Alginolyticus Virulence: An Overview

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ABSTRACT
Introduction: Marine habitats harbor the gram-negative, halophilic Vibrio alginolyticus. Usually connected with aquatic life, it poses a serious health risk to humans, especially seafood and marine workers. Marine economic animals like prawns, fish, and shellfish can develop serious illnesses with rapid progression and significant fatality rates. Pathogenic relatives such as Vibrio cholerae and Vibrio parahaemolyticus, which carry genes for several virulence factors, have inherited its virulence, thereby increasing its clinical importance. No effective treatments exist to stop V. alginolyticus dissemination and infection; therefore, studying its virulence mechanisms is crucial to understanding its pathogenicity and improving prevention and therapy.
Objectives: To analyze the virulence factors and antibiotic resistance mechanisms of Vibrio alginolyticus to improve prevention and control strategies for managing infections in aquaculture and public health settings.
Methods: This study is a literature review synthesising findings from recent research on V. alginolyticus. Sources include peer-reviewed articles, clinical studies, and microbiological research focusing on virulence factors and antibiotic resistance. Data collection involved searching databases like PubMed, Scopus, and Web of Science, filtering for studies on V. alginolyticus's virulence mechanisms, antibiotic resistance, and impacts on public health and aquaculture. We selected key studies to gain a comprehensive understanding of the pathogenic characteristics and resistance profiles of the bacterium.
Results: The results show that V. alginolyticus uses many ways to be strong, like quorum sensing, making virulence proteins, secreting haemolysin, and making biofilms, which make it more dangerous to humans and marine animals. Several resistance genes and efflux systems contribute to its antibiotic resistance, thereby impeding the effectiveness of treatment. Studies also show that biofilm formation and quorum sensing facilitate survival in hostile environments, making infections more challenging to manage.
Conclusions: Understanding the virulence factors and antibiotic resistance mechanisms of Vibrio alginolyticus is crucial for developing effective control strategies. This bacterium's rapid spread and severe impact on marine life and human health necessitate new approaches in aquaculture management and clinical interventions. Enhanced monitoring and targeted research are essential to curb its spread and prevent infection, ultimately improving aquaculture productivity and reducing public health risks associated with this pathogen.

1. Introduction

Vibrio alginolyticus is a Gram-negative bacterium that is part of the genus Vibrio and family Vibrionaceae, widespread in aquatic and marine ecosystems and presents serious health concerns to aquatic life and people through local food chains (Ahmed, Rafiquzaman, Hossain, Lee, & Kong, 2016; Hackbusch, Wichels, Gimenez, Döpke, & Gerdts, 2020). It is considered one of the most dangerous Vibrio species and is pathogenic to people and marine life (Citil, Derin, Sankur, Sahan, & Citil, 2015). Each year, these harmful bacteria cause billions of dollars in economic losses and many health scares, which has sparked rising international alarm. Recent years have seen a surge in the study of V. alginolyticus pathogenic processes. This allowed for the discovery of important virulence factors like hemolysins (Jia, Woo, & Zhang, 2010), polar and lateral flagella (Tian et al., 2008), serine proteases (Rui et al., 2009) and outer membrane protein A (OmpA) (Bunpa et al., 2020). As this species belongs to the Vibrio genus, V. alginolyticus has several characteristics in common with other microorganisms in the same family. These characteristics include its appearance, surface antigens, genomic sequences, virulence components, and early signs of infection (Bakeeva, Drachev, Metlina, Skulachev, & Chumakov, 1987; Zhou et al., 2013). According to reports, V. alginolyticus is resistant to ampicillin, vancomycin, cephalothin, cefuroxime, streptomycin, tetracycline, and amikacin (Kang, Shin, Jang, Jung, & So, 2016; Krishnika & Ramasamy, 2013). Therefore, bacteria resistant to antibiotics have the edge over their hosts regarding survival. Vibrio species' genetic information of plasmid origin often determines antibiotic resistance (M. Kurdi Al-Dulaimi, Abd. Mutalib, Abd. Ghani, Mohd. Zaini, & Ariffin, 2019).

