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# Virulence factors and mechanisms of *Aeromonas hydrophila* infection in catfish Siluriformes: a review and bibliometric analysis

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**ABSTRACT**

*Aeromonas hydrophila*, a gram-negative bacterium belonging to the Aeromonadaceae family, has significantly impacted global catfish production within the Siluriformes family, resulting in economic losses. Despite being recognized as part of the normal flora found in water systems and fish organs, the pathogenicity of *A. hydrophila* is often activated by the expression of virulence genes encoding toxins. To systematically gather reliable scientific studies on the virulence of *A. hydrophila* in catfish Siluriformes, the PRISMA method was employed, utilizing the Scopus database. Following the application of inclusion and exclusion criteria, a total of 66 documents were scrutinized, and a bibliometric analysis was conducted using VOSviewer. Based on the keyword analysis, aerolysin toxin emerged as the most prevalent virulence factor, appearing in 64% ($n$ = 42) of the papers, followed by hemolysin, enterotoxin, and biofilm. The analysis also revealed that the primary organs associated with infection were the kidney and liver, recognized as immune secretion organs, followed by the gills, intestine, skin, and mucus, identified as entry points. The major clinical signs of *A. hydrophila* infection included hemorrhage, lesion, ulceration, and septicemia. This study elucidates the scientific consensus on the infection mechanisms of *A. hydrophila*, covering entry points, adherence, and invasion. The ranked entry points and virulence factors identified herein offer valuable insights for designing novel treatments or preventive measures in catfish farming.

*Keywords:* Catfish; Siluriformes; *Aeromonas hydrophila*; Virulence; Aerolysin; VOSviewer

## 1. Introduction

The order Siluriformes comprises one of the most varied vertebrate orders in the world, with 500 genera belonging to 39 families and 4019 presently validly documented species [1]. Particularly catfish are considered a popular aquaculture product for human consumption and Malaysia is one of the countries well known for its production of African catfish from the family Clariidae for the global market [2]. *However, this catfish species around the world have been dealing with the same freshwater disease caused by A. hydrophila* a gram-negative bacterium from the family Aeromonadaceae

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that inhabits freshwater and brackish water. It causes a wide range of conditions in fish and can easily be found in normal conditions as it is known as normal flora [3].

Virulence is described as a pathogen's degree of pathogenicity, which is assessed by its capacity to penetrate and reproduce within the host [4]. The genes and proteins that are involved in pathogenicity are known as virulence factors from surface or capsule proteins that influence cell and tissue morphology to tiny adaptor proteins that modify cell-signalling pathways are all examples of virulence factors [5]. The synthesis release of virulence factors such as adhesins, cytotoxins, hemolysins, lipases, and proteases, as well as the ability to build biofilms, utilise particular metabolic pathways and mediate virulence factor expression via quorum sensing, all contribute to pathogenesis in *A. hydrophila* [6].

The infection of the bacteria can be identified through several clinical signs, including chronic ulcerative syndrome and acute hemorrhagic septicemia. These bacteria invade internal organs, causing pyknotic nuclei, early stages of necrosis in the liver, degradation of renal tubules, and widened sinusoidal space in the kidneys along with enlargement of the epithelial region in the intestine [7]. Enlargement and congestion in the kidney as well as pale liver and gills turned out to be the significant clinical signs on internal organs that can be observed through *C. gariepinus* infected with *A. hydrophila* [8]. Considering *Aeromonas* spp. are widespread in freshwater habitats and freshwater fish are constantly at risk of infection. Moreover, *Aeromonas* spp. may potentially endanger public health, particularly those who come in contact with infected fish [9].

Bibliometric analysis is a way of measuring, tracking, and analysing academic literature that employs a set of quantitative approaches [10]. It lists the writers' publications, the most prestigious journals, as well as the methodology employed and the conclusions reached [11]. WoS, Scopus, GS, MA, and Dimensions are software used to effectively aggregate databases for bibliometric evaluation, whereas scientific mapping generators include VOSviewer, Bibexcel, BiblioShiny, CiteSpance, SciMAT, and several more [12].

This review aimed to: (1) Compile reliable and sufficient scientific studies on the virulence of *A. hydrophila* in catfish Siluriformes family; (2) Identify the main mechanisms of virulence in catfish Siluriformes; and (3) Postulate and rank the main mechanisms based on bibliometric analysis using VOSviewer. Finally, considering the widespread *A. hydrophila* antibiotic resistance, novel drug targets will be identified to aid the development of new treatments or management approaches.

## 2. Material and method

### 2.1. Literature search

A systematic review and a bibliometric analysis were carried out according to a previously published article [13]. The systematic review followed the PRISMA method [14], while the bibliometric analysis was done using VOSviewer [15]. The keywords "Aeromonas AND hydrophila AND virulence AND catfish OR Siluriformers" were used to obtain literature from the Scopus database up to 31 December 2022. A total of 69 documents were detected and these articles were subjected to inclusion and exclusion criteria. Documents such as review, conference, and non-English articles were excluded. A final list of 66 documents was included in this review. The data of all the documents were exported in CSV format for the bibliometric analysis.

