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Diagnostic methods for identification of various disease-causing infectious pathogens infishes.

Purabi Panda, Department of Life Science, GIET University(20PGLS015.purabipanda@giet.edu)
Sagarika Patel, Department of Life Science, GIET University (20PGLS022.sagarikapatel@giet.edu)
Ankita Behera, Department of Life Science, GIET University(20PGLS027.ankitabehera@giet.edu)
Jasmin Bhuyan, Department of Life Science, GIET University(20PGLS001.jasminbhuyan@giet.edu)
Netaji Behera, Department of Life Science, GIET University(20PGLS014.netajibehera@giet.edu)
Suman Polaki, Assistant Professor, Department of Biotechnology, GIET University (drsuman@giet.edu)

***Abstract*- India with 7.7% pitch into the global fish production rate and stands in the 4th rank globally in fish product exportation , boost economic through international business and within but this million-dollar assignment affects the sequel economical agitation due to infectious diseases. This review aims in controlling the diseases through various strategies like Histological diagnostic method, Microbiological diagnostic method, Molecular diagnostic method, Immunological diagnostic method. To aggravate commercial status, by identification of disease with its treatment by characterization tools, is required to detect and analysis the causative agents. Rather than treating disease, it would be more preferable to give proper environmental condition to prevent the outbreak of any disease.**

***Keywords*−Fish-aquaculture, Fish-production, Global rank, Fish-disease, Causative agent, Methodology, Action**

I. INTRODUCTION

Fishes, aquatic dweller of water bodies, an important organism spends its life in the major of the planet Earth. These are one of the largest group of vertebrates, approximately 34, 200 species discovered according to fish database, performing a major set of biological activities in ecosystem [14]. The major exotics and cultured species found in India are Indian oil Sardines (*Sardinella longiceps)*, Hilsa (*Tenualosa ilisha*), Catfishes (*Plotosus canius*), Mackerel (*Rastrelliger kanagurta)*, Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), Silver carp (*Hypophthalmichthys molitrix*), etc. exported and cultured effectively [20]. Fishes are rich in micro-nutrients and rich in Retinol, Calciferol, Tocopherol, Phyto menadione and B complex supplements. So, if the diseases are controlled then the additives can be extracted easily for mankind. In aquaculture, fishes are frequently affected by diseases due to insufficient nutrient availability. This can cause health hazard to mankind and massive financial loss either through reduced meat quality or fish mortality. Diseases such as velvet disease infecting gills in marine fishes, white spot disease infect fins as well as gills and many other diseases infect various parts of fishes [19]. Various effective applications canbe taken to stop its intensity. The epidemiological study is the most effective applications which helps to improve the health of aquatic lives. This review examines diseases in fishes and methodology uses in determination of fish diseases in aquaculture practices in India both indigenous and laboratory based with future developmental scope in this sector.

II. DISEASE OF FISHES

India, being carp country has established popular position in world ranking status. India's major indigenous carps found are Indian oil Sardines, Hilsa, Catfishes, Mackerel, Catla, Rohu, Mrigal, Silver carp, Grass carp, Carpio, Scampi, Pangasius and Tilapia, etc. produced in bulk amount and tonnes are being exported to other counties. The stability of fish culturing is a major concern which is often disturbed by Diseases, habitat destruction, limitation of resources and other environmental factors. Natural stability of fish when hampered acts as an aid to diseases. Diseases of fishes occur by contagious organism such as the parasite, Bacteria, viruses, protozoan (Table1)and Metazoans parasite. Among all diseases- causing pathogens, bacterial disease is most frequented diagnosed in eggs; young fingerlings results decrease in the production rate of fishes (Table 2). Parasitic diseases are major concern for the fish production due its ability to multiple in rapid speed which increases its mortality rate doubling the natality rate of fishes. In the same category the multiplication of fungal disease-causing pathogens occurs to infect the host (Table 4). Fishes undergo various infectious and viral diseases like carp edema virus, koi rana virus, herpesvirus-2, etc. There is effect of sessional variation on the disease severity (Table 3). In most situations, the environmental conditions or seasonal variations in aqua cultured fields, shows its impact on the life of fishes. Like, the parasitic diseases are mostly seen during rainy and post-winter season.so farmers are advisable to take extra care during the variation period, i.e., mostly post-rainy and winter seasons. If the care is taken with proper preventive measures this can enhance quality and quantity of the fishes [20]. The detailed information of various kind of diseases with their pathogens and treatments are mentioned in aquaculture sector. The table also gives information about the stages which is being infected by the pathogen.

