Pande Gde Sasmita Julyantoro Revised 5

By Diah Ayu Satyari Utami

WORD COUNT	4943	TIME SUBMITTED	28-JUN-2024 07:26PM
		PAPER ID	109918771

MINIREVIEW ON SUSTAINABLE ANTIVIRULENCE STRATEGY FOR AQUACULTURE

ABSTRACT

The increasing occurrence of antibiotic-resistant bacteria is one of the major challenges currently faced by the aquaculture sector. Ineffective applications of antibiotics to treat bacterial diseases, leading to the need for alternative strategies to address the problem. The antivirulence approach is a highly promising strategy that aims to stop pathogenic bacteria from causing harm to the host by disrupting their virulence mechanisms. This approach involves understanding the mechanisms of bacterial pathogenicity that can be developed into new therapeutic methods. There have been numerous advancements in combating bacterial infections, such as disrupting host-pathogen communication and inhibiting quorum sensing (QS). Antivirulence therapy offers a significant advantage as it specifically targets bacterial virulence without imposing excessive pressure on bacterial growth, reducing the risk of resistance development. This review outlines the limitations of antibiotic use and presents current insights into bacterial pathogenicity mechanisms and antivirulence strategies in aquaculture. It particularly highlights the impact of host-pathogen signaling via catecholamines, stress hormones, and QS mechanisms in certain aquaculture-pathogenic bacteria. The influence of host stress hormones on pathogen growth and virulence is noteworthy. Quorum sensing (QS) is known to regulate the expression of certain virulence genes in response to bacterial density by releasing and detecting a small signal molecule called autoinducers. This review further explains various strategies to interfere with QS mechanisms, including inhibiting signal molecule biosynthesis, using QS antagonists, chemical inactivation, or biodegradation of QS signals. These promising strategies have been considered as the first step and proof of concept of antivirulence strategies to prevent disease outbreaks in aquaculture.

KEYWORDS: ***Antibiotic resistant; quorum quenching; quorum sensing; vibriosis; virulence factors***

ABSTRAK: Review pendekatan antivirulensi berkelanjutan terkini untuk akuakultur

Meningkatnya jumlah bakteri yang resisten terhadap antibiotik merupakan salah satu tantangan besar yang saat ini dihadapi oleh sektor akuakultur. Penerapan antibiotik yang tidak efektif untuk mengobati penyakit akibat bakteri, menyebabkan perlunya strategi alternatif untuk mengatasi masalah tersebut. Strategi antivirulensi dianggap sebagai salah satu alternatif yang menjanjikan di mana bakteri patogen dapat dicegah menyerang inang dengan mengganggu mekanisme virulensinya. Strategi ini memerlukan pemahaman tentang mekanisme patogenisitas bakteri yang dapat dikembangkan menjadi pendekatan terapi baru. Beberapa pendekatan telah dikembangkan, seperti mengganggu sinyal patogen inang atau menghambat quorum sensing (QS). Keuntungan utama dari terapi antivirulensi adalah hanya menghambat virulensi bakteri tanpa memberikan tekanan kuat pada pertumbuhan dan proliferasi bakteri, sehingga mengurangi kemungkinan terjadinya resistensi. Tinjauan ini merangkum permasalahan penggunaan antibiotik dan pengetahuan terkini tentang mekanisme patogenisitas bakteri dan strategi antivirulensi dalam akuakultur yang berfokus pada sinyal patogen inang melalui hormon stres katekolamin, dan mekanisme QS pada beberapa bakteri patogen akuakultur. Hormon stres inang secara signifikan mempengaruhi pertumbuhan dan virulensi patogen, sedangkan QS diketahui mengatur beberapa ekspresi gen virulensi sebagai respons terhadap kepadatan bakteri dengan melepaskan dan mendeteksi molekul sinyal kecil

yang disebut autoinduser. Tinjauan ini lebih lanjut menjelaskan beberapa strategi untuk mengganggu mekanisme QS, baik dengan menghambat biosintesis molekul sinyal, penerapan antagonis QS, inaktivasi kimia, atau biodegradasi sinyal QS. Strategi yang menjanjikan ini telah dianggap sebagai langkah pertama dan bukti konsep strategi antivirulensi untuk mencegah wabah penyakit pada budidaya perikanan.

KATA KUNCI: factor virulensi; quorum quenching; quorum sensing; vibriosis; resistensi

INTRODUCTION

Aquaculture refers to the farming of aquatic animals and plants in environments that can be natural or controlled, including marine, brackish, or freshwater settings. This activity includes the production process, such as breeding in hatchery to rearing and harvesting market-size products in ponds, tanks, cages, or raceways (FAO, 2024). Aquaculture fulfills multiple roles, including the cultivation of aquatic species for human consumption, ornamental species for the aquarium trade, and other species used in pharmaceutical, nutritional, and biotechnology products. Aquaculture stands as the fastest-growing sector in animal food production (Anderson et al., 2017) and plays a vital role in the economic development of both developed and developing nations. With a global fish production of around 179 million tonnes and a first-sale value of approximately USD 401 billion in 2018, aquaculture contributed 82 million tonnes valued at USD 250 billion, showcasing its significant economic impact (FAO, 2020). As global capture fishery production remains unchanging while the human population continues to grow, aquaculture is the key to meeting the increasing demand for safe and high-quality aquatic food in the future.