## 3. Results

### 3.1. Overall keyword mapping

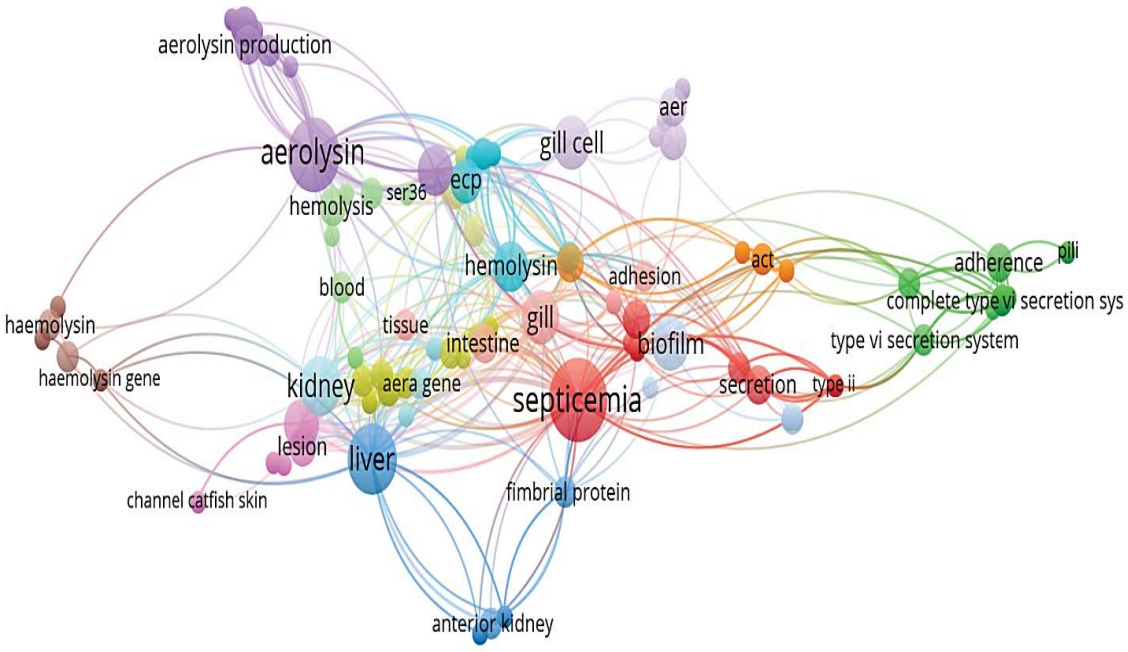


Fig. 1. Network visualisation of the correlation between virulence factors, organ and clinical sign.

*3.1.1. Major virulence factors of Aeromonas hydrophila infection*

Table 1
Keyword of the major virulence factors of *Aeromonas hydrophila*

| Main keyword | Keywords | Occurrences |
|---|---|---|
| Aerolysin | Aerolysin, aerolysin activity, aerolysin expression, aerolysin mediated cell death, aerolysin production, aerolysin toxin, aerolysin virulence gene, aerolysin induced cell injury, inhibiting aerolysin, recombinant aerolysin, virulent factor aerolysin, proaerolysin, proaerolysin coding gene, first recombinant proaerolysin, functional recombinant proaerolysin, recombinant proaerolysin, unprocessed proaerolysin, *aer*, *aera*, *aera* gene, gene *aera* | 42 |
| Haemolysin/ Haemolysis | Bhaemolysin, extracellular haemolysin, haemolysin, haemolysin gene, haemolysis, haemolysis activity, haemolytic activity, hemolysin, hemolysis, hemolytic activity, inhibited haemolysis, *hlyA* gene | 24 |
| Enterotoxin | Cytolytic enterotoxin, cytotonic enterotoxin, cytotoxic activity, cytotoxic potential, heat-labile cytotonic enterotoxin, heat stable cytotonic enterotoxin, heat-stable enterotoxin, *act*, *ast*, *ast* gene, *alt* | 16 |
| Biofilm | Biofilm, biofilm assay, biofilm cell, biofilm formation, biofilm grown *vah*, biofilm growth, biofilm sample, biofilm secretome | 13 |

*3.1.2. Organ infected with Aeromonas hydrophila in catfish Siluriformes*

Table 2
Keyword of the major organs infected with *Aeromonas hydrophila* in catfish Siluriformes

| Main keyword | Keyword | Occurrences |
|---|---|---|
| Kidney | Anterior kidney, kidney, kidney tissue | 10 |
| Liver | Liver | 10 |
| Gill | Gill, gill cell | 7 |
| Skin | Skin, channel catfish skin | 6 |
| Intestine | Intestinal content, intestine, full gastrointestinal tract | 5 |
| Mucus | Mucosal defense, mucosal factor, mucosal surface, mucus | 6 |

*3.1.3. Clinical signs of catfish Siluriformes infected with Aeromonas hydrophila*