TABLE-1: Some Protozoan infected fish diseases, their causative agents with affected parts and treatment

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Danio rerio & Anabantoidei	*Oodinium spp.*	Velvet or Rust	Fins, gills, skin	Copper at 0.2 ppm & Acriflavine 0.2% solution Quinine hydrochloride	[25]
Cyprinus rubrofuscus & *carpio*	*Costia necatrix* & Protozoa	Costia & White spot disease (all stages)	Skin, Body, fins, gills		
Paracheirodon innesi	Sporozoa (*Plistophora hyphessobryconics)*	Neon tetra disease	Bright bluegreen Neon stripe & skin	No cure or Destruction of infected fish	[18]
Colossoma macropomum	Protozoa	Lymphocystis	Fins, body		

TABLE-2**:** Common Bacterial Fish Diseases, their causative agents with affected parts and treatments

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Cyprinus carpio L.	*Shewanella putrefaciens Acinetobacter spp., Aeromonas spp. Or chryseobacterium spp.*	Acinetobacter infection (All stages of life cycle)	Fins, tail, body	Acriflavin or monoacrin 0.2% solution 1% antibiotic Penicillin, chloromycetin, Kanacyn, erythromycin, minocycline	[25], [18]
Carassius auratus, Oncorhynchus mykiss, Samonidae family, Cyprinidae family, Ghadus morhua	*Eubothrium, Flexibacter columnaris, F. branchiophilum, F. psychrophilum, Kocuria rhizophila*	Columnaris disease, rainbow trout fry syndrome (all stages of life)	Mouth, gill, skin abdomen		
Scaled fishes	Aeromonas bacteria/ *Pseudomonas fluorescens*	Dropsy/Scale protrusion	Body cavity/tissues/Scales	Chloramphenicol/ tetracycline	

TABLE 3**:** Viral-Borne Fish Diseases, their causative agents with affected parts and treatments

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Anguilla spp.	European eel Angulla Anguilla	Endogenous viral element disease	Lethargy, skin, fins, internal organs.	Temperature control	[29]
Carassius auratus, Rubrofuscus carpio	Viral hemorrhagic septicemia virus, Rhabdovirus carpio	Viral hemorrhagic septicemia, spring Viremia of carp	Gills, eyes, skin, abdomen swim bladder, intestines	No specific treatment or cure	

Table 4**:** Fugal Affected Fish Diseases, their causative agents with affected parts and treatments

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Labeo rohita, L.bata Puntius sophore,	Achlya and Dictyuchus sp.	Achlya encyst and zoospores	Mouth	Malachite green	[12]
Carassius auratus	*Branchiomyces sanguinins & demigrans*	Branchiomycosis or gill rot	Gill tissue	Avoidance, treatment with calcium oxide and copper sulfate	

Table 5: Common Parasitic Fish Diseases, their causative agents with affected parts and treatments

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Silurus glanis, Cyprinus carpio	Gyrodactylus sp.	Gyrodactylide, Skin fluke	Caudal, peduncle,skin, gills, scales.	Praziquantel, Levamisole-HCl & Metrifonate	[16], [27], [28],
Mugil cephalus	Dactylogyrus and Gyrodactylus	Dactylogyroxsis and Gyrodactylosis (Adult)	Gills, excessive mucus secretion and Caudal peduncle, skin, gills, scales	3% NaCl bath, Acetic acid bath, Formalin bath, Piric acid bath	

Table 6: Common Crustacean in Fish Diseases their causative agents with affected parts and treatments

SPECIES (SCIENTIFIC NAME)	CAUSATIVE AGENT	DISEASE (STAGES IT AFFECT)	PARTS AFFECTED	TREATMENT	REF.
Carassius auratus	*Argulus japonicas, A.foliaceus, A.siamensis, A.bemgalensis , Lernaea cyprinacea, L.hardingi*	Argulosis (adult), Lernaeasis (adult and juveniles)	Skin, fin and belly region	NaCl bath treatment (2-3%), potassium permanganate(2-5mg/l), gammaxene treatment	[2]

