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Fisheries and Aquaculture Journal

Mini Review

A Mini Review on Technological Advancement of Diagnostic and Therapeutic Measures in Disease Control Used in Aquaculture

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ABSTRACT

Aquaculture plays a crucial role in the global economy as its demand increases over the years. However, infectious diseases pose the greatest challenge to aquaculture as they would cause billions of dollars in economic losses annually. To reduce these impacts, it is necessary to ensure the health of aquaculture with scientifically proven technologies. Advances in science are leading to a new perspective to better understand the nature of infectious diseases and to apply this knowledge to develop novel and effective diagnostic techniques (molecular diagnostics, biosensors, spectroscopy and nanoparticles) and therapeutic agents (vaccines, gene silencing methods, etc.). In this review, we would like to present some current technologies for diagnostic methods, preventive measures and therapeutics to control infectious diseases in aquaculture.

Keywords: Diagnosis; Technologies; Biosensor; Vaccines; Therapeutics aquaculture

INTRODUCTION

Aquaculture is the process of breeding various aquatic organisms in a controlled aquatic environment, including ocean, river, lake and ponds. The demand for fish and crustacean's increases as these foods are the sources of protein for human consumption [1]. Besides that, it could produce various products that are used in feed, fuel, cosmetics, pharmaceuticals, food processing as well as to restore threatened and endangered aquatic species [2]. According to the latest FAO report on The State of World Fisheries and Aquaculture, aquaculture production reached 114.5 million tonnes of live weight in 2018, at a cost of approximately USD 263.6 billion. In this total production, aquatic animals accounted for 82.1 million tonnes (USD 250.1 billion), 32.4 million tonnes of aquatic algae (USD 13.3 billion) and 26000 tonnes of ornamental shellfish and pearls (USD 179 000) [3]. Despite the impressive expansions, aquaculture faces several challenges that affect seafood production, including destructive habitat conversion, disease, water and soil pollution, harmful algal blooms, and climate change [4]. Diseases are a critical problem in aquaculture that must be addressed to prevent further threats to aquatic species. Therefore, this review focuses on recent technological advances in diagnostic and potential therapeutic methods that can be used to combat diseases in aquaculture.

DIAGNOSTIC AND THERAPEUTIC MEASURES IN AQUACULTURE

Molecular diagnostic approach (multiplex PCR and LAMP)

Polymerase Chain Reaction (PCR) and real-time PCR (RT-PCR) are the gold standard for molecular detection of pathogens in aquaculture [5,6]. However, multiplex PCR produces undesirable, non-specific primer extension products that result in mismatch amplicons being amplified, producing false positive results [7]. As a solution, Dual-Priming Oligonucleotide (DPO)-based multiplex PCR (DPO-mPCR) was developed by [8] to reduce these drawbacks of conventional multiplex PCR. Lately, this DPO-mPCR has been used for the detection of Vibrio species [9] and shrimp viruses [10]. This method has high sensitivity and specificity but is dependent on an expensive thermo cycler and requires technical expertise.

Loop-mediated Isothermal Amplification (LAMP) is an innovative method developed by Japanese researchers that could

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Received: 10-Jan-2022; Manuscript No. FAJ-22-43551; **Editor assigned:** 12-Jan-2022; PreQC No. FAJ-22-43551(PQ); **Reviewed:** 26-Jan-2022; QC No. FAJ-22-43551; **Revised:** 3-Jan-2022, Manuscript No. FAJ-22-43551(R); **Published:** 07-Feb-2022; DOI: 10.35248/ 2150-3508.22.287

Citation: Chan YY, See SNA, Bhassu S (2022) A Mini Review on Technological Advancement of Diagnostic and Therapeutic Measures in Disease Control Used in Aquaculture. Fish Aqua J.13: 287.

overcome the limitations of the PCR method. In 2000, LAMP was developed to amplify the target sequence at a single temperature using a water bath or a heat block [11]. The end-product of LAMP can be observed with the naked eye by color changes or turbidity [12], yielding a rapid and in-field diagnostic assay that has been used to detect viruses [13,14] and bacteria [15]. The sensitivity of the LAMP assay is reported to be at least 10-fold higher than conventional PCR.

Biosensor diagnostic approach

Biosensors are an emerging tool for pathogen diagnosis in aquaculture. An average biosensor consists of three main components: Bio receptor, transducer and signal processor [15]. The bioreceptor immobilizes a bio recognition layer to bind analytes (DNA, protein, enzyme, antigen or antibody, etc.). A transducer detects the stimulus interaction and converts it into electrical signals. The data obtained are then quantified by predetermined algorithms and presented in a user-friendly analog-digital result. Biosensors are grouped and classified according to their conversion mechanisms, which can be optical, electrochemical, electrical or piezoelectric [14].