Table 3
Keyword of the major clinical signs of *Aeromonas hydrophila* infection in catfish Siluriformes

| Main keyword | Keywords | Occurrences |
|---|---|---|
| Haemorrhage | Fatal haemorrhagic ulcer, gastric haemorrhage, haemorrhage, hemorrhage, severe hemorrhagic dermatitis | 7 |
| Dermal lesion | Lesion, dermal lesion, dermal scraping, dermomuscular lesion, muscle lesion | 7 |
| Ulceration | Ulcer, ulceration, ulcerative tissue, severe skin, severe skin ulceration, severe skin | 6 |
| Septicemia | Septicemia, severe motile aeromonas septicemia | 2 |
| Necrosis | Necrosis | 2 |
| Anemia | Anemic fish | 1 |
| Wound infection | Wound infection | 1 |

## 4. Discussion

### *4.1. Aerolysin*

According to the result of the keyword from VOSviewer, aerolysin is the initial major virulence factor with the highest occurrences among all the literature obtained. Aerolysin toxins were secreted by the type ii secretion system, which allows the adhesion of the bacteria into the tissues. *A. hydrophila* secreted amylase, DNase, glycerophospholipid cholesterol acyltransferase (GCAT), and proteases other than aerolysin through the type ii secretion system (T2SS) [16]. Gram-negative bacteria are prevalent with the T2SS, which consists of 12–15 proteins with a component found in both membranes since it is a general system that plays significant functions in environmental organisms and opportunistic diseases [17,18].

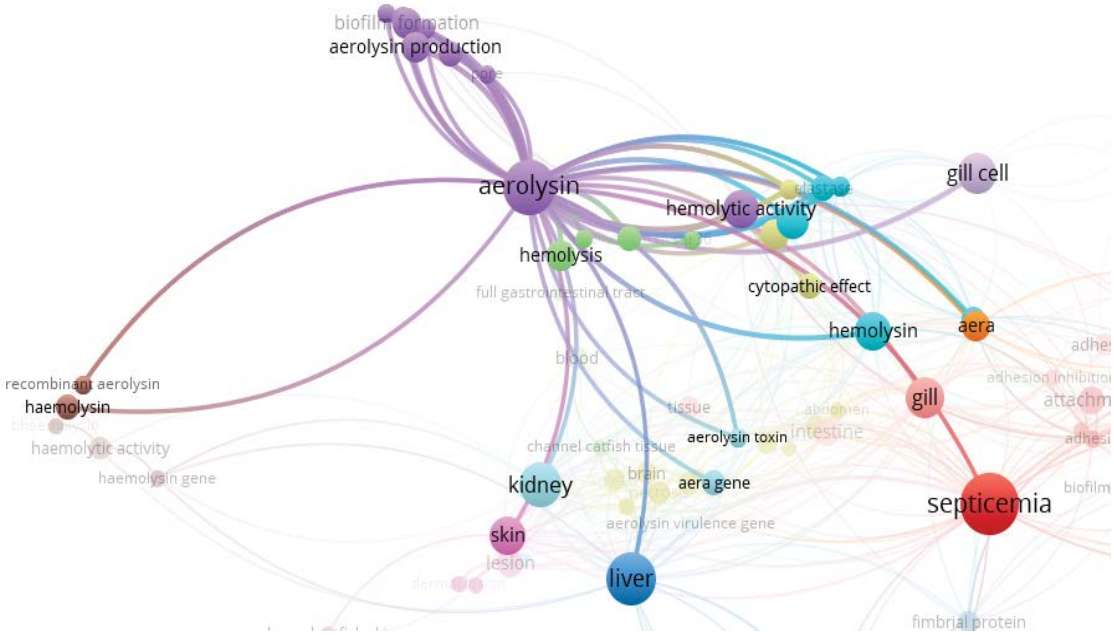


Fig. 2. Network visualisation of the correlation between the keyword 'Aerolysin' with organ and clinical signs of the *Aeromonas hydrophila* infection.

Extracellular Aeromonas protease has been shown to transform the protoxin of aerolysin into a pore-forming toxin that is more hemolytic than the protoxin. Aerolysin toxin, which induces holes in cytoplasmic membranes, and proteases are among the physiologically active extracellular products (ECPs) produced by *A. hydrophila* [19]. Aerolysin toxin releases inactive precursors known as proaerolysin that have been proven to be crucial in *A. hydrophila* pathogenesis and appear to be water-soluble [20]. This toxin can cause tissue damage by generating a hole on the surface of target cells and is also toxic to many mammalian cells [21].

Research by Nhinh et al. [22] on the identification of virulence genes from *A. hydrophila* disease outbreaks, *aerA* and *act* virulence genes were frequently detected with higher prevalence. Degradative enzymes such as protease and lipase were encoded by the genes, causing the breakdown of cell tissues and the formation of holes in the intestine and stomach, followed by hemorrhagic and septicemia in the organ. Skin and muscle lesions due to protein secretion are also shown to have a significant effect on the infected fish due to aerolysin activity. Six out of 35 infected *C. gariepinus* with MAS symptoms in Indonesia have tested positive for the existence of *aerA* gene, which is a virulence factor for MAS infection [23]. Thus, aerolysin has been found as a pathogenic *A. hydrophila* strain marker. Bacterial biofilm development leads to antimicrobial resistance and is a cause of recurrent and ongoing infections in aquatic animals [20].

