scholar.google.com/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), ScienceDirect (https://www.sciencedirect.com/), and Scopus (https://www.scopus.com/) for studies published between January 2020 and December 2024. The search will use the following terms: (("*Aeromonas hydrophila*" OR "*A. hydrophila*") AND ("antibiotic" OR "antibiogram" OR "antimicrobial resistance" OR "resistance" OR "multidrug resistance" OR "antimicrobial susceptibility testing" OR "multidrug resistance" OR "genotypic determinants" OR "genotype") AND ("virulence factors" OR "virulence genes") AND ("fish" OR "shellfish" OR "shrimp" OR "seafood"), with no language restrictions. To ensure a comprehensive collection of studies, several keyword combinations will be used for each relevant topic area. This approach will aim to include as many studies as possible, covering a wide range of research on *A. hydrophila*, antimicrobial resistance, and various seafood types. The characteristics of the studies included, such as the year of publication, seafood type, geographical location, and antimicrobial susceptibility testing methods, will be documented.

During the review process, the CoCoPop framework will be used to address conditions (prevalence of bacteria and AMR, bacterial isolation and confirmation, and antimicrobial susceptibility testing), context (geographical distribution), and population (various seafood types). This framework will guide the research questions on the prevalence of *A. hydrophila* (Munn et al., 2018).

Participant or population The review will focus on studies involving seafood animal species as the primary participants. Specifically, the review will address Aeromonas hydrophila isolated from various seafood types, including fish, shrimp, and shellfish. These participants will encompass both wild-caught and farmed species, sourced from diverse aquaculture environments or natural water bodies. The review will include studies that examine seafood species from different geographical regions to assess the prevalence of A. hydrophila, its antimicrobial resistance (AMR) patterns, and associated virulence factors. No restrictions will be placed on the specific species of fish, shrimp, or shellfish, provided that A. hydrophila has been isolated from them. The review will also include studies that analyze the phenotypic and genotypic characteristics of A. hydrophila from these seafood species.

Intervention Not applicable.

Comparator Not applicable.

Study designs to be included This review will include cross-sectional studies that report on the prevalence of Aeromonas hydrophila, antimicrobial resistance patterns, and virulence factors in seafood such as fish, shrimp, and shellfish. It will also consider experimental studies assessing antimicrobial susceptibility testing of A. hydrophila isolates, using methods like disk diffusion and molecular techniques. Additionally, epidemiological studies documenting geographical trends and A. hydrophila outbreaks, as well as surveillance reports on its occurrence in aquaculture and seafood, will be included to provide comprehen.

Eligibility criteria For this systematic review, eligibility will be limited to peer-reviewed original research papers published in reputable scientific journals, each assigned a DOI. These journals will be required to be indexed in recognized databases such as Google Scholar (https://scholar.google.com), PubMed (https://pubmed.ncbi.nlm.nih.gov), Science Direct (https://www.sciencedirect.com), and Scopus (https://www.scopus.com). Selected articles will focus on assessing the prevalence, virulence potential, and antimicrobial resistance of A. hydrophila isolated from seafood species, regardless of whether the seafood is wild-caught or farmed in aquaculture. Only studies that are accessible for comprehensive analysis and adhere to stringent reporting standards will be deemed eligible. It will be essential that these studies provide clear descriptions or appropriate references for the methodologies employed in bacterial isolation and

identification, antimicrobial susceptibility testing (AST), and virulence factor detection. Additionally, the findings, discussions, and conclusions in these studies will be required to be clearly presented and structured in a manner that allows for thorough evaluation and critical appraisal. These stringent criteria will be consistently applied during the selection process to ensure that only the most relevant and high-quality studies are included in the review.

Information sources The information sources for this systematic review will primarily consist of reputable electronic databases, including Google Scholar (https://scholar.google.com), PubMed (https://pubmed.ncbi.nlm.nih.gov), ScienceDirect (https://www.sciencedirect.com), and Scopus (https://www.scopus.com). These databases will be systematically searched for peer-reviewed articles published between January 2020 and December 2024.

Main outcome(s) The outcomes of this systematic review and meta-analysis will focus on key areas related to Aeromonas hydrophila in seafood. The primary outcome will assess the prevalence of A. hydrophila across different seafood species, such as fish, shrimp, and shellfish, based on studies published between January 2020 and December 2024. Prevalence rates will be reported as percentages or proportions with confidence intervals (CIs). The secondary outcomes will examine antimicrobial resistance (AMR) patterns, analyzing both phenotypic and genotypic characteristics of resistance to various antibiotic classes, with effect measures such as odds ratios (OR) and relative risks (RR) for multidrug resistance (MDR). Additionally, the review will explore virulence factors in A. hydrophila isolates, including toxin production and hemolysin activity, documented based on detection methods like PCR. These factors will be categorized and reported as frequencies or proportions. Finally, the review will evaluate the public health impact by assessing the risks of A. hydrophila transmission through seafood consumption and tracking trends in AMR and virulence over time. Effect measures, including risk ratios (RR), will be used to quantify human infection risks. The synthesis of these outcomes will offer comprehensive insights into the burden of A. hydrophila in seafood and its implications for public health.