Despite the rapid growth of the global intensive aquaculture industry, it continues to grapple with significant challenges in controlling infectious bacterial diseases. The aquatic environment, unlike the terrestrial environment, provides an ideal breeding ground for pathogenic bacteria, posing a threat to the health of aquatic species. This translates to highly unpredictable survival rates, especially in the early stages of aquaculture species (Irsath et al., 2023). Infectious diseases caused by viral, bacterial, and eukaryotic pathogens significantly

hamper aquaculture production, leading to industry-wide losses exceeding US$6 billion annually. Notably, sectors like shrimp farming suffer severe economic and social impacts, with total losses surpassing 40% of global capacity (Stentiford et al., 2017).

In aquaculture, the impact of infectious diseases is significant, causing both economic losses and animal welfare problems. To combat this issue, farmers often rely on chemical compounds as antimicrobial agents to treat bacterial diseases. These agents include disinfectants, anthelmintic agents, and commonly used antibiotics (Danner & Merrill, 2005; Zhou et al., 2020; Schar et al., 2020). Antibiotics play a crucial role in treating various bacterial infections (Defoirdt, 2013; Thiang et al., 2021). However, the emergence and spread of antibiotic-resistant bacteria and resistance genes, as well as the presence of antimicrobial residues in aquaculture products and the environment, have raised concerns about the use of antibiotics in this industry. The challenge of diseases caused by antibiotic-resistant bacteria is also significant. Therefore, it is paramount to develop alternative methods and techniques to control pathogenic bacteria for the sustainable development of the aquaculture sector.

ANTIBIOTICS PROBLEM IN AQUACULTURE

In the realm of aquaculture, antibiotics are essential for fighting bacterial diseases, just as they are in human medicine and terrestrial animal production. These applications are categorized as therapeutic, prophylactic, or metaphylactic. Table 1 lists various classes of antibiotics utilized in aquaculture, along with examples of pathogenic bacteria in aquaculture exhibiting (multi)resistance. Instead of directly injection into the adult aquaculture animal, the use of antibiotics in aquaculture is commonly added to the feed, which is then delivered to the animal by placed in the rearing water, whereas in some cases, antibiotics may be added directly to the water. Almost every aquaculture farmer uses antibiotics to protect their culture animals from diseases. A wide range of antibiotics, encompassing over ten different types including

chloramphenicol, trimethoprim, gentamicin, tetracyclines, tiamulin, quinolones, and sulfonamides, were deployed (Schar et al., 2020). The common antibiotics used are also different in different countries; for instance, oxytetracycline, oxolinic acid, chloramphenicol, furazolidone, nitrofurans, and erythromycin (Suprapto et al., 2015), and oxytetracycline, florfenicol, trimethoprim-sulfamethoxazole, sarafloxacin, and enrofloxacin (Prena et al., 2020).

Table 1. Various classes of antibiotics used in aquaculture and examples of multiple antibiotic-resistant aquaculture pathogenic bacteria

Class of antibiotic	Mode of Action	Examples	Country	References
Aminoglycosides*	Inhibiting bacterial protein synthesis by binding to bacterial ribosomes	Streptomycin	Vietnam	Dung et al., 2008
		Neomycin	China	Liu et al., 2017
	Inhibit protein synthesis	Kanamycin	Greece, Italy, France, Egypt	Pepi et al., 2021; El-Gohary et al., 2020
Glycopeptides*	Inhibit bacterial cell wall biosynthesis	Teicoplanin	China	Zhao et al., 2024
Ansamycins*	Inhibit bacterial RNA synthesis	Rifamycin	China, the Philippines and Vietnam	Lulijwa et al., 2020
Amphenicols**	Inhibit protein synthesis	Florfenicol, Thiamphenicol and florfenicol, amphenicols	Chile, South Korea, Turkey, China, Viet Nam, Chile, Korea and Portugal	Jang et al., 2018
	Interfering with bacterial protein synthesis	Chloramphenicol	Chile	Saavedra et al., 2018
Beta-lactams*	Inhibit bacterial cell wall biosynthesis	Amoxicillin	Australia	Algammal et al., 2022
		Ampicillin	Vietnam, Thailand, Malaysia, Indonesia	Suyamud et al., 2024; Teo et al., 2000
Fluoroquinolones**		Enrofloxacin	Spain and Portugal	Avendano-Herrera et al., 2008
Macrolides**	Inhibit the synthesis of protein by bacteria	Erythromycin	China	Broughton et al., 2009
Nitrofurans**	binds bacterial DNA which leads to the gradual inhibition of monoamine oxidase	Furazolidones	Greece	Smith & Christofilogiannis, 2007
	inhibit the citric acid cycle, the synthesis of DNA, RNA, and protein	Nitrofurantoin	Taiwan	Liu et al., 1997
	Inhibit bacterial cell wall biosynthesis	Furazolidone, nitrofurantoin, nitrofurazone and furaltadone	China, Vietnam, Korea and Portugal.	Bondad-Reantaso et al., 2023
Quinolones*	Interfere with bacterial DNA replication and transcription	Oxolinic acid, enrofloxacin, ciprofloxacin,	China, Philippines, Vietnam, South Korea, Egypt, Thailand, Brazil	Tendencia & de la Pena, 2001; Lulijiwa et al., 2020