Consuming aquatic food items that haven't been fully cooked may lead to human infections, which can lead to symptoms including diarrhoea and gastroenteritis, involving V. alginolyticus leads to septicemia and skin

infection (Osunla & Okoh, 2017). If humans come in contact with V. alginolyticus through contaminated seawater, it quickly spreads to soft tissues, the ear, and superficial wounds (Citil et al., 2015). Numerous diseases in aquatic animals are caused by V. alginolyticus, including corneal opaqueness and exophthalmia in Epinephelus, melanosis in Rachycentron canadum, septicemia in Sparus aurata, enormous death rates in Tapes decussatus (Dong et al., 2020). More significantly, it has been shown that V. alginolyticus is the main cause behind deadly outbreaks of Vibriosis in several marine organisms (Kashinskaya et al., 2018; Mello, Trevisan, Danielli, & Dafre, 2020). For instance, the embryonic oyster is susceptible to Vibriosis, which significantly effects the aquaculture sector due to the huge death rates of larvae (Kehlet-Delgado, Häse, & Mueller, 2020; Oberbeckmann, Wichels, Wiltshire, & Gerdts, 2011).

The virulence factors enhance pathogens' ability to colonize and adhere to the hosts, facilitating the infections' ability to cause harm to host tissues. Virulence factors stimulate protease synthesis in Vibrio, and a biofilm protects the pathogen from the host immune mechanism and antibiotics (Waters & Bassler, 2005). The virulence factors in V. alginolyticus have been reported by (Gargouti et al., 2015), including tdh and trh and (Najwa, Daniel, Mat Amin, & Effendy, 2015), including toxR, ompK, and a protease such as collagenase.

The widespread saprophytic microbial member V. alginolyticus has been isolated from a variety of marine species. It is quite concerning how common it is for V. alginolyticus to possess many virulence genes in its genome. This literature review provides phathogenicity, virulence factors, and an understanding of their mechanisms. As a result, it is essential to keep an eye on how harmful bacteria react to different antibiotics to know which antibiotics are still effective in treatment.

2. Epidemiology and environmental factors of V. Alginolyticus

further research needs to be done to determine the way of infection and spread of this species, water is most likely one of the transmission routes. It's unclear whether this microbe is naturally occurring in the aquatic environment or if it just makes fish sick when the opportunity arises; both questions remain unanswered.

However, according to the findings of certain studies, strains of these bacteria could serve as a possible reservoir for many of the virulence genes found in other Vibrio species that live in aquatic environments. Which, when exposed to seawater, has the potential to lead to the emergence of wound contaminations, gastrointestinal illnesses, and sepsis in human beings (Lafisca, Pereira, Giaccone, & Rodrigues, 2008; Masini, De Grandis, Principi, Mengarelli, & Ottaviani, 2007). A study by (Li, Zhou, & Woo, 2003) evaluated the pathogenic mechanism and the invasion routes of this bacteria in Sea bream Sparus sarba. The results showed that infected Sea bream had lower red blood cell amount, haemoglobin, serum glucose, hematocrit, and protein. V. alginolyticus might cause Vibriosis death by destroying circulatory and immune system components. The V. alginolyticus pathogenic processes have been studied mostly in marine creatures like Epinephelus awoara (Chang et al., 2012), Pagrus majo (J. Ye et al., 2008), Haliotis diversicolor supertexta (Wu, Wang, Chan, & Li, 2011) and erythropterus (S.-H. Cai et al., 2010). A study by (X.-F. Liu et al., 2014) performed the pathogenic analysis of V. alginolyticus on a mouse model. The outcomes revealed that the infection with V. alginolyticus caused severe damage to both the lungs and the liver and altered IL1, IL-6, and hematological changes.

Moreover, V. alginolyticus might be identified from skin illnesses in human beings. These infections are often brought on by exposure to marine water (Scheftel, Ashkar, Boeri, & Monteil, 2006). earlier research has demonstrated the possible health threats linked to non sterile products, for example, alginate gels derived from marine seaweed that are homemade for the clean-up of wounds. These materials can implicate V. alginolyticus infections as a source of severe wounds, particularly amongst susceptible groups such as people of older ages and those with conditions that place them at an increased risk (Reilly, Reilly, Smith, & Baker-Austin, 2011). Similarly, V. alginolyticus was secluded from the pus of the ear and was shown to be accountable for an infection of conjunctivitis and opacification of the sphenoid sinus and tissue necrosis (Lopes et al., 1993).

