Bacterial Pathogens Associated with Fish Diseases and Nutritional Losses

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Introduction

Fish diseases and infections are caused by various pathogenic bacteria, viruses, fungus or parasite. Most of the causative agents associated with fish diseases are saprophytic and are widely distributed in aquatic environment. A variety of microorganisms are pathogens of fish and may be present on the external body surface or within the tissues of healthy fish. Therefore, consumption of infected fish sometime creates a micro biological health hazards to the people in Bangladesh.

For this reason prevention of fish diseases is becoming increasingly important specially in topics but the information of bacterial pathogens of fish are extremely fragmentary and often neglected. So, in most disease outbreak rapid identification will expedite information needed to start therapy[2] and so there is a continuing demand and a definite requirement of rapid microbial such techniques for extension research in fish and fishery services. The aim of this paper is to provide technical informations and methods for accurate determination of bacterial pathogens with minimal time, efforts and equipment s for preventing the important nutritional losses.

Materials and Methods

These fish are specimens whose appearance or behavior indicate the presence of an infection due to contamination by bacteria. It is important to examine infected tissue from inside the fish. The fish are specimens whose appearance or behaviour indicate the presence of an infection due to contamination by bacteria. It is important to examine infected tissue from inside the fish. The fish should be freshly killed to avoid overgrowing by pathogens or saprophytes. The deep-seted tissues are less likely to be populated with extraneous organisms, such as bacterias which would otherwise be troublesome in surface lesions. All tissues sample should be pretreated with alcohol to avoid possible contamination while examining the specimen. But care must be taken to ensure that the sterilant does not penetrate into the tissues and kill all pathogens.

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In the absence of any visible sign of disease these fish are taken from the same population as diseased stock and submitted for simultaneous examination. The usual procedure is to kill the living fish by decapitation anesthetic overdose immediately before examination. Benzocaine (0.28 dissolved in 5 ml acetone and added to 8 litres of water is an effective and relatively inexpensive agent for the purpose.[3]

Sampling

Appearance of fish will normally indicate the appropriate tissues for examination. Many diseases give rise to visible lesions on tissues, particularly, ulcers, on the external surface of the body similarly if fins, tails or gills how pathological changes material from these sites are selected. When a septicemia condition is selected for examination.

Samplings are made by inserting a sterile inoculating loop into the selected lesion or tissue, when external lesions are to be sampled, The surface is needed to be cleared with a hot scalpel and the inoculating loop inserted through the surface into the diseased edge of the elsion. Disinfectant should be applied to external lesions before sampling.

Samples taken from fish may be examined by wet and stained preparation. Wet preparations are useful for the quick presumptive diagnosis of myxobacterial infections, including bacterial gill diseases and some forms of fin and tail rot.[4]

In identification of Myxobacteria can be confirmed by a series of culture stained smear techniques may indicate the presence of bacteria in affected tissues, specially for the diagnosis of bacterial kindney diseases of fish.[5] Cultural methods are required to identify bacterial infections of fish. These are necessary because different bacteria may give to similar sign of disease (e.b. ulcers, granulomas or septicemia) and it is also common to encounter mixed infections which can be recognised only by the cultural methods.[6]

Isolation of Bacteria

There are general approaches to the isolation of fish pathogens. One is non inhibitory media and the other is selective media specially for the recovery of a group of a single organism. The selective approach is comparatively less time consuming and less expensive. In the non selective approach media which will support the growth of a wide range of organisms are used. This may result in a considerable amount of time and materials for isolation and identification methods.

Results and Discussions

Bacterial Pathogens-Their Nature, Description and Isolation

A variety of microorganisms, including bacterial fungi, viruses and protozoa are

pathogens of fish. In the present paper only the bacterial fish pathogens have been described.

A. Gram-negative Organism

Gram-negative rods were first reported as a proven cause of fish disease during 1897. Emphasis on aquaculture of maximum fish production for minimum water volume has resulted in the elucidation of gram negative fish pathogens. The emergence of these organisms has been further enhanced by the ever-growing problems of water pollution due to expending industrialization and increasing human population.