### 4.2. Hemolysin

The second highest occurrence of major virulence factors of *A. hydrophila* is hemolysin toxin that correlates with the gills, skin, septicemia, and hemorrhage. Hemolysin is a toxin that causes the lysis of erythrocytes by disrupting the cytoplasmic membrane of the cells [24]. It is an exotoxin composed of lipids and protein that assists in the degradation of the red blood cell membrane, causing anemic conditions in the fish due to the low oxygen concentration transported by the erythrocytes.

This cytolytic toxin facilitates the lysis process, enabling the bacteria to consume essential nutrients, such as iron, from the host. *A. hydrophila* is beta-haemolysis which is also toxic to epithelial, endothelial, and phagocytic cells in the intestine. The most prevalent gene detected from the *A. hydrophila* on this toxin is *ahh1* an extracellular heat-labile gene in single or conjunction with another haemolysis gene with 77% out of 128 isolations [25].

Hemolysin toxin often causes haemorrhagic septicaemia on the skin, gills, and intestine of the fish which have reached the final phase of the infected fish and highly. An infected fish with clinical sign of hemorrhagic, septicemia, and ulceration were isolated, and perform hemolysin test confirm that the *A. hydrophila* were related to the disease infection [26].

### 4.3. Enterotoxin

Cytotoxic enterotoxin or *act genes* that were correlated with aerolysin toxin are the third highest occurrence after hemolysin and consist of heat-stable (*ast*) and heat-liable (*alt*) genes [27]. The *alt* genes stimulate fluid accumulation in the intestine, whereas (*ast*) induces CHO cells to dilate and elicit intestinal excess fluid. A test on *A. hydrophila* virulence genes isolation, *aerA*, and *act* genes is the most frequently discovered with an 80% rate out of 236 infected fish samples without the presence of any *ast* gene [22]. This toxin specifically targets the intestine to degrade the epithelial tissues of the organ causing lesions and ulceration on the epithelial tissue.

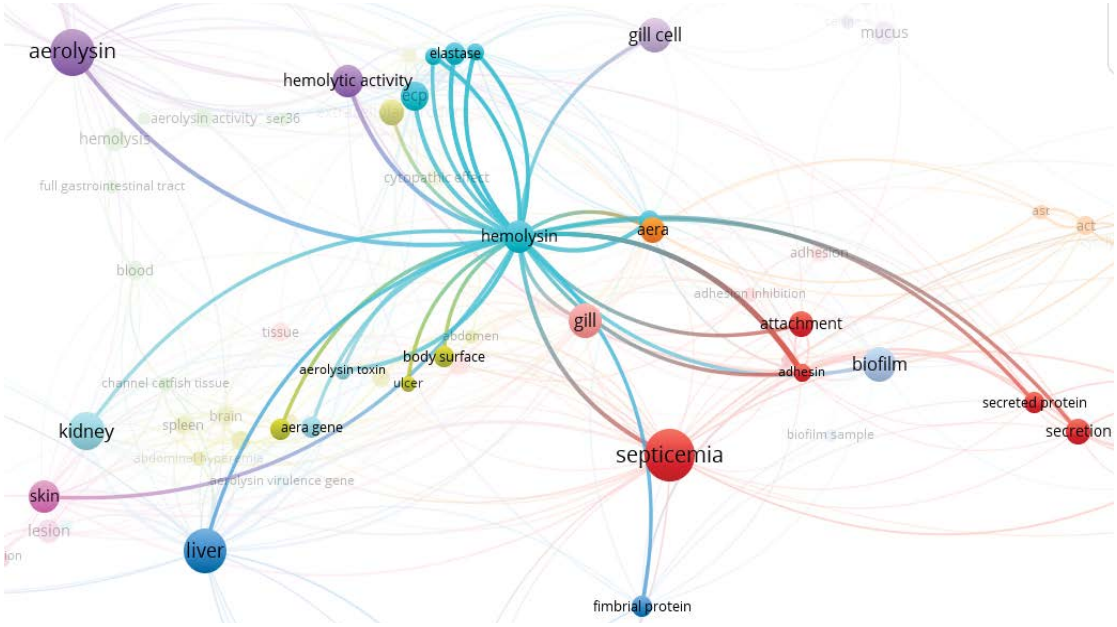


Fig. 3. Network visualisation of the correlation between the keyword 'Hemolysin' with organ and clinical signs of the *Aeromonas hydrophila* infection.