As V.alginolyticus has been connected to several diseases affecting fish, crabs, and mollusks in marine environments. (Beleneva, Maslennikova, & Magarlamov, 2004; Gómez-León, Villamil, Lemos, Novoa, & Figueras, 2005; Zhu, Sun, & Wang, 2019). This is consistent with the increasing stress that human activity places on shoreline areas as well as Vibrio's genetic and phenotypic adaptability, which enables it to adapt quickly to changing climatic circumstances. (Fischer-Le Saux, Hervio-Heath, Loaec, Colwell, & Pommepuy, 2002) According to (Kahla-Nakbi, Besbes, Chaieb, Rouabhia, & Bakhrouf, 2007) research, V. alginolyticus may live in salted water without its typical host or food source. Under particular circumstances, this bacterium may infect fish. According to its temperature-dependent survival rates, the opportunistic pathogen V. alginolyticus most likely inhabits watery settings. There are places where the bacteria can multiply, which could

lead to the discharge of huge quantities of the bacteria into the environment once the germs have infected fish that are susceptible to Vibriosis.

3. Virulence and phathogenicity in V alginolyticus

Even though the process through which V. alginolyticus triggers disease is not yet completely identified, various variables that contribute to the pathogen's virulence have been identified. V alginolyticus contains polar and lateral flagella. Various behaviour, including adhering to surfaces, biofilm production, swimming, and swarming, have been linked to these flagella (Chen et al., 2017; Kitaoka et al., 2013). In bacterial transcriptome profiling, RNA-sequencing has seen the widespread application, and it offers the possibility of determining the expression amounts of many genes in a single trial. This can be done to better understand the molecular genetic processes at work for bacteria (Mandlik et al., 2011) . According to the results of a study, strains of V. alginolyticus likely to have multiple homologous of the virulence genes of V. parahaemolyticus and V. cholerae, including toxR, tlh, and VPI. This indicates that Vibrio alginolyticus could be an important source of several recognized virulence genes of other Vibrio species in the marine system. It is likely that distinct virulence-associated genes are dispersed throughout ambient Vibrio and that these genes are found in environments including water (Melissa B. Miller, Skorupski, Lenz, Taylor, & Bassler, 2002).

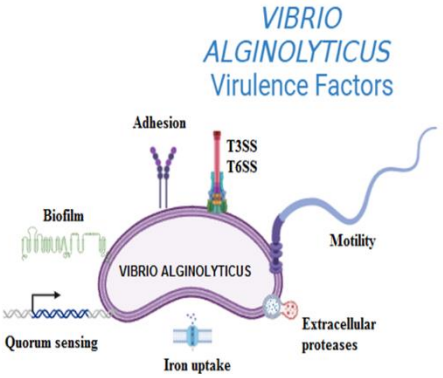


Figure 1: Virulence Factors For Vibrio Alginolyticus

3.1 Motility as virulence factor

Many bacteria that infect people, animals, and plants use their flagella to move around. During some stages of their life cycles, motility is crucial for many pathogens, and virulence and motility are frequently closely related by intricate regulatory networks (Josenhans & Suerbaum, 2002). The basal body, filament, and hook are the three components that make up the bacterial flagellum. This organelle is responsible for the movement of the bacterial cell. The construction of flagella is a logistical process that requires transporting thousands of protein sub units across considerable gaps, apart from the MS ring, a basal body component found inside the membrane. Other components must be transferred via the export mechanism and assemble in the correct sequence without outside assistance (Wang et al., 2015). According to a study, the 2-DE findings and MALDI-TOF mass spectrometry analysis identified OmpA as an over-expressed protein in the disease-causing V. alginolyticus strain (Bunpa et al., 2020). Moreover, data indicated that mutation of the ompA gene was substantially associated with the reduction of V. alginolyticus' swarming capacity, biofilm formation, and phathogenicity (Bunpa et al., 2020).

3.2 Adhesion as virulence factor

Adhesion are key virulence aspects that assist host colonization and allow bacteria to resist mucosal secretions and peristalsis clearance. Bacteria produce adhesion and may be found in various environments (Stones & Krachler, 2015). The adhesion found on the surfaces of most commensal and harmful bacteria facilitate associations with receptors of eukaryotic host cells. They may be categorized as fimbrial, which refers to long and polymeric structures; fimbrial, which refers to structures that are short and either monomeric or trimeric; and polysaccharide adhesion groups (Taylor, Fitzpatrick, Islam, & Maxwell, 2019). The adherence of bacteria is affected by environmental factors, But the underlying mechanism of this impact is still unclear. The expression levels of flrA, flrB and flrC were remarkably down regulated in adhesion-deficient V. alginolyticus strains

grown in the presence of Cu2+, Pb2+, Hg2+, and low pH. Silencing these genes resulted in defects in adhesion, motility, flagellar assembly, biofilm formation, and polysaccharide production (Luo et al., 2016). A study was performed using in-vitro adhesion, gene silencing and qRT PCR to examine the association of adhesion of V. alginolyticus with genes of type II secretion system including yajC, secA, secD, secF, and yidC. After the gene silencing, the activation of target genes and bacterial adherence decreased significantly, indicating that these genes played roles in the bacterial adhesion of V. alginolyticus. Temperature, salinity, pH, and nutrient deprivation greatly impact genes' expressions (Guo et al., 2018).