III. DIAGNOSTIC METHODS OF FISH DISEASE

According to reports, we found that there is experience of economic scarcity in field of aquaculture around 1980s due to the substantial increase in the rate of diseases in shrimp. This affects the socio- economic life of the people as they felt insecurity in their professional life. In order to invoke confidence in farmers, government launched blue revolution around 1980s. Under "Neel Kranti Mission" leadby government of India, there is not only improvement in fishery sector but also evolution in food and nutrition sector [24]. This mission improves knowledge of farmers about the use and misuse of drugs by introducing various diagnosis methods which leads to proper therapies and chemotherapeutic procedures.

A. METHODS

This method is more fragile and employed to verdict fish disease. This includes Polymerase Chain Reaction (PCR), Restriction Enzyme digestion, probe hybridization, Enzyme Linked Immuno-Sorbent Assay (ELISA), In-situ hybridization and Microarray. The symptomatic and asymptomatic pathogens can be detected through this technique, so that the, disease onset can be prevented [31]. On the basis of diagnostic, the procedure can be divided in to three parts: -

a) CULTURE DEPENDENT

It is a traditional method to compare the phonotypical data which is obtained by culture of pathogen from pathological materials [3].

b) CULTURE INDEPENDENT

It is faster easier than the traditional method for disease detection. It provides better potential with sensitive. Their use has presented a high rate of accuracy and speed with specificity in reporting pathological, phonotypical, morphological(fig.1) and microbiological character of the pathogen [16]. If the disease symptoms and signs are noticeable easily than the diagnosis shows positive with molecular techniques gives strong evidence towards the presence of pathogen.

c) SEROLOGY

Serological diagnostic based on the presence of specific immunoglobulin or crucial increase in level of specific immunoglobulin in following 7-10 days(fig.1). The popular serological techniques used cell agglutination using polyclonal antisera, Latex agglutination), etc. provide information of origin and phenotypic character of pathogen after acute stage and useful in screening ceramic fluid of species for epidemiological study and other health purpose. It has certain limitations like specific antigen use which determine the pathogen.

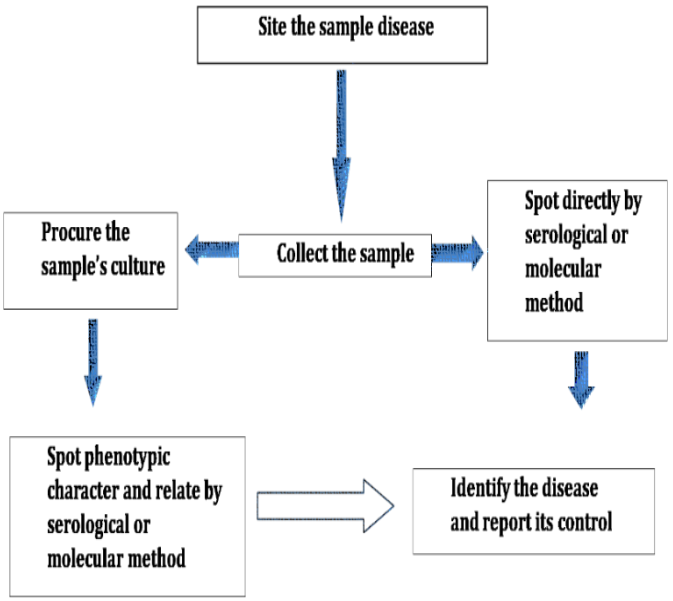

Fig.1: Systematic procedure followed after the collection of samples for identification of disease and the methods followed for the detection of the disease.

The disease diagnostic method classified as follows: -

B. Histological Diagnostic Method

In this, sample fixed in aqueous fixative to preserve structural and chemical constituents of fish cells/tissue. A sequence of graded alcohol is used to prevent the cell/tissue from dehydration. The tissue is treated with a substance which is the mixture of dehydrating fluid and embedding agent. Haematoxylin and Eosin (H&E) and gram stain are used for identification and differentiation between various microbial pathogens. The periodic acid-Schiff used to detect fungal infection in the tissue by the emergence of red/pink colour stain. The Immuno-histochemical staining method used to detect viruses by viral antibody results in the coloured product which can be seen under the light microscope [9].