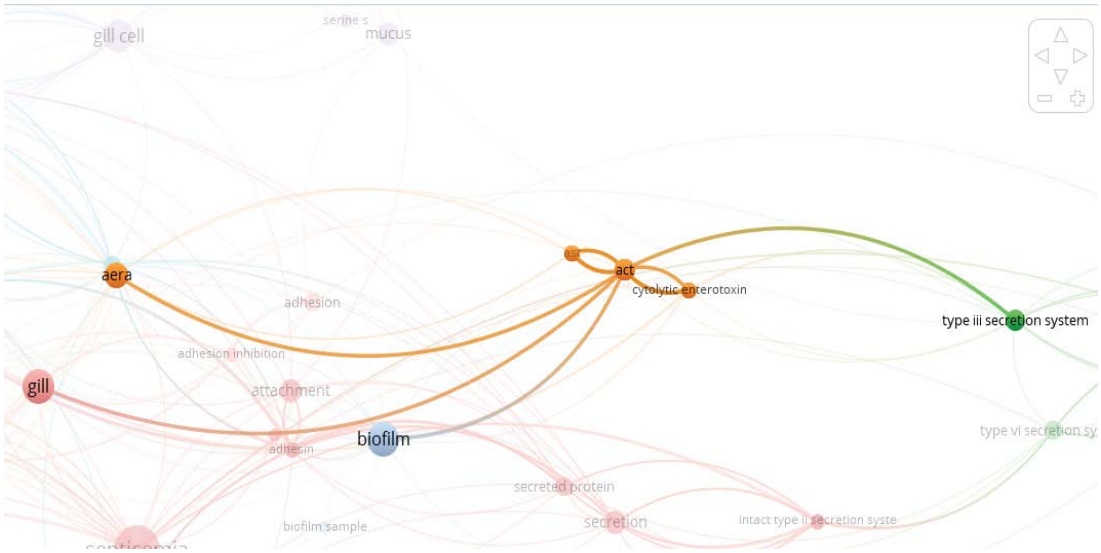


Fig. 4. A map from VOSviewer analysis on the correlation between the keyword 'Enterotoxin' with organ and clinical signs of the *Aeromonas hydrophila* infection.

Chopra et al. [27] discovered that cholesterol, which inhibited *act*'s cytotoxic effect, is necessary for the toxin's contact with this cellular membrane. Considering the differences, either cytotoxic enterotoxin genes or *aerA* genes have been demonstrated to be triggered by adhesion to the host cell membrane, followed by oligomerization and pore formation.

### 4.4. Biofilm formation

Another virulence factor for *A. hydrophila* infection is a biofilm, which is a physical barrier generated by the bacteria when they need protection from an unstable environment. The map shows the correlation between the biofilm formation with aerolysin, hemolysin, adhesion, fimbrial protein, and other virulence factors of *A. hydrophila.* This biofilm would also happen to be associated with the organs of adhesion, like the intestine and gills. This is because biofilm formation is a process through which bacteria adhere to a surface such as an organ tissue then proliferate by producing extracellular polymers that strengthen the adhesion [28]. The formation of the capsule-like membrane that consists of polysaccharides, water, protein, lipids, and bi-polymers will affect the growth rate, gene transcription, and structure

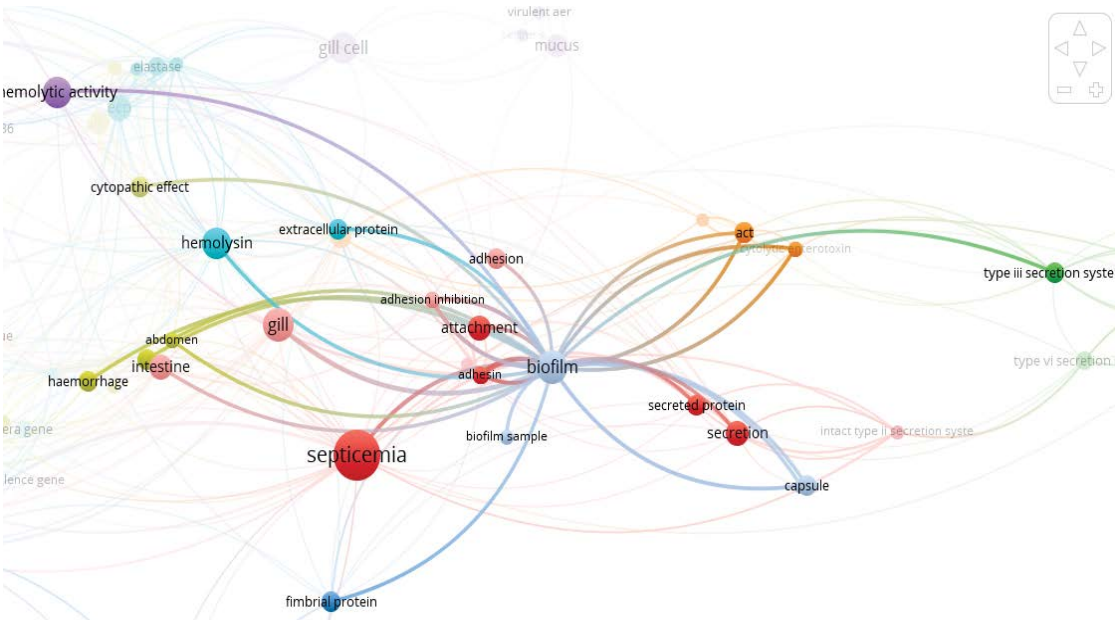


Fig. 5. Network visualisation of the correlation between the keyword 'Biofilm formation' with organ and clinical signs of the *Aeromonas hydrophila* infection.