According to a study, the V. alginolyticus treated with stress conditions such as low pH, and higher amounts of mercury, lead and copper ions lead to decreased adhesion capability together with altered expression levels of multiple genes, which suggested that genes (genes related to adhesion) might play an important role in adhesion mechanism (W. Kong et al., 2015). Strains of V. alginolyticus explicitly adhered to the mucus of gilthead sea bass and gilthead sea bream, polystyrene and glass surfaces at different levels of adhesion. Therefore, the research suggests that V. alginolyticus has a strong ability to adhere to biotic as well as abiotic surfaces, and they colonize vulnerable fish, and the skin might serve as an entry point in the fish (Snoussi et al., 2008). Hence, the adhesion is the determining factor in the bacterium's capacity to detect and attach to host cells and tissues, and it is the major target for researchers seeking to produce vaccinations that inhibit this binding process (Bann, Dodson, Frieden, & Hultgren, 2002).

To achieve new indications for the mechanism(s) triggering adhesion regulation in V. alginolyticus, a study described the RNA-sequencing in V. alginolyticus that were cultivated under stress situations (such as low pH, Cu, Pb, and Hg), together with normal environments. This was done to compare the gene expression patterns of V. alginolyticus cultivated in normal and stressful conditions. Using RNA-sequencing and bio informatics, we determined that each of these stress treatments has the potential to drastically impact the pathway responsible for flagellar assembly (Wendi Kong et al., 2015).

3.3 Quorum sensing

The process of regulating the expression of genes about changes in the density of a cell population is described as quorum sensing (QS) (M. B. Miller & Bassler, 2001). The build-up of signaling factors in an environment causes a significant threshold level to be reached, at which point the signaling pathway is stimulated. This stimulation leads to the transcriptional regulation of functional genes and QS molecules syntheses themselves, where the term "auto inducers" (AI) originates. Firstly, the intra-species interaction (among bacteria that are phylogenetically similar to each other) with the N-acyl-homoserine lactones (AHLs) or AI-1, which is a characteristic shared by Gram-negative bacteria, and cholerae autoinducer-1 (CAI-1), which is unique to certain Vibrio species. Both types of molecules are found within Vibrio species (Henke & Bassler, 2004; Melissa B. Miller et al., 2002; Papenfort et al., 2017). Secondly, inter-specie interaction is communication between species or amongst microbes using the AI-2, which is extensively dispersed throughout microbes (Keller & Surette, 2006; Xavier & Bassler, 2003). Bacteria that are capable of QS generate and emit chemical signal molecules known as auto inducers, The modification in the expression of genes may occur due to identifying a minimum threshold stimulatory concentration of an auto inducer (Jiang & Li, 2013). Both Gram-negative and Gram-positive bacteria employ communication pathways using QS to monitor a broad array of biological activities. Symbiosis, virulence, competency, conjugation, antibiotic synthesis, sporulation, motility, and biofilm production are a few of the mechanisms that fall under this division (M. B. Miller & Bassler, 2001).

Signal transmission in Vibrio spp. corresponds intracellularly through a common two-component phosphorelay signaling pathways cascade composed of LuxO and LuxU (VanO and VanU) (Milton, 2006). Despite this, signal transduction pathways in Vibrio spp. are very similar and typically include a membrane and an intracellular receptor (LuxN/LuxR), that is a two-component phosphorelay protein (LuxU/O), and a master regulator. The LuxM synthases are more prevalent than the LuxI synthases amongst Vibrio species (Girard, 2019). Recent research on the genetic component of regulation of QS in V. alginolyticus has mainly concentrated on the potential consequences of LuxR type genes (such as the gene related to virulence, Hfq) and QS signaling transcriptional regulators, such as the motility-regulated extracellular protein Pep and the "colony phenotype inter mediated protein" valR (Cao et al., 2011; H. Liu et al., 2011). Even though the LuxR type homolog of V. alginolyticus is capable of inducing changes in colony phenotype and regulating flagellar production in relation to biofilm production, the genes have not been found in the V. alginolyticus (Chang, Jing-Jing, Chun-Hua, & Chao-Qun, 2010). In a study, 11 unique AHLs were described after being created by 47 distinct strains of V. alginolyticus (J. Liu et al., 2017b).