Researchers from Iran used a fluorescence-free electrochemical genosensor for detection and biomonitoring of Viral Hemorrhagic Septicemia Virus (VHSV) that infects fish [15]. The electrochemical biosensor is a sensor that detects current through chemical reactions (redox reaction) or electrochemical parameters. In this method, hybridization of the target sequence with the probe was measured using cyclic voltammetry, differential pulse voltammetry and Electrochemical Impedance Spectroscopy (ELIS). In addition, the screen-printed electrode is a small electrochemical cell coated with different ink (carbon, gold, platinum) for field analysis. This small and disposable device has been used for screening V. parahaemolyticus [15] and determining the sex of Arowana fish [11]. Other researchers have used electrochemical methods to trace malachite green (antibacterial agent), a dye toxic to mammalian cells [12].

As a piezoelectric sensor, the Quartz Crystal Microbalance (QCM) is an extremely mass-sensitive sensor that detects pathogens through Nano scale biomolecule (receptor-ligand) interaction. This QCM immunosensor has been used for the detection of aeromonas hydrophila and VHSV, which are found in fish species. Both QCM immunosensor and indirect ELISA methods are immobilized with antibodies, but QCM has the advantage of sensitivity, rapid label-free response, and real-time monitoring (frequency shift) over indirect ELISA. The frequency shift is associated with the mass change of the binding interaction.

Spectroscopy-based diagnostic approach

Matrix-Assisted Laser Desorption/Ionization (MALDI) and mass analyzer is a Time-Of-Flight (TOF) analyzer and has been used to identify 47 vibrio species led by an Italian team [11]. The gold standard for species identification is sequencing of the 16S rRNA gene [12], but due to the high similarity of the genome, the discriminatory power is not sufficient in the identification of vibrio species. In another study, MALDI-TOF mass spectroscopy was used to evaluate the virulence of V. tapetis isolates [13].

Nanotechnology diagnostic approach

In nanotechnology, magnetic-based nanoparticles have been applied in aquaculture wastewater treatment to remove nitrate, phosphate, biological oxygen demand, and heavy metals. Portuguese researchers also used this technique to remove antibiotics from aquaculture wastewater to circumvent the problem of bacterial resistance. Moreover, Luminex bead array technology using magnetic microspheres beads (Bio-Plex assay) also has been implemented in detection of salmon anaemia virus. A sensitive and specific electrochemical electrode containing graphene quantum dots and gold nanoparticles was developed to detect White Spot Syndrome Virus (WSSV). Quantum dots are fluorophores that can couple with biosensors such as aptamer DNA to detect cyanobacterial toxins.

Genome sequencing approach

Third-generation nanopore sequencing using the MinION platform complemented by PCR was performed to analyse the genomes of Salmonid Alphavirus (SAV) and Infectious Salmon Anaemia Virus (ISAV). This is a rapid, genome-wide analysis of fish viruses that examines the origin and spread of disease outbreaks. Routine genomic studies also allow researchers to infer evolutionary trends, pathogenicity, drug interactions with aquatic pathogens and transmission patterns at the epidemiological level through second or third generation sequencing. Summary of these diagnostic methods are shown in Table 1.

Diagnostic methods	Analysis time	Advantages	Disadvantages
DPO-based multiplex PCR	≤ 4 h	More sensitive and reliable than conventional PCR, multiplexing	More expensive
LAMP	≤ 2 h	More sensitive and selective than conventional PCR, simpler in-field analysis, no thermocycler needed	Reagents are costly, qualitative results (yes-no assay), multiplexing requires complex procedures
RT-PCR/qPCR	≤ 2 h	Very sensitive	Require qPCR instrument, costly reagents
Electrochemical (SPE)	≤ 1 h	Rapid, sensitive, selective, portable, cost-effective, near	Require instrument, singleplex,

		real- time analysis, quantitative	
QCM	≤ 10 min	Rapid, sensitive, selective, cost-effective, real-time analysis, quantitative, label-free	Require instrument, singleplex
Aptasensor	≤ 2 h	Have high specificity and affinity - based oligonucleotide probes recogn-ize whole cell and protein, real-time analy-sis	Require labelling low availability aptamers compa-red to monoclon-al antibody
DNA-based Schottky diode	≤ 10 min	Rapid, label-free free, no ligand immobilization, cost - effective, sample prepara-tion is simple, Broad field of applications	Database is limited
Spectroscopy-based	Few minutes up to several hours	Very sensitive and selective	Require high-end and bulky instruments, high cost
Bio-Plex assay with magnetic microspheres	≤ 3 h	500 reactions in a single reaction compared to 96 reactions in ELISA, rapid than ELISA, as sensitive as RT-PCR, can use on non-lethal sample as screen-ing material (plasma)	Require bulky and expensive instruments, high cost, high require technical expertise
Biosensor-based QDs	Dependent on type of biosensor	Excellent fluorophores, resistant thermal and photochemical reactions, biocompatible, simple manufacturing	Low fluorescence quantum yield which requires surface passivation process (coating)

Table 1: Comparison of latest diagnostic methods used in aquaculture.