		norfloxacin, nalidixic acid, ofloxacin, levofloxacin, enoxacin, sarafloxacin and flumequine		
Sulphonamides**	Prevent bacterial growth and multiplication	Sulphadiazine	India	Das et al., 2009
Tetracyclines**	Inhibit the synthesis of protein by bacteria	Tetracycline	China, Vietnam, South Korea, Thailand, Brazil, Malaysia	Shah et al. (2014); Lo et al. (2014); Algammal et al. (2022), Suyamud et al., 2024
		Oxytetracycline	Canada	FDA, 2022; McIntosh et al., 2008
		Doxycycline	Brazil, Finland, Chile, Taiwan Province of China, Vietnam, Bangladesh, Korea, South Africa, Tunisia, and Portugal.	Bondad-Reantaso, et al., 2023

*Commonly act as bactericidal agents, causing bacterial cell death
**Commonly act as bacteriostatic agents; restrict growth and multiplication

The increase in bacterial diseases in intensive aquaculture farming has led to an increase in the use of antibiotics. The use of antimicrobials in the aquaculture industry has been documented, which may contribute to the rise of antimicrobial resistance, carrying potential consequences for animal-, human-, and ecosystem health (Defoirdt et al., 2011; Schar et al., 2020). The overuse of antibiotics in aquaculture has led to harmful effects in many farms worldwide. The most common way antibiotics are given to aquaculture animals is by mixing them with specially formulated feed. However, this method is not very effective because fish and other aquaculture animals do not effectively break down antibiotics. As a result, about 75 percent of the antibiotics fed to the animals are excreted into the water (Burridge et al., 2010). These leftover antibiotics can be ingested by wild fish and shellfish, and the rest can remain in the sediment. These leftover antibiotics can lead to the selection of antibiotic-resistant bacteria, changing the composition of the sediment's microflora (Cabello, 2006). A study found that Vibrio harveyi strains with multiple resistances caused mass mortality in Penaeus monodon larvae (Karunasagar et al., 1994). The resistance gene determinants in aquatic antibiotic-resistant bacteria have the potential to be transmitted to terrestrial bacteria through horizontal gene transfer, including to human and animal pathogens (Miller & Harbottle, 2018; Sorum, 2006; Ishida et al., 2010). This has been observed in Salmonella enterica serotype Typhimurium and Vibrio cholerae (Cabello, 2006; Miller & Harbottle, 2018; Sorum, 2006; Defoirdt et al., 2011).

Another significant issue is the difficulty in determining the current dose of antibiotics used in aquaculture due to variations in distribution and registration systems across different countries (Burridge et al., 2010). In 1994, about 500-600 metric tons of antibiotics were used in shrimp farm production in Thailand (Moriarty, 1999). Antibiotic use varies greatly between countries, with Norway using 1 gram per metric ton of production and Vietnam using up to 700 grams per metric ton (Smith, 2008). It is crucial to address the limited data availability that

hinders our understanding of antibiotic usage and content in the aquaculture sector. Research by Heuer et al. (2009) and Smith (2008) highlights the challenges in obtaining a complete overview of this issue. The presence of residual antibiotics in commercialized aquaculture products due to this problem creates unpredictability and poses a risk to human health. Overuse of antibiotics in aquaculture has been linked to the unnoticed intake of these substances by humans consuming aquaculture animals such as fish and shrimp (Cabello, 2006). This unnoticed intake can lead to allergies and toxicity, which are difficult to diagnose due to the lack of information about antibiotic content in the aquaculture products.

From an aquatic environment perspective, the effect of excessive usage and large residual amounts of antibiotics on the normal flora and plankton in the aquatic environment can result in changes in the diversity of the aquatic microbiota by eutrophication because of high input of N, C, and P from non-ingested feed and feces in the water. Moreover, the heavy use of antibiotics is also capable of altering ecological equilibrium at the microorganism level, such as indicated by algal blooms and anoxic environments that have a big influence on the higher levels of consumers such as fish and humans (Cabello, 2006).

Those cumulative issues led to a significant restriction in the use of antibiotics in the aquaculture industry in numerous countries (Cabello, 2006; Defoirdt et al., 2011). This restriction is not only strict regulations including prescription and proscription on the use of antibiotics, but also on the presence of antibiotic residues in aquaculture products. However, some countries that lack adequate regulation on antibiotic usage in aquaculture often face a problem in the worldwide trade of their aquaculture product. Their export product was rejected because the antibiotic residues were over the limit of Maximum Residue Levels (MRLs) that are applied by many importing countries that have more strict regulations (Scalia-Bruce, 2023). Intensive studies are underway to find alternative methods to protect aquaculture animals from pathogenic bacteria due to the negative impact of using antibiotics in this sector. One alternative

strategy to replace antibiotic usage in aquaculture is by preventing the pathogenic bacteria from attacking the host without the need to kill them, called the antivirulence strategy. This strategy targets non-essential pathways of bacterial metabolism; therefore, it does not pose a strong pressure on the pathogen, making it unlikely to develop resistance. Indeed, this strategy needs a comprehensive understanding of the virulence mechanism by which pathogenic bacteria cause disease in aquaculture animals (Defoirdt, 2013).