C. MICROBIOLOGICAL DIAGNOSTIC METHOD

In this method, bacterial diseases can be identified easily understerile condition and cultured in non-specific media for diagnosis purpose, such as: -

D. METHODS OF STAINING

The common methods used in staining bacterial pathogen are Gram staining, Acid-fast or moderate acid-fast stain, fluorescent staining, Giemsa stain, India-ink (colloidal carbon stain), haematoxylin stain and Gomori-Wheatley stain used to detect bacteria and fungi, parasites in tissue, cells, phagocytes and blood, intra-cellular inclusion in the virus, intra-cellular bacteria encapsulated fungal diseases and intestinal protozoa. Selective media use for culturing media used for the growth is selected specific for organism which stops the growth of other microbial organism like Thiosulphate Citrate Bile saltSucrose agar (TCBS) media for vibrio [15].

E. BIOCHEMICAL TESTING

A biochemical testing method, a differential medium test in which the organism determine the ability to detect the production of enzyme tryptophanase, organism producing acetyl methyl carbinol from fermented glucose, catalase enzyme converts H_2O_2 to O_2 and H_2O or reduce NO_3^- to NO_2^- using NO^- reductase, on the basis of Sulphur and fermented carbohydrates and determine the production of decarboxylase, which contains nutrients, dextrose pyridoxal, and pH indicators, i.e., Bromocresol purple and cresol red. The testing includes Indole testing, Methyl red and Voges-Proskauer, Catalase test, Citrate test, Oxidase test, Reduction test, Urease test, Coagulase test, Casease Test, Oxidase test, Reduction test, Urease test, Coagulase test, Casease Test, Triple-Sugar Iron Agar, Decarboxylation Test. The formation of colour indicates the positive result, that is, the presence of positive bacteria indole, pyruvic acid and ammonia. (*E. coli*) [6].

F. *POLYMERASE CHAIN REACTION:* -

It is a scientific clinical technique, widely used for forensic studies by medical aspirants, researchers and clinical technicians. This process is very sophisticated analysis of DNA amplification, gene and genome by gel electrophoresis. Due to its effectiveness, it used in monitoring, analyzing and controlling of closet disease or various stages of disease in the sample by fluorescent dye specific sequenced DNA vector. Due to its highly sensitive nature, any contamination states even in the trace amount, decept the result of experiment. In this with the help of primers and DNA polymerase, there is the addition ofnon-specific DNA sequences of nucleotides in the desired sequence [5].

G. MICROARRAY: -

It is used for measuring the nucleic acid sequence concentration in the specific sequence solution for detection of the DNA or RNA unique sequence various microarray methods used, i.e., DNA microarray; self-assembled array; in-situ, synthesized array and spotted array. In comparison to traditional, detection of nucleic acid hybridization technique, the above microarray methods are highly sensitive, effective, and low cost and help in rapid detection with low background support. It is used in detection of multiple sequences and large-scale estimation of the disease testing [5].

H. IN-SITU HYBRIDIZATION

The efficacious research molecular tool, used in clinical practices for improving the knowledge of infectious and necrotic disease and diagnostic methods. It is helpful in detection of the low expressed gene within the tissue [30].

I. CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS (CRISPR) SYSTEM

With the development of metabolic and genetic engineering, industries are producing a high quantity of strains with broad prospectus for industrial production due to this science developed a new technique for promoting bacterial cell engineering. The technique known as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR- associated proteins system is high-grade technique that helps in switching genetic expression by modifying genes. It mainly focuses on DNA sequences, which modified and make use of small fragments of RNAs to run the Cas-nuclease. In case of bacteria or prokaryotes, CRISPR-Cas system act as a specific immune system and protects themselves from different viral infections. According to the character of Cas protein CRISPR/Cas system Type I, II and V identifies and splits DNA sequences while function of Type IV is not properly identified but have Csf1 signature protein. Type III acts on both RNA and DNA. CRISPR Type I system, target ssDNA molecule with its signature protein Cas3. Type II CRISPR/Cas system containing Cas9 protein helps in formation of blunt ended DNA molecule. Multiple subunit class-1 CRISPR/Cas system binds to nascent strand with the help of Cas10 protein. Type V CRISPR/Cas system contain single subunit with Cas12 protein helps in formation of staggered ds stranded DNA molecule. CRISPR-Cas9 is not only useful in genome editing but also in biomedical applications, agricultural, transcriptional and therapeutics.