Quality assessment / Risk of bias analysis The risk of bias in the eligible studies will be assessed using the Joanna Briggs Institute (JBI) critical appraisal tools, which are specifically designed for cross-sectional and other epidemiological study

designs (Munn et al., 2015). For cross-sectional studies, the tools include nine key categories on the checklist for studies reporting prevalence. These categories focus on aspects such as inclusion criteria, the reliability and accuracy of the measurements, and the management of confounding factors. The risk of bias will be classified based on a total score out of 9, with scores of 7-9 indicating low risk, 4-6 suggesting moderate risk, and 3 or lower representing high risk of bias. Two authors (JO and SA) will independently evaluate the risk of bias, and any discrepancies will be resolved through discussion with a third investigator (SJ). This process ensures a rigorous and transparent assessment of study quality.

Strategy of data synthesis To examine the prevalence of A. hydrophila and phenotypic and genotypic resistant A. hydrophila, the number of positive samples/isolates will be divided by the total number of samples/isolates. Differences in the prevalence of A. hydrophila and resistant A. hydrophila among various aquatic animals, specifically fish, shellfish, shrimp, and others, will be estimated using a meta-analysis with a random-effects model. The results will be presented as a pooled estimate of prevalence with a 95% confidence interval (C.I.). Heterogeneity among eligible studies will be assessed using the I^2 index based on the Q statistic, and categorized as >25% (low heterogeneity), 25-75% (moderate heterogeneity), and >75% (high heterogeneity) (Higgins et al., 2003).
Publication bias will be assessed visually using a funnel plot and tested with Egger's test. A contour-enhanced funnel plot will be used to determine whether funnel plot asymmetry is due to publication bias or other factors. If asymmetry is found due to publication bias, the trim-and-fill method will be applied to correct the effect estimate. All statistical analyses will be conducted using Stata 18 (StataCorp LLC, TX, USA).

Subgroup analysis Subgroup analyses will be performed to explore potential sources of heterogeneity based on sample characteristics such as time of publication, geographical distribution, aquatic animal types, and antimicrobial classes. Pooled prevalence estimates will be derived from data in at least two individual studies to enhance the robustness of the findings. To visualize the prevalence of A. hydrophila, data will be imported into ArcGIS to create mapping.

Sensitivity analysis A sensitivity analysis will be conducted to assess the robustness and reliability of the pooled estimates in this systematic review

and meta-analysis. This analysis will involve excluding studies with a high risk of bias, as identified using the Joanna Briggs Institute appraisal tool, to determine their impact on the overall prevalence estimates of A. hydrophila and antimicrobial resistance (AMR). Geographical variations will be examined by excluding studies from specific regions, and the influence of individual seafood types (fish, shellfish, shrimp) on the estimates will be assessed by removing them from the analysis. Additionally, the resistance patterns will be tested by focusing on specific antimicrobial classes to evaluate their influence on the results. Heterogeneity will be addressed by comparing random-effects and fixed-effects models, especially if substantial heterogeneity ($I^2 > 75\%$) is observed. The impact of smaller studies will also be examined by excluding them to assess potential skewness in the results. Publication bias will be further evaluated using the trim-and-fill method to adjust for asymmetries detected through funnel plots and Egger's test. The sensitivity analysis results will be compared with the primary findings to ensure the conclusions are robust and reliable, with any significant changes discussed in relation to study limitations and sources of bias.

Language restriction Yes only studies published in English will be included.

Country(ies) involved Justice Opare Odoi-Ghana/ Chulalongkorn University/Animal Research Institute, Ghana; Saran Anuntawiru- Thailand/ Chulalongkorn University; Saharuetai Jeamsripong- Thailand/Chulalongkorn University.

Keywords Aeromonas hydrophilla, antimicrobial resistance, multidrug resistance, prevalence, resistance genes, virulence factors.

Contributions of each author
Author 1 - Justice Opare Odoi - He will contribute to the design of the study, the development of the systematic review protocol, and the search strategy. He will also be involved in the data collection, screening, and extraction process, as well as interpretation and drafting of the manuscript.
Email: opareojk@gmail.com
Author 2 - Saran Anuntawiru - She will be actively involved in the screening and selection of eligible studies, data extraction, and ensuring the accuracy of the collected data and review of the manuscript.
Email: pond_saran@hotmail.com
Author 3 - Saharuetai Jeamsripong - She conceptualize the study and ensure that all stages of the systematic review and meta-analysis are executed effectively. Additionally, she will supervise data collection, statistical analyses, and interpretation of results. Additionally, she will supervise data collection. She will lead the drafting, revision and submission of the manuscript.
Email: saharuetai.j@gmail.com