VIRULENCE MECHANISM OF AQUACULTURE PATHOGENS

Virulence Factors

Infection by pathogenic bacteria triggers the activation of diverse virulence factors that are crucial for the pathogen to invade and harm its host. These essential virulence factors include gene products that play a role in adhesion, motility, host tissue degradation, toxin secretion, iron acquisition, and defense against host immunity (Defoirdt, 2013). Some studies showed that several factors have an influence on virulence factor expression, such as regulated by quorum sensing mechanism, bacterial cell-to-cell communication (Natrah et al., 2011; Yang & Defoirdt, 2014), and increased by the presence of catecholamine stress hormones in media containing serum (Pande et al., 2014; Yang et al., 2014). Virulence factors play a critical role in the infectious cycle of pathogenic bacteria. These factors facilitate the entry of the pathogen into the host, as well as the establishment and multiplication of bacterial cells. They also help the bacteria evade host defenses, cause damage to host tissues and cells, and eventually exit the host.

The virulence factors of pathogenic bacteria have different mechanisms to infect their target hosts. Some important mechanisms, including bacterial motility, adhesion, production of lytic enzymes, chemotaxis, biofilm formation, siderophores, production of extracellular polysaccharides, iron acquisition, and secretion systems, have been reported (Defoirdt, 2013).

Bacterial motility, adhesion, and chemotaxis play crucial roles in successfully infecting a host by colonizing and adhering to the host surface (Yang & Defoirdt, 2014). The production of extracellular polysaccharides (EPS) and biofilm formation enhances bacterial resistance to phagocytosis, providing protection from antimicrobial agents (Chen et al., 2010). Moreover, the production of lytic enzymes, such as hemolysins, proteases, lipases, and chitinases, is essential for breaking down host tissues and enabling the pathogen to obtain nutrients and spread through tissues (Finlay & Falkow, 1997). Additionally, the iron acquisition mechanism is vital for thriving within the iron-repleted environment of a host. Many pathogenic bacteria can acquire iron via siderophores, and the secretion system is instrumental for transporting virulence factors out of the cell (Defoirdt, 2013).

Regulations

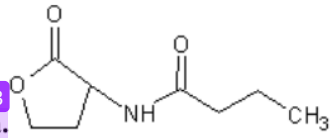
Understanding how bacteria regulate their pathogenicity is crucial for developing effective alternative treatments for bacterial diseases in aquaculture. Identifying the gene products responsible for bacterial pathogenicity and how these virulence factors are controlled is essential. Pathogenicity is not strictly species-dependent but rather a characteristic of specific bacterial strains, with some being highly virulent and others not. The relationship between the presence of virulence genes and bacterial pathogenicity is not always clear. In pathogenic bacteria, the production of virulence factors is tightly regulated, involving mechanisms such as cell-to-cell communication (quorum sensing) and ToxR, and is also influenced by host factors (Natrah et al., 2011; Ruwandeepika et al., 2012).

Quorum Sensing (QS)

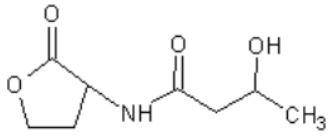
Quorum sensing is a crucial regulatory mechanism in bacteria. It allows them to coordinate gene expression based on their population density using small signal molecules called

autoinducers. This system was initially discovered in luminous marine bacteria such as Vibrio fischeri and Vibrio harveyi (Fuqua et al., 1996; Miller & Bassler, 2001), but similar systems were later found in many other bacteria (Bassler, 1999). Quorum sensing systems can use N-acyl-homoserine lactones (AHLs) as signal molecules, or multi-channel signaling (Defoirdt et al., 2011). AHLs of different species typically contain invariable lactone rings connected to variable acyl side chains with 4 and 18 carbons. This acyl chain can have an oxo or hydroxyl substitution at the third position (Figure 1). Some aquaculture pathogens like Yersinia ruckeri, Aeromonas hydrophila, Edwardsiella tarda, Aeromonas salmonicida, and Vibrio anguillarum use AHLs as signal molecules, while the multi-channel quorum sensing system is found in vibrios such as Vibrio harveyi and Vibrio vulnificus (Milton, 2006; Natrah et al., 2011).

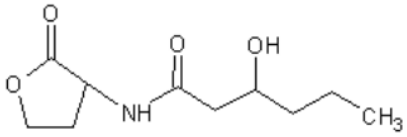
a.



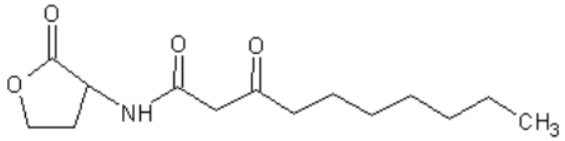
b.



c.



d.



e.