PREVENTION, THERAPEUTIC AND FUTURE ASPECTS OF AQUACULTURE

For the last decade, synthetic biology has emerged from genetic engineering, molecular biology and microbiology, to design, redesign, manufacture or modify biological systems, genetic materials (DNA or RNA), proteins and living organisms for useful purposes. Currently, synthetic biology is applied in various technologies for disease control and therapy in aquaculture. These include immunostimulants, vaccines, antimicrobial and antiviral biomolecules, and gene silencing techniques (RNAi and CRISPR), which are discussed in more details below.

Immunostimulants

Disease control using various diagnostic measures alone would not be sufficient to maintain aquaculture health, but a combination of preventive strategies could better control disease outbreaks. First, aquaculture farmers could adopt appropriate biosecurity measures to minimize the risk of disease outbreaks by implementing restrictions in facilities, maintaining high water quality using Biofloc technology, and other measures [10]. Biofloc technology uses the abundant microbiota to detoxify excess ammonia-nitrogen waste through waste-nutrient cycling in water.

Vaccines

Vaccines are used to trigger the immune system by recognizing and combating the invasion of the host by pathogens, thus providing protection through the production of antibodies. In aquaculture, vaccines used to date include inactivated vaccines, live attenuated vaccines, nucleic acid-based vaccines, peptide subunits and recombinant vectors. Inactivated vaccine is produced by completely killing the pathogens through heat, radiation, or chemicals [5].

Interestingly, there is a new approach to vaccine development called codon deoptimization. This method uses silent mutations in the viral gene sequence without altering amino acids and recodes the codons in the viral genome that are less preferred by hosts. This method was first used to reduce protein synthesis of poliovirus, resulting in attenuation of its virulence.

Potential therapeutics

Recently, the aquaculture industry has used the approved antimicrobial products as therapeutants for farmed fish, but overuse of these products may lead to drug resistance in aquaculture over time. Therefore, knowledge of pathogen life cycle closely related to host cell pathways or molecular pathways is crucial and contributes to the development of effective treatments against infectious diseases. A more environmentally friendly and great alternative therapy to treat aquaculture pathogens without the use of antimicrobial drugs is light emitting diode (LED) technology. In a recent study, blue LED has been successfully used to inactivate seven major bacterial pathogens that could infect fish and shellfish [9,8].

The clustered regularly inter-spaced short palindromic repeats (CRISPR)-associated nuclease 9 (Cas9) systems is another novel gene silencing method that can be used to modify, repair, or knock out specific genes in hosts. This system uses the designed single guided RNA (sgRNA) to complementarity bind to the target DNA sequence, followed by the Cas9 enzyme as "molecular scissors" to cut and disrupt the target sequence or edit the target sequence by introducing the external donor template. In aquaculture, this technique is mainly used to edit various genes related to immunology, breeding, nutritional regulation, sex determination, pigmentation and fertility of aquatic animals, but few studies on disease resistance. For example, the CRISPR-Cas9 technique was first used to knock out the grass carp Junctional Adhesion Molecule-A (gcJAM-A) gene and reduce Grass Carp Reo Virus (GCRV) infection.

DISSCUSSION

Aquaculture is becoming increasingly important in the global economy as demand grows. Infectious illnesses are the most serious threat to aquaculture, since they would result in billions of dollars in yearly economic losses. Science is leading to a fresh viewpoint on the nature of infectious illnesses. Diseases are a serious concern in aquaculture that must be tackled if aquatic species are to be protected. Magnetic nanoparticles have been used in the treatment of aquaculture effluent. Quantum dots are fluorophores that can detect cyanobacterial toxins by combining with biosensors like aptamer DNA. Magnetic microsphere beads and Luminex bead array technologies have also been used. To create, construct, produce, or change biological systems, synthetic biology has arisen from genetic engineering, molecular biology, and microbiology. Synthetic biology is now used in a variety of aquaculture technologies for disease management and therapy, including as fish feeding and feeding equipment. Vaccines work by triggering the immune system to recognise and battle pathogen invasion of the host. Inactivated vaccines live attenuated vaccines, nucleic acid-based vaccines, peptide components, and recombinant vectors have all been employed in aquaculture.

CONCLUSION

In conclusion, many diagnostic tools and prevention measures have been implemented to curb infectious diseases outbreak in the aquaculture industry, but few diagnostic tools are available to farmers in the field to diagnose multiple diseases. Moreover, reliable prevention and treatment methods are still under intense research and debate, including their efficacy, toxicity, consumer perception of GM products, commercialization and sustainability aspects. Thus, further studies should be conducted to determine the immune responses and mechanisms of these treatments and therapeutic agents. In addition, the ethical and biosafety aspects should be taken into consideration by conducting risk assessments and public perception surveys.

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