J. IMMUNOLOGICAL DIAGNOSTIC METHOD

This immunological diagnostic for quantitative and qualitative analysis of pathogen in aquaculture. Due to the advancement in this technique, there is discovery of the monoclonal antibody-based method which improves the

experimental result and new pathogenic diseases are 6being researched. The various diagnostic method: -

a) BY AGGLUTINATION TECHNIQUE

The binding of specific antibodies and the antigens of bacteria or the particulate matter, i.e., latex particlesis called the agglutination test. It is used for identification of serum antibodies with a known antigen interpreting the relation between the antibodies and antigen. This type of diseased diagnostic techniques used for detecting diseases like tetanus, yersiniosis, leptospirosis, brucellosis and tularaemia. This is sensitive technique to detect an antibody to infectious mononucleosis, mycoplasma antibodies, etc.

b) AGAR GEL IMMUNO-DIFFUSION TEST

In this, antibody and preferred antigen is diffused in wells of agar plate. A circular ring of precipitation is been visible when a proper concentration of antigen and antibody communicate with each other.

c) FLUORESCENT ANTIBODY TEST

This test exclusively uses for antigen quantitative estimation by annexation of fluorescein. Here the antigen-antibody reaction is sensitive by the addition of fluorescent dye, which detect the presence of pathogen in the sample [31].

d) ENZYME LINKED IMMUNOSORBENT ASSAY

There are various methods of ELISA for detection of antigen-antibody interaction. Dot ELISA, a quantitative ELISA test performed in small number of an antibodies immobilized, binding to nitro-cellulose while other antibodies bind to HRP (Horse-Radish Peroxidase). At first, a sample antigen reacted with an inactivated antibody and HRP-linked antibody. To determine the concentration of linked antibody, the stripes are incubated in hydrogen peroxide and tetramethylbenzidine. Horseradish peroxidises acts on hydrogen peroxide by hydrolysing tetramethylbenzidine to tetramethylbenzidine oxide which give a blue colour product. This blue-coloured product precipitate on the strip. This method is used in the analytic laboratories to diagnose diseases as enzyme activity is directly proportional to antigen concentration.

IV. EFFECTIVENESS AND SCOPE OF DEVELOPMENT

Under the blue revolution programme, restructured activities enhances development of fish and fisheries sector. So, there is a drastic change in diagnostic methods for analysis of aquatic pathogens and probe-based diagnosis increase effectiveness in diagnosis of fish diseases. The pathogen genomes upgrade biological studies and analysis antigen and antibody interaction in target sites. Polymorphism improve specificity, sensitivity, and diagnostic efficiency of techniques useful in epidemiological studies and identify cause of outbreak. Considering the above factors government of India have to launch many missions to enhance the quality and quantity of marine life which stabilizes fish culturing by improving productivity of water using the recommended stocking mixture to enhance growth of fishes, regular surveillance on fishes' health, creating surveillance programs which would be effect for farmers, periodical studies on the effectiveness of the pathogen could help in taking the preventive measures for cultivation of the particular species.. Various efforts have taken to improve the growth of fishes in counties sanctuaries, wetlands and other water bodies like usages of probiotic, prebiotics and synbiotics. This review literature basically gives the knowledge towards fish diseases with the methods to detected and eradicate it. It also gives suggestion on improving the researches on using natural based methods for better yield and increase the productivity by reducing disease breakdown in the aquatic field.

CONCLUSION

Fishing is the primary activity of agriculture- based counties, million dwellers of the country are directly or indirectly dependent of this sector. After the commencement of the mission there is immense change in thissector, i.e., from traditional way to advanced methods. Resulting in increase in the production from single- digit tonnes to triple-digit tonnes expanding the field to multiple folds. But then also disease-causing pathogens a problem in the aquaculture system globally which can't be uprooted. The disease occurs in result of intensive culture practices without knowledge of host pathogens and environmental challenges. Control of disease is complex and mostly depends on various methods of detection, diagnosis, prevention, treatment and general management of fishes' health. In this work, we have focused on disease status and control measures 'influence for sustainable development production.

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