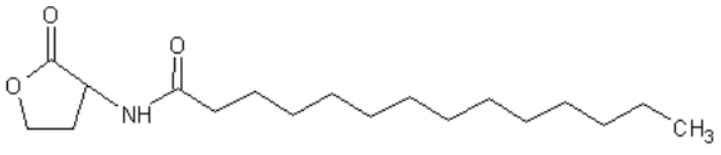


Figure 1. Chemical structures of different AHL molecules produced by different aquaculture pathogenic bacteria species: (a) N-butanoyl-L-homoserine lactone produced by *Aeromonas hydrophila* and *Aeromonas salmonicida*, (b) N-(3-hydroxybutanoyl)-L-homoserine lactone produced by *Vibrio campbellii*, (c and d) N-(hydroxyhexanoyl)-L-homoserine lactone and N-(oxododecanoyl)-L-homoserine lactone, both produced by *Vibrio anguillarum*, (e) N-tetradecanoyl-L-homoserine lactone

The quorum sensing system in Vibrio campbelli strains strains BB120 (=ATCC BAA-1116) is one of the most intensively studied (Lin et al., 2010) (Figure 2). This bacterium uses three different signals: Harveyi Autoindicer-1 (HAI-1), Autoinducer-2 (AI-2), and Cholerae Autoinducer 1 (CAI-1) (Cao & Meighen, 1989; Chen et al., 2002; Higgins et al., 2007).

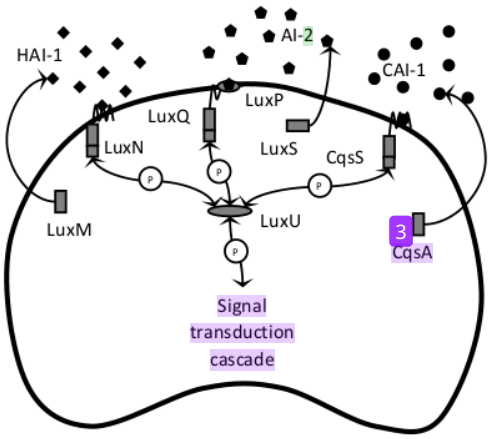


Figure 2. Quorum sensing in *Vibrio harveyi*. The LuxM, LuxS and CqsA enzymes synthesize the autoinducers HAI-1, AI-2 and CAI-1, respectively. These autoinducers are detected at the cell surface by the LuxN, LuxQ and CqsS two-component receptor proteins, respectively. Detection of AI-2 by LuxQ requires the periplasmic protein LuxP. The receptors feed a common phosphorylation/dephosphorylation signal transduction cascade regulating the expression of target genes. (P) denotes phosphotransfer.

Discoveries in the detection of autoinducers by surface district membrane-bound, two-component receptor proteins have led to a better understanding of how these proteins initiate a signal transduction cascade controlling the production of the transcriptional regulator protein LuxR (Taga & Bassler, 2003). The significant role of quorum sensing in regulating the

expression of various virulence factors in pathogenic bacteria and influencing virulence towards different hosts in vivo has garnered substantial attention and research efforts focused on developing techniques to disrupt quorum sensing (Defoirdt et al., 2008; Pande et al., 2013) are underway.

Host factors

Consideration of host factors is crucial in understanding the expression of virulence in bacterial infections. Several metabolism products and stress levels of the host play a significant role in the success of bacterial infections. It has been established that host stress can diminish the activity of the host defense system, thus influencing the outcome of host-microbe interactions (Vicente-Santos et al., 2023). Moreover, new findings indicate that infectious bacteria have evolved specialized detection systems to identify stress hormones produced by their host, which may have a potential link to the heightened virulence of pathogens (Sarkodie et al., 2019).

Catecholamines Stress Hormones

Research has primarily honed in on the impact of stress hormones such as adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine on the growth and virulence of pathogenic bacteria in the host gut tissues (Freestone et al., 2008). It has been demonstrated that these hormones can influence the growth, motility, biofilm formation, and/or virulence of intestinal pathogens like Escherichia coli and Salmonella spp. (Verbrugghe et al., 2012). Notably, catecholamines have been shown to enhance the growth and virulence of the human pathogenic Vibrio parahaemolyticus in serum-based media (Nakano et al., 2007). This suggests that the response of bacteria to catecholamines may be important in the virulence of aquaculture pathogenic bacteria, as the host organisms produce these hormones. Norepinephrine and

dopamine significantly induced virulence in two aquaculture pathogenic bacteria, Vibrio campbellii and Vibrio anguillarum, by increasing motility and growth in media containing serum (Pande et al., 2014). The addition of serum accurately mimics the iron-limited host environment, where transferrin, a high-affinity ferric-iron-binding protein, regulates iron availability (Freestone et al., 2008). Current literature supports the mechanism wherein catecholamines create complexes with transferrin, leading to the reduction of ferric iron (Fe3+) to ferrous iron (Fe2+). This process weakens the bond between iron and transferrin, making iron available for bacterial use (Lyte, 2014; Freestone, 2013). In addition, some studies reported that catecholamines enhanced the motility of *E. coli*, *Salmonella typhimurium*, *Campylobacter jejuni*, and the common aquaculture pathogen *Edwardsiella tarda* and *Vibrio harveyi* (Bearson & Bearson, 2008; Cogan et al., 2007; Kendall et al., 2007; Wang et al., 2011; Yang et al., 2014).