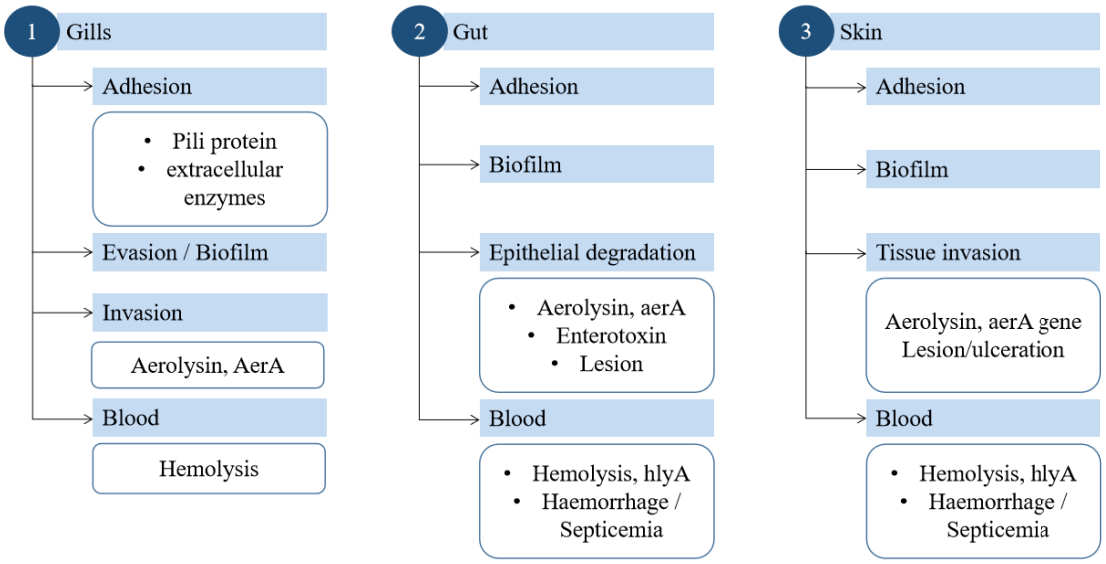


Fig. 6. Proposed and ranked mechanisms of *Aeromonas hydrophila* in catfish Siluriformes.

of the bacteria. This will further influence the ability to invade the host's immune system [29,30].

Biofilm formation is led by the quorum sensing system which is the main mechanism that could be the indicator of resistance genes for the identification of suitable antibiotics and drug treatments [20]. A study on the bio-control measure of drug resistance by Nithin et al. [31] indicates the resistance and susceptibility rates of *A. hydrophila* against multiple drugs using the cells of biofilm. Furthermore, the isolate formed biofilms, which may help the bacteria resist antibiotic treatment and boost their adaptation to the environment [31]. In different studies, *A. hydrophila* may form the biofilm barrier from chitin substrates as free cells when the essential nutrient for the process is depleted.

### *4.5. Proposed mechanisms of A. hydrophila in catfish Siluriformes*

#### *4.5.1. Route 1: gills and skin*

The initial potential entrance point for *A. hydrophila* virulence might be through the gills. This is because *A. hydrophila* is known as a normal flora that can be found on any surface and in any normal condition of the water system, as well as in their freshwater habitat [32]. A study

conducted by Muduli et al. [33] on the pathogenic potential of *A. hydrophila* by isolating the gills of 50 healthy freshwater fish found that 20 were biochemically recognized as *A. hydrophila* using Aerokey-II. This shows that the gills could be the main entry point of the bacteria infection, which might be through the filtration of oxygen from the water system or the bacteria itself swimming to the gills as it is the only open system in the fish.

The activity of the fimbrial protein, or W-pili protein, which occurred to be attached to the tissue of the gills and skin by motility, was fundamental for bacterial adhesion [34]. Chemotaxis, motility, and adhesion are all key virulence factors to locate the host and adhere to it, ECP is crucial for invasion [35]. Once the bacteria are attached to the gills and skin, the initial immune defenses in the mucus will attenuate or kill the bacteria to prevent an invasion. The bacteria require a safe environment in which to proliferate and adapt to their surroundings before invading the tissue and forming the biofilm. Physical barriers such as biofilm aid the bacteria in evading the antibacterial attack, as well as the host immune system. After successful evasion of the host immune, the bacteria await for favourable conditions to generate new persistent infections and invade [30].

The main virulence infection for the invasion process is an aerolysin pore-forming toxin. This toxin will create a pore by secreting the enzyme that will degrade the tissue of the host gills, causing lesions and ulcerative conditions [22]. The wound caused by the toxin may then be taken over by the secretion of hemolysin toxin, which is essentially one of the key virulence factors in *A. hydrophila* and is secreted through the type II secretion system [18]. The toxin might perform a hemolytic activity in the blood capillary that will degrade the red blood cells leading to the anaemic condition in the fish. Once the bacteria perform hemolysis in the blood, it will cause hemorrhagic and septicemia in the fish, leading to low survival chances.

#### *4.5.2. Route 2: gut and intestine*

The final entry point that could be proposed is through the ingestion of feed that ends up in the intestine or stomach of the fish. However, the pathogenicity of the bacteria should be determined by the gene expression activity of the virulence factors. Once the bacteria reach the intestine, they will start to attach to the tissue surface through adhesion and biofilm formation, just as they do on the gills and skin. However, an intestine condition that is unfavorable for the bacteria due to the low pH condition could kill the bacteria itself, which the biofilm helps to protect to evade, reproduce, and improve the vulnerability towards the immune system.