Additionally, the study investigated the production of AHLs and their influence on the control of V. alginolyticus biofilm development. Furthermore, it suggested that 3-oxo-C10-HSL (belongs to the homoserine lactone category, which contains N-octanoyl-homoserine lactone) has a functional role in the production of biofilms, as well as validated the existence of AHLs and identified the predominant types of AHL signals generated by the V. alginolyticus strains. Furthermore, it was shown that temperature was an important factor in the control of the aforementioned processes. A total of 11 different AHLs were found, with the highest amounts coming from 3-OH-C4-HSL, 3-oxo-C10-HSL, and 3-oxo-C14-HSL, respectively. In addition, it was found that exogenous 3-oxo-C10-HSL at moderate concentrations (10 and 20 M) might initiate or promote biofilm development and modify its structure in V. alginolyticus, but at high concentrations (40 and 100 M), biofilm formation was not substantially enhanced and was even prevented. Furthermore, in V. alginolyticus, the concentration and temperature both had a role in the regulation induced by exogenous 3-oxo-C10-HSL. (J. Liu et al., 2017a, 2017b).

3.4 Biofilm as a virulence factor

The term “biofilm” refers to an accumulation of microbial accretions that are encased in a matrix and may attach to either biological or non-biological surfaces. The development of biofilm, which involves the production of a wide variety of chemicals, is a key contributor to the pathogenesis of a significant number of illnesses, which may affect both people and animals (Hall-Stoodley, Costerton, & Stoodley, 2004). Adherence to bacterial cells towards host tissues is important in inducing disease and/or biofilm development. Adhesion factors are surface proteins that attach with the receptors on extracellular matrix proteins or eukaryotic cell surfaces (Stępień-Pyśniak, Hauschild, Kosikowska, Dec, & Urban-Chmiel, 2019). It is widely believed that the capacity of recognized pathogenic Vibrio spp. to produce biofilms is necessary for the pathogenesis of these bacteria (Sheng, Gu, Wang, Liu, & Zhang, 2012). The production of biofilms by pathogenic strains of Vibrio spp. will contaminate water and aquatic creatures, negatively impacting human health and likely resulting in an epidemic of Vibrio sickness (Beshiru & Igbinosa, 2018). For instance, according to a study, the cholera patients’ stool samples included both biofilm-like clumps and planktonic V. cholerae, the latter of which was much more contagious: It was able to cut the incidence of cholera by 48% if particles larger than 20 microns in diameter were removed from water (Yildiz & Visick, 2009). Biofilm-producing microorganisms, such as Vibrio alginolyticus, create extracellular polymeric substances (EPS) before surface colonisation and biofilm formation.

Once created, the biofilm structure enables bacteria to spread and protects themselves against adverse environmental conditions, including excessive salinity and antibiotics (Prakash, Veeregowda, & Krishnappa, 2003; Suhartono, Ismail, Muhayya, & Husnah, 2019). Researches on biofilm production in human infection are still few, despite indications that biofilm-producing V. alginolyticus strains are superior to planktonic strains at inducing non-specific immune responses and greater immunological responses in young tiger shrimp (Sharma et al., 2010). Most bacterial infections are accompanied by bacterial biofilms, which are an essential factor leading to bacterial resistance. Through various methods, biofilms cause bacteria to become far more resistant to antibiotics and disinfectants than planktonic bacteria (Römling & Balsalobre, 2012). Different substrates of EPS (extracellular polysaccharides) that V. alginolyticus secretes contribute significantly to keeping biofilm structures stable and strong. They are also engaged in biofilm assembly and the toxic properties of biofilms (H. Cai, Yu, Li, Zhang, & Huang, 2022).

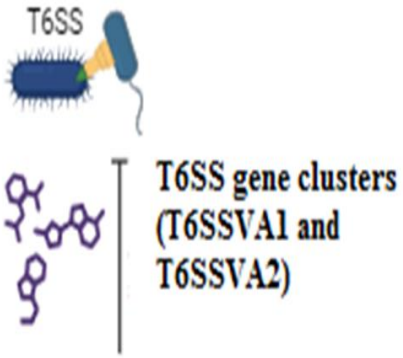
3.5 Type III secretion systems (T3SS)