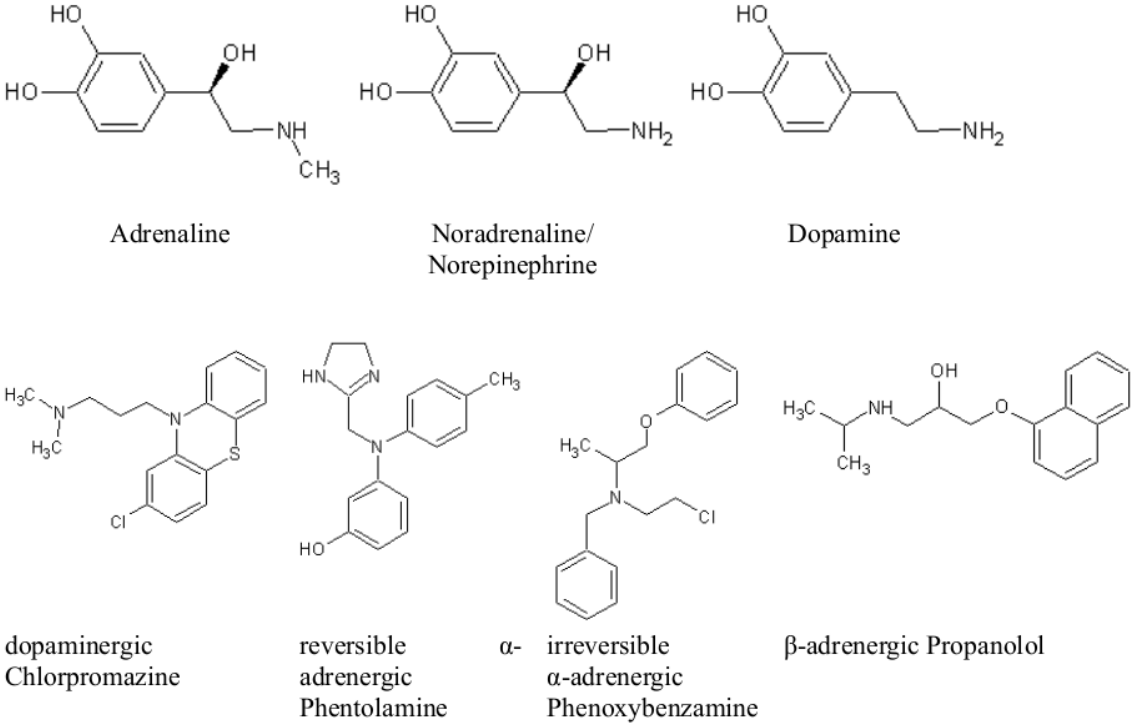


Figure 3. Chemical structure of the catecholamine hormones (adrenaline, noreadrenaline/norepinephrine and dopamine) and eukaryotic receptor

antagonist (chlorpromazine, phentolamine, phenoxybenzamine and propranolol).

The research has found that the antagonist of eukaryotic catecholamine receptors can counteract some of the effects of catecholamines (Pande et al., 2014) (see Figure 3). The eukaryotic dopamine receptor antagonist effectively neutralized the motility-inducing effect of dopamine in the aquaculture pathogen V. campbellii. Additionally, the α-adrenergic receptor antagonists phentolamine and phenoxybenzamine successfully counteracted the motility-inducing effect of norepinephrine. In contrast, the β-adrenergic receptor antagonist propranolol had minimal to no impact (Pande et al., 2014). Other research has shown that both α-adrenergic and β-adrenergic receptor antagonists were capable of blocking the response of E. coli O157:H7 to norepinephrine and epinephrine (Sperandio et al., 2003). Moreover, the virulence of V. campbellii was found to increase towards the larvae of giant freshwater prawn Macrobrachium rosenbergii when they were pre-treated with catecholamines, without affecting the growth of surviving larvae. Nevertheless, the effects of catecholamine receptor antagonists on in vivo virulence were less definitive compared to the in vitro experiments (Pande et al., 2014). These findings highlight the potential of receptor antagonists in modulating the effects of catecholamines and merit further exploration for their potential applications.

Mucin, bile salts, and cholesterol

The expression of virulence factors in bacteria can be influenced by various host factors, including stress hormones, mucin, bile salts, and cholesterol (Defoirdt, 2013; Li et al., 2014). Studies have shown that these host factors can increase the virulence factors of Vibrio anguillarum, including protease activity, flagellar motility, biofilm formation, and exopolysaccharide production, without affecting the growth of the bacterium towards

gnotobiotic sea bass (Dicentrarchus labrax) larvae (Li et al., 2014). Additionally, bile has been found to trigger the production of virulence factors such as type III secretion system-related protein, hemolysins, and capsular polysaccharide in Vibrio parahaemolyticus (Hsieh et al., 2003).

Interference virulence factors

Several innovative methods have been developed to combat bacterial infections in aquaculture without relying on antibiotics, by targeting virulence factors. This antivirulence strategy involves disrupting the regulation of virulence factor expression, impacting multiple virulence factors simultaneously, or specifically inhibiting a particular virulence factor (Defoirdt, 2013). Efforts have been made to interfere with virulence regulatory mechanisms, such as inhibiting bacterial quorum sensing with quorum sensing disrupting agents (Lu et al., 2022; Zhou et al., 2020; Defoirdt et al., 2012; Natrah et al, 2012; Pande et al., 2013), and interfering with bacterial detection of host catecholamines stress hormones by the QseC receptor (Rasko et al., 2008). Additionally, specific virulence factors have been inhibited by blocking bacterial secretion systems with acylated salicylaldehyde hydrazones and thiazolidinones (Baron, 2010), and inhibiting bacterial pili formation, known as pilicides, with bicyclic 2-pyridones (Clatworthy et al., 2007). Nonetheless, this strategy is yet to be tested against aquaculture pathogens and requires further exploration.