However, in the intestine and stomach, it requires more than aerolysin to degrade the epithelium cells with the help of enterotoxin-like *act*, *ast*, and *alt* genes. This is because the layers of epithelium consist of a lot of enzymes that will protect the organ from external infection. The *alt* genes stimulate fluid accumulation in the intestine, whereas (*ast*) induces CHO cells to dilate and elicit intestinal excess fluid. In a test on *A. haemorrhage* virulence genes isolation, *aerA* and *act* genes are the most frequently discovered by 80% rate out of 236 samples of infected fish without the existence of any *ast* gene [22]. This toxin specifically targets the intestine to degrade the epithelial tissues of the organ, causing lesions and ulceration on the tissues. Once, the intestine is vulnerable to bacteria, it might proceed for hemolytic activity by the secretion of hemolysin toxin to degrade the membrane of red blood cells causing wound infection, septicemia, and haemorrhage of internal organs leading to high mortality risk [26].

## 5. Conclusion

This review summarized the virulence mechanisms *A. hydrophila* utilises to infect catfish from the family Siluriformes. The major virulence factor of *A. hydrophila* is aerolysin which enables the bacteria to invade the fish via the skin route and epithelial surface by causing ulceration. The ulcer could lead to the translocation of bacteria into the blood capillary. Then, hemolysins could increase disease severity by causing hemorrhage and septicemia. The second main route of infection is the intestine where enterotoxin also acts as a pore-forming toxin. This information is key to preventing *A. hydrophila* infection or to identify novel drug targets for new treatments. This review recommends that new *A. hydrophila* control measures should focus on creating new agents that can inhibit the expression of aerolysin or anti-toxins that can neutralize it.

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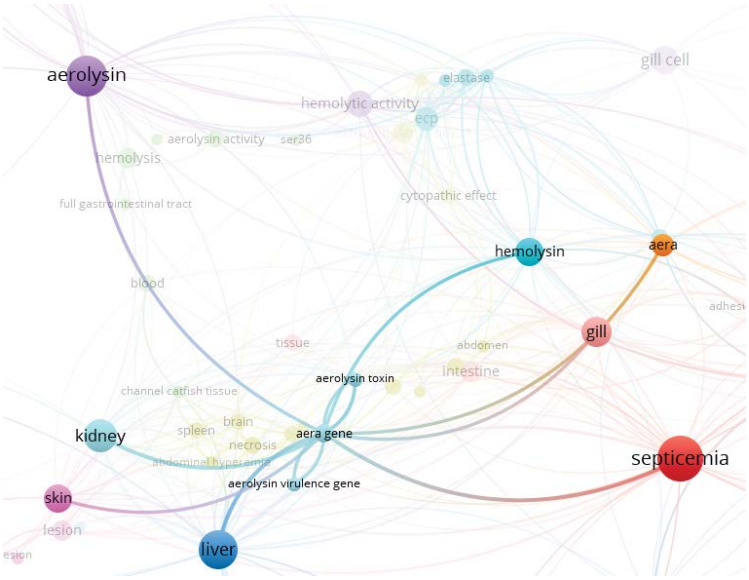
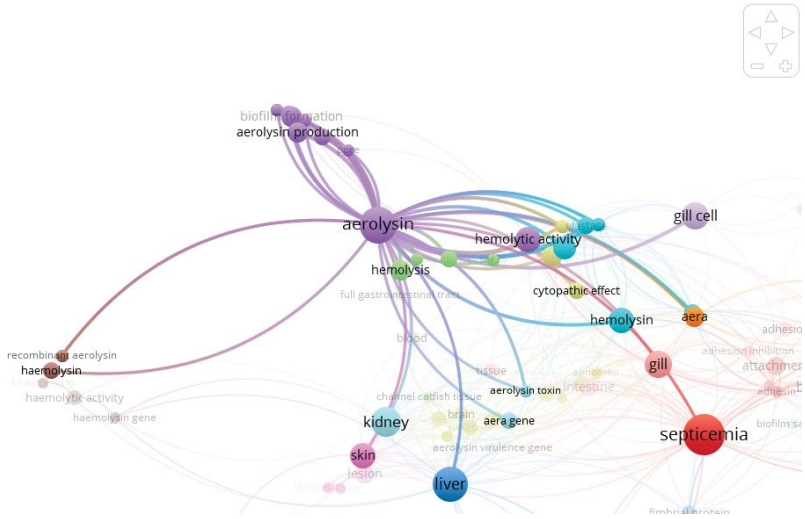
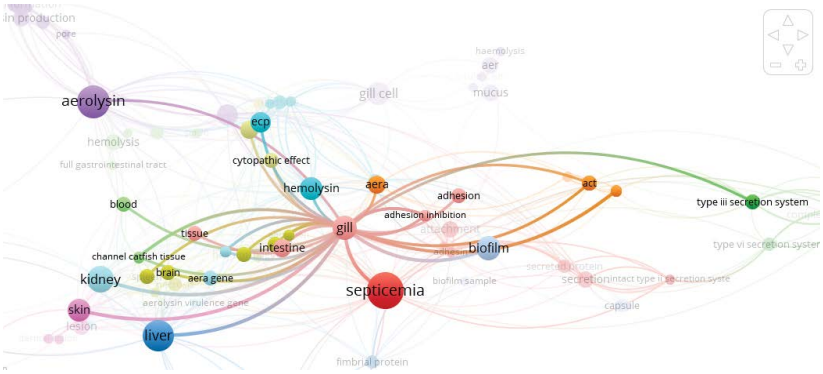
## Appendices