T3SSs are complex microbial constructs that offer gram-negative bacteria a distinctive phathogenicity system that allows the pathogens to transfer the effector proteins directly into the host cell’s cytoplasm, evading the extracellular environment (Coburn, Sekirov, & Finlay, 2007). Even though the effector proteins of various T3SS pathogens are distinct, they share a number of pathogenic mechanisms, such as interference with the host cell’s cytoskeleton to enhance attachment and Cytotoxicity interference with cellular trafficking pathways, invasion, and barrier malfunction. In human infections and animal models, the activity of the T3SSs corresponds strongly with the course and outcome of the infection (Coburn et al., 2007). A study identified various pathways that promote cell death in fish cells following infection with the ZJO strain of V. alginolyticus. Fish cells infected by the bacteria needed its type III secretion system (T3SS) to rapid death of infested cells. Dying cells displayed apoptotic characteristics, including membrane blebbing, DNA breakage, and nuclear condensation. Further research revealed that T3SS of the ZJO strain activated caspase-3, showing that infection with V. alginolyticus quickly triggers T3SS-dependent death in fish cells. As indicated by the release of lactate dehydrogenase and the absorption of a membrane-impermeable dye, infection with the ZJO strain also caused membrane pores

development and the discharge of cellular contents from infected fish cells. However, apoptosis suppression did not prevent ZJO-infected cells from releasing their cell components or folding, suggesting that infection with V. alginolyticus may induce distinct T3SS-dependent processes that result in the death of fish cells (Zhao et al., 2010). Research indicated that the T3SS of V. alginolyticus destroyed mammalian cells in a manner remarkably similar to how it killed fish cells, but instead of apoptosis, autophagy was triggered during T3SS-mediated cell death (Zhao et al., 2011).

3.6 Type VI secretion systems (T6SS)

The type VI secretion system (T6SS) is extensively distributed among Gram-negative virulent and symbiotic bacteria and is used for interbacterial competition to eliminate competitive species directly. The T6SS is a contact-dependent nanomachine that employs a contractile function to deliver effector proteins directly inside target cells, where they trigger toxic events. T6SSs are adaptable and may target neighbouring host cells or bacterial antagonists (Basler, Ho, & Mekalanos, 2013; Serapio-Palacios et al., 2022). It mainly consists of three complexes: membrane complex, contractile tail, and baseplate (Amaya et al., 2022). In V. alginolyticus two T6SS gene clusters (T6SSVA1 and T6SSVA2) have been identified (Ma et al., 2012).



Figrue 2 : Vibrio Alginolyticus T6SS gene clusters .

Numerous pathogenesis-related and non-pathogenesis-related traits have been discovered to be regulated by T6SS that are also susceptible to the precise control of other regulatory mechanisms, including QS, the two-component regulatory system, alternative sigma factors, and histone-like proteins (Bernard, Brunet, Gavioli, Lloubes, & Cascales, 2011; Cascales, 2008; Ishikawa, Rompikuntal, Lindmark, Milton, & Wai, 2009; Mougous, Gifford, Ramsdell, & Mekalanos, 2007; Renzi, Rescalli, Galli, & Bertoni, 2010). The hemolysin coregulated protein (Hcp1) expression, one of the defining characteristics of T6SS, is thoroughly regulated in V. alginolyticus bacteria (Mougous et al., 2006). It was demonstrated that the expression of Hcp1 depended on the growth phase and that Hcp1 production peaked during the exponential phase. The QS regulators LuxO and LuxR favorably and negatively controlled the expression of Hcp1, respectively. In addition, it was discovered that the alternative sigma factor RpoN and the enhancer-binding protein VasH, encoded by the T6SSVA1 gene cluster, were essential for Hcp1 expression. In addition, LuxR, RpoN, and VasH can favorably regulate the expression of additional T6SS genes. It was revealed that the expression of T6SS in V. alginolyticus was controlled by QS (Sheng et al., 2012).

3.7 Extracellular proteases:

The term “serine proteases” refers to a wide set of enzymes that activate proteolytic degradation at the serine catalytic site (Almonte & Sweatt, 2011). The research regarding the virulence of V. alginolyticus have suggested that the proteolytic enzymes act as significant virulence components, such as serine protease (Hernández-Robles et al., 2016). The Vibrio species are known to produce extracellular proteolytic enzymes that break down different protein substrates for nutrition. However, it has been shown that these enzymes (proteolytic enzymes) released by human pathogenic Vibrio species have been known to regulate virulence (Miyoshi, 2013). V. alginolyticus has been previously described as having two extracellular proteases, alkaline serine protease A and collagenase (Deane, Robb, Robb, & Woods, 1989; Takeuchi et al., 1992). The role of serine protease has been identified as an exotoxin that is deadly for prawns, shrimps and fish (Aguirre-Guzmán, Mejia Ruíz, & Ascencio, 2004; Balebona et al., 1998; Lee, Yu, & Liu, 1997). Collagenases are some of the proteolytic enzymes produced by Vibrio spp. (one of the most important protease-generating bacteria) (Salamone, Nicosia, Ghersi, & Tagliavia, 2019). In the MEROPS(proteases, proteinases and proteolytic enzymes) database, Vibrio