Quorum sensing inhibition

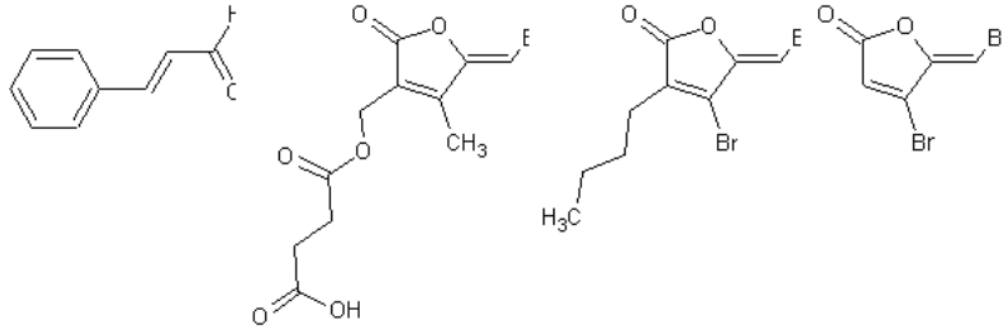
The increasing understanding of bacterial pathogenesis has led to efforts to inhibit bacterial cell-to-cell communication mechanisms known as quorum quenching. Quorum sensing inhibition, which is a key area of study in antivirulence strategies, can be achieved through various methods including inhibiting signal molecule biosynthesis, using quorum sensing

antagonists, chemically inactivating and enzymatically degrading quorum sensing signal molecules, and using quorum sensing agonists (Defoirdt et al., 2004; Kalia, 2013). Further exploration of these different methods is needed to identify the most effective approach for treating bacterial diseases across various fields, including aquaculture.

Researchers have successfully reduced the production of quorum sensing signal molecules by using substrate analogs (Defoirdt et al., 2004). For example, S-adenosylcysteine, an analog of S-adenosylmethionine, has been found to decrease the activity of Pseudomonas aeruginosa LuxI RhlI by up to 97% (Parsek et al., 1999). S-adenosylmethionine, a substrate for the homoserine lactone moiety utilized by homologs of V. fischeri LuxI protein, plays a crucial role in the biosynthesis of Gram-negative AHL signal molecules (Whitehead et al., 2001). This research highlights the potential use of S-adenosylmethionine analogs as specific inhibitors of quorum sensing, offering a targeted approach without disrupting essential processes in prokaryotic and eukaryotic organisms (Defoirdt et al., 2004).

Quorum sensing antagonists can effectively block the transmission of signal molecules, offering a promising strategy to control virulence factors in aquaculture pathogens. One type of quorum sensing antagonist is long-chain natural AHLs produced by bacteria. These AHLs have been shown to reduce the production of virulence factors in aquaculture pathogens such as Aeromonas hydrophila and Aeromonas salmonicida, and protect burbot (Lota lota) larvae from infection by these pathogens (Natrah et al., 2012). Another type of quorum sensing antagonists is synthetic quorum sensing inhibitory AHL analogs, such as halogenated furanones, brominated thiophenones, and cinnamaldehyde (Benneche et al., 2011; Brackman et al., 2008; Janssens et al., 2008) (Figure 4). Furanones specifically disrupt AHL-mediated quorum sensing by interacting with the LuxR-type AHL receptor, reducing the amount of LuxR available as a transcriptional regulator (Menefield et al., 2002). Furanones and thiophenones have also been shown to block the multichannel quorum sensing systems of vibrios by

decreasing the DNA-binding activity of the master regulator LuxRvh (Defoirdt et al., 2007). Studies have demonstrated the protective effect of these antagonists against vibrios in both fish and crustacean larvae (Defoirdt et al., 2006; Pande et al., 2013). However, it's crucial to note that while these compounds offer significant benefits, caution should be exercised as halogenated furanones can be toxic to higher organisms at specific concentrations.



(a) (b) (c) (d)

Figure 4. Chemical structure of some quorum sensing-disrupting compounds. (a) Cinnamaldehyde, (b) The brominated thiophenone (*Z*)-4-((5-bromomethylene)-2-oxo-2,5-dihydrothiophen-3-yl)-4-oxo-butanoic acid. (c) The natural furanone (*5Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone, produced by the red marine algae Delisea pulchra. (d) The synthetic derivative (*5Z*)-4-bromo-5-(bromomethylene)-2(*5H*)-furanone

A further method is by chemical inactivation of quorum-sensing molecules. The quorum sensing signal can be chemically inactivated by yielding the cognate acyl homoserine via alkaline hydrolysis at pH ≥ 8 (Decho et al., 2011). and by oxidized halogen antimicrobials for 3-oxo-substituted AHLs (Michels et al., 2000). The signal inactivated by oxidizing indicated that treating culture water with strong oxidizing agents, such as ozone, for removing the signal molecules of pathogen quorum sensing system might be useful as an anti-infective strategy in aquaculture (Defoirdt et al., 2004).