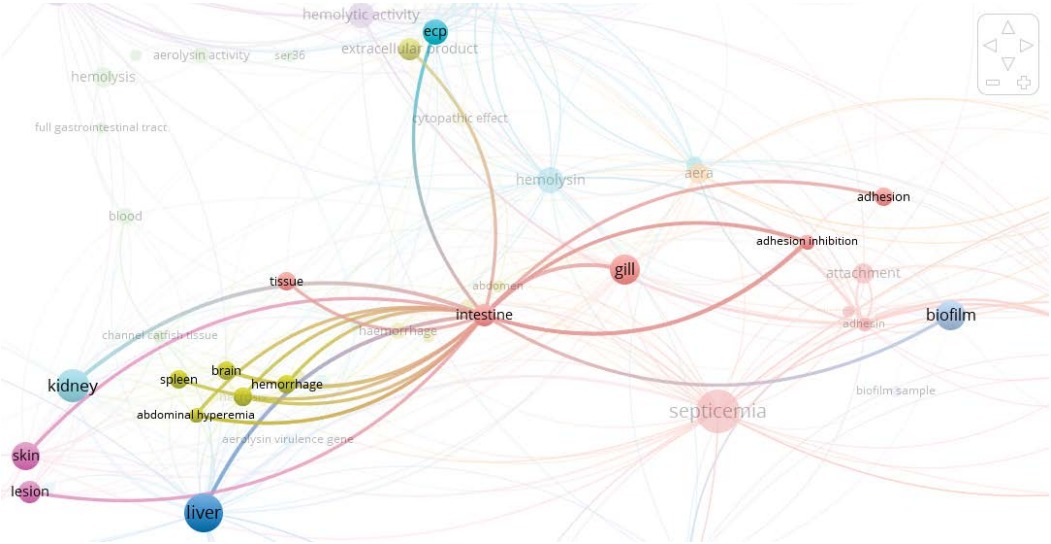

Appendix 1: Network visualization of the correlation between the keyword of *aerA* gene with clinical signs and organs



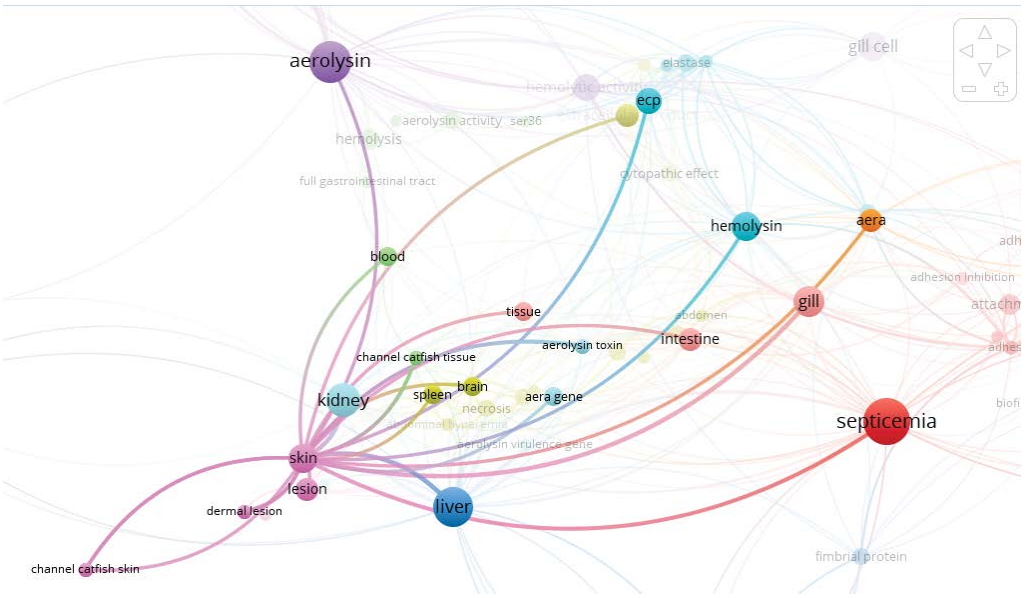

Appendix 2: Network visualisation of the correlation between another aerolysin keyword with clinical signs and organs




Appendix 3: Network visualisation of the correlation between the keyword of gills with virulence factors and clinical signs




Appendix 4: Network visualization of the correlation between the keyword of intestine with virulence factors and clinical signs




Appendix 5: Network visualization of the correlation between the keyword of skin with virulence factors and clinical signs