collagenases are zinc metalloproteinases(As a class of zinc-dependent metalloproteinases, collagenases are responsible for the breakdown of collagen substrates and are generated normally by a wide range of organisms, including most mammals and many microorganisms.) classed as subfamily M9A. Collagenases are zinc-dependent metalloproteinases that breakdown collagen substrates and are generated spontaneously by most mammals and many microorganisms (Rawlings, Barrett, & Finn, 2016). It is known that a variety of variables, such as collagen and its peptides, high molecular weight fragments, and oxygen, influence the synthesis of collagenase in V. alginolyticus (Salamone et al., 2019).

3.8 Iron uptake as a virulence factor

Iron is one of the necessary micro nutrients for all live cells, but biological accessibility under physiological and functional conditions is extremely restricted and insufficient to keep cells developing. However, pathogenic bacteria have advanced mechanisms to absorb iron from their hosts (Skaar & Raffatellu, 2015). Many Gram-negative and Gram-positive pathogenic bacteria have been investigated for their iron absorption methods (Krewulak & Vogel, 2008). These bacteria have a range of approaches to circumvent the iron restriction in their hosts. However, they all have systems that are highly conserved across a wide variety of taxonomic groups, as described by (Sheldon, Laakso, & Heinrichs, 2016) (host has developed ways to protect its iron stores from invading bacteria, but the competent infections have developed equally sophisticated methods to circumvent the host's nutritional defence of iron.). The construction of siderophores, which are small iron chelators that are synthesized by bacteria for the scavenging of iron from the nearby iron-binding proteins by creating a Fe(III) complex, and the use of heme groups directly are the two of their systems that have received the most attention from researchers (Richard, Kelley, & Johnson, 2019). The quantity of iron taken into the bacterial cell is highly regulated to prevent hazardous reactions. Therefore, the activation of every system involved in iron uptake is tightly controlled by iron via the iron-dependent transcriptional regulator (Fillat, 2014; Porcheron & Dozois, 2015).

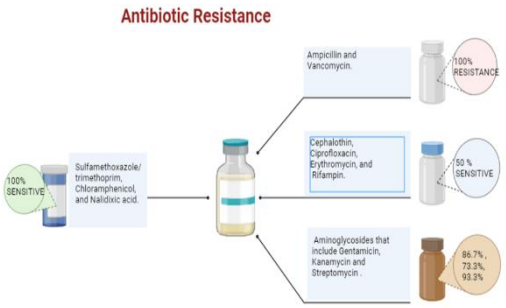
Siderophores such as anguibactin and piscibactin have been recognised as crucial virulence components in Vibrio and Photobacterium infections that lead to diseases in fish (Lemos & Balado, 2020). Outer membrane proteins OMPs, are among the factors that perform essential functions in adapting to changes in the external environment. This ability includes reacting to iron restriction, osmolarity, antibiotics, and acid stress (Lin, Wu, Li, Wang, & Peng, 2008; Sato et al., 2000; Xiong et al., 2010; Xu et al., 2005). Under the conditions of iron restriction, the expressions of OMPs are upregulated by bacteria to act as receptors for iron-siderophores and heme compounds for the transport of iron (Andrews, Robinson, & Rodríguez-Quiñones, 2003). Studies have shown that ompU is an important porin present in Vibrio species that perform an essential function in colonisation and adhesion with resistance to antimicrobial peptides (Duperthuy et al., 2010; X. Liu et al., 2015). It has been demonstrated that bacterial pathogens recognize iron-limiting situations and react accordingly by up regulating virulence genes and systems of iron acquisition (Zughaier, Kandler, & Shafer, 2014). The capacity to absorb dissolved iron ions from the environment is crucial for developing most bacteria in an iron-limited setting. Bacteria have adapted iron absorption systems, including iron carriers, to do this. It is common for genes involved in producing and transporting iron carriers to be located on chromosomes or plasmids. Two different classes of enzymes catalyze the production of iron carriers: non-ribosomal peptide synthase (NRPS) and NRPS-independent synthase (NIS) (Challis, 2005; Crosa & Walsh, 2002). Vibrio alginolyticus, as well as other pathogenic bacteria, have established two iron uptake processes: one decimates erythrocytes in host tissues with toxins and then fetches iron ions from heme released by erythrocytes, the second generates higher-affinity iron carriers known as siderophores which can transfer iron absorbed from transferrin and lactoferrin to bacterial cells for their use (Hider & Kong, 2010). It is widely believed that the strong iron carrier-mediated iron uptake mechanism is a key virulence factor among pathogenic Vibrio species (Biosca, Fouz, Alcaide, & Amaro, 1996).