Quorum sensing can be effectively disrupted through enzymatic breakdown of signal molecules. This method is commonly utilized to interfere with AHLs quorum sensing signals

(Kalia & Purohit, 2011). Enzymatic degradation, based on the AHL structure, can be facilitated by deaminase lactonase, acylase, and decarboxylase (Hong et al., 2012).

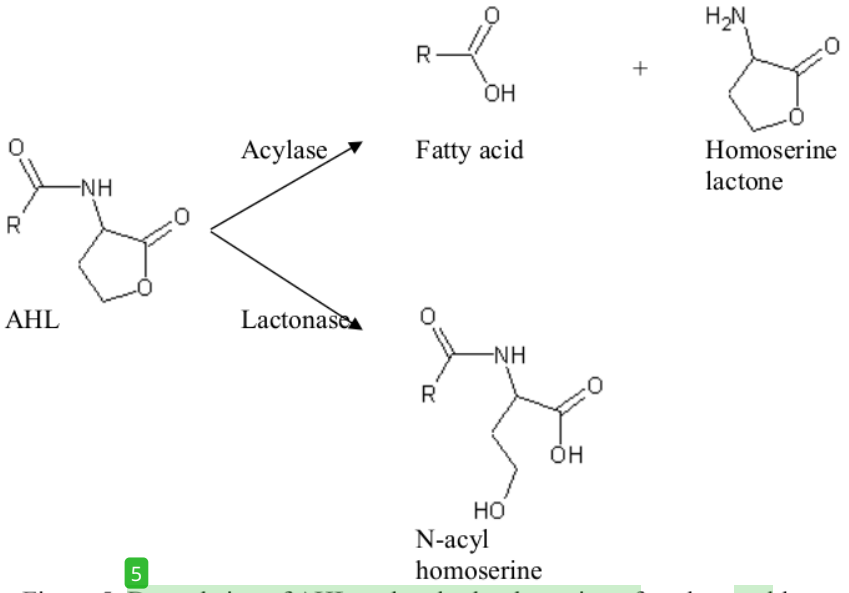


Figure 5. Degradation of AHL molecules by the action of acylase and lactonase enzyme.

The lactonase enzyme plays a crucial role in breaking down AHLs by cleaving the ester bond of the lactone ring, which are important for binding with specific transcriptional regulators (Dong et al., 2000). This enzyme is encoded by the AHL-inactivating activity (AiiA) gene and is found in various Bacillus species (Dong et al., 2002). Similarly, the AHL acylase enzyme contributes to a significant reduction in the effectiveness of the signaling molecule by breaking the peptide bond of the lactone ring, releasing a fatty acid and homoserine lactone (Fast & Tipton, 2012).

The use of AHL-degrading enrichment culture to break down AHL-signal molecules has been found beneficial in certain aquaculture animals (Tinh et al., 2007; Cam et al., 2009; Nhan et al., 2010). AHL-degrading enrichment cultures can be developed by using media that contain AHLs as the primary carbon and/or nitrogen source (Tinh et al., 2007). Pure strains of AHL-

degrading Bacillus sp. have been isolated from this enrichment culture (Defoirdt et al., 2011). Therefore, bacteria with the ability to break down quorum sensing signal molecules may be useful as a new type of probiotics for aquaculture.

Future Perspectives

In our comprehensive review, we provided an insightful analysis of antibiotic challenges and offered compelling alternative strategies for effectively controlling bacterial infections in aquaculture. The antivirulence strategy stands out as a particularly promising method for combating diseases caused by aquaculture pathogenic bacteria. One of its key advantages lies in its significantly lower potential for bacterial resistance development compared to antibiotics. This is due to the fact that the selective pressure exerted is confined to the pathogens through specific killing by phages or targeted disruption of quorum sensing in specific environments. This is a marked contrast to the broad impact of conventional antibiotics, which also affect harmless and beneficial bacteria (Defoirdt et al., 2011).

To ensure success, aquaculture's antivirulence strategy requires precise targeting of specific pathogens in their respective environments. Hence, a comprehensive understanding of the virulence mechanisms of aquaculture pathogenic bacteria is imperative. Recent scientific breakthroughs have unveiled promising antivirulence strategies, such as disrupting bacterial cell-to-cell communication and host-pathogen signaling. Further exploration of these mechanisms holds great potential for advancing disease treatment. A deeper grasp of this knowledge could pave the way for innovative biocontrol methods to combat bacterial diseases and infections, offering a sustainable alternative to antibiotics in aquaculture.

Pande Gde Sasmita Julyantoro Revised 5

ORIGINALITY REPORT

18%
SIMILARITY INDEX

PRIMARY SOURCES

1	biblio.ugent.be Internet	764 words — 15%
2	mts.intechopen.com Internet	45 words — 1%
3	core.ac.uk Internet	37 words — 1%
4	onlinelibrary.wiley.com Internet	36 words — 1%
5	libstore.ugent.be Internet	34 words — 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 1%
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 10 WORDS