4. Relationship of virulence factors with antibiotic resistance

Several extracellular components of Vibrio contribute to its phathogenicity, including QS, biofilm production, mobility etc. (Frans et al., 2011; Nakhamchik, Wilde, & Rowe-Magnus, 2008; Natrah, Defoirdt, Sorgeloos, & Bossier, 2011). Resistance to bactericidal processes is an additional significant factor contributing to the phathogenicity of Vibrio species. These bacteria have developed antimicrobial resistance due to the extensive use of antibiotics in aquaculture systems, human medicine, and agriculture during the last few decades (Cabello et al., 2013). Numerous antimicrobial drugs that are therapeutically indicated for medicinal reasons, including aminoglycosides, cephalosporins, quinolones, tetracycline, and folate pathway inhibitors, are very susceptible to Vibrio species (Elmahdi, DaSilva, & Parveen, 2016). The persistent misuse of antibiotics has significantly accelerated the growth of drug-resistant microorganisms, including various Vibrio species (Manyi-Loh,

Mamphweli, Meyer, & Okoh, 2018). From a biological perspective, virulence and resistance mechanisms are required for bacteria to live in hostile environments. Virulence mechanisms are required to defeat host defence systems, and the evolution of antimicrobial resistance is important for pathogenic bacteria to overcome antimicrobial therapy and adapt thrive in intense (wrong word)settings. Immune defence mechanisms and antibiotic pressure are constraints for the survival of the microbial species since they severely restrict the population's growth ability and reduce microbial diversity (Feldman & Laland, 1996; Martínez & Baquero, 2002). The majority of virulence and resistance determinants have been distributed among species via horizontal gene transfer; the transfer of DNA fragments is probably the primary genetic means of distribution of virulence and resistance genes, even though some other systems, such as adaptive mutations may also be involved (Burrus & Waldor, 2004; Handel, Regoes, & Antia, 2006). Moreover, antibiotic resistance is often linked to infection and phathogenicity, such as biofilm-producing microbes (Patel, 2005). Other similarities between virulence and resistance involve the direct participation of efflux pumps, cell wall changes, and two-component systems that stimulate or suppress the transcription of numerous genes responsible for resistance and virulence (Beceiro, Tomás, & Bou, 2013).

Antibiotic overuse has resulted in several strains resistant to a single antibiotic or a combination of medicines. The recommended medications for treating Vibrios are fluoroquinolones, tetracyclines, third-generation cephalosporins, and aminoglycosides. (Daniels & Shafaie, 2000). However, Vibrio alginolyticus appearance with tet plasmids for tetracycline resistance (Kitiyodom, Khemtong, Wongtavatchai, & Chuanchuen, 2010) and pVAS3-1 (L. Ye et al., 2016) plasmids for β--lactamase resistance is dangerous. A study reported that 100% of the isolates were resistant to Ampicillin and Vancomycin. Cephalothin, Ciprofloxacin, Erythromycin, and Rifampin also showed intermediate resistance. Sulfamethoxazole/trimethoprim, Chloramphenicol, and Nalidixic acid showed 100% sensitivity. Aminoglycosides that include Gentamicin, Kanamycin and Streptomycin also showed sensitivity in 86.7% , 73.3% and 93.3% cases respectively (Kang et al., 2016).



Figrue 3 : Vibrio Alginolyticus Antibiotic Resistance .

5. Conclusion

To summarize, V. alginolyticus infection is caused by the combined action of many virulence factors, consistent with pathogenic bacteria's general mechanism. During the invasion and growth in the host, V. alginolyticus may induce tissue injury, resulting in significant illness outbreaks in aquatic animals and humans. Long-term antibiotic or drug usage has been observed regarding the creation of drug-resistant strains, making disease treatment difficult. As revealed for other pathogenic Vibrios, these factors may allow the bacterium to penetrate the host and cause tissue injury to access nutrition sources essential for growth and multiplication. However, more research on the virulence factors and transmission of antibiotic resistance genes on V. alginolyticus is required to learn more about its prospective threat to public health.

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