The importance of obtaining pure cultures before carrying out identification tests are emphasized. Mixed infection give mixed cultures and, therefore, purification by subculture is essential to obtain a correct identification of isolates. So, the gram reaction is the first characterization test applied to isolates obtained from fish.[8]

Pseudimoans anguilisptics

Pathogenic members of the genus pseudomonas primarily affect marine and fresh water fish. A syndrome referred to as'red spot disease' has caused great economic losses in cultured Japanese eel. The causative agent, P. anguilliseptics is responsible for potential hae-morrhage on external structures such as mouth, opercula and ventral side of body as well as internal haemorrhage in kidney, liver and in peritoneum.[10]

Pseudomonas may be cultured from the blood, liver, spleen or kidney. Primary isolation can be accomplished by bovine, sheep or horse blood agar containing media (BHBA) incubated at 20-25°c for one week. These bacteria were considered as an oxidase positive rod which degraded gelatin, negative for indole production and arginine dehydralase and were neither oxidative nor fermentative for glucose 11. Other members of these genus, such as P. chloroaphis, are soil and water organisms and may be associated with disease problems as either primary or secondary pathogens.[11]

Vibrio

Several species of vibrio including the genera V. Anguillarum. damsela, alginotyticus have been associated primarily with disease in fish, result in generalised septicaemia often associated with ulceration of external surface and fins. Vibrio damsela was first described as a new pathogen of damsel fish [12] and also reported to produce 'Red Pest' in eels.

Isolation of these pathogenic organism can be successfully accomplished using tryptone soya agar (TSA) or BHBA, supplimented with 1% to 3% W/V) Nacl at 22°C[13]

A number of vibrio isolating media have been formulated. Two such media marine 2216 E agar and TCBS

(Thiosulphate Citrate Bile Salt) agar are used primarily for selection of vibrio which is the causative agent of cholera, dysentry and food poisoning to man. distinguishing characteristics include : nitrate reduction (+), arginine dehydrolase (+), vogesproskaur freaction (+), and utilization of alanine (+) and vibriostatic compound of 0/129 sensitivity.

Aeromonas

A haemorrhagic disease of cultured fish is caused by Aeromonas 14. The genus Aeromonas is divided into two distinct groups as motile and non-motile. Motile Aeromonas were first reported as being associated by haemorrhages of gill and fins along with absenses or ulcers[15] with swollwn kidney and liver with ascitic fluid[17]. Similarly motile aeromonas have been associated with tail/fin rot for many years. At the present time, there are 4 species of motile aeromonas, eg. A. hydrophila, A. caviae, A. sobria, A. veronil. However only a. hydrophila is pathogenic for fish[18].

Typical A. salmonicida and A. hydrophila are usually isolated easily on either TSA or BHIA. Presently, there is no widely used selective isolation media avilable for A. salmonicida is usually grown in NA or TSA agar in 24-48 hrs, at incubation temperatures of 20-25° C[19]. Diagnosis of fresh isolates has been achived by examination of the phenotypic traits. The presence of gram-negative motile, fermentative rods, which produce catalase, oxidase and arginine dihydrolase but not lysine or ornithine decarboxylase is regarded as diagnostic of Aeromonas hydrophila.

Acinetobacter

Poald & Hastein (1980) investigated a case of mortality among Atlantic salmon.[21] The fish showed ulceration of skin and at the pelvic and analfins and began to dig [22]. From this infected fish haemolytic, non-acid fast rods were isolated on blood agar medium containing 0.5% Nacl. It was reported that these organisms may be related to Acinetobacter Moraxella [22, 23].

Cytophaga

Several species of Cytophaga have been associated with disease in fish. C. aychrophila has been well documented as a fish pathogen[24] as the causal agent for peduncle disease. Pacha[25]. (1968) studied Cytophaga extensively by identifying several isolates grown on Cytophaga agar. It was reported that they produced brightyellow pigmented colonies with thin spreading margins which are strictly aerobic and flexible rods. The description, however, matches Flexibacter more closely than Cytophaga. It should be emphasised that Cytophaga and Flexibacter are common inhabitants of aquatic ecosystem.

Yarsinia

A haemorrhage disease of cultured salmonida, known as Enteric Redmouth (ERM), as first described in the Hagerman valley, Idaho, and USA[27] was known to be caused by Yarsinia. Haemorrhage occur in the muscle of mounth and in fin base of the infected fish[28]. This type of disease is known as Blood Spot Disease[29].

Primary isolation of Yarsinia may be made on TSA incubated at 20-25 C for 24—28 hr. In this media colonies of Yarsinia appear as yellow colour.

Flavobacterium

There has been many report of Flavobacterium infections in fish [30,31].This disease was characterized by slowly developing granulomaotous lesion accompanied by a high death rate[32] This disease was named as the 'Red Ride' in marine fish [33]. The incidence of the disease was associated with proliferation of phytoplankton. These organisms were nonmotile, aerobic, catalase and oxidase positive, gelatin and starch were degraded but not cellulose or chitin. Nitrates were not reduced, nor was H_2 or indole produced but glucose, fructose and sucrose were utilize [34]. There was an outbreak of Flavobacteriosis, which first apeared in Japan during 1977 during 1977[35,36]. This disease cnaracterized by generalized septicalmia. This organism was isolated using TSA supplimented with 0.5% Nacl, which produced yellow orange pigmented colonies after 24 hr. incubation at 18-20°C [24].

Proteus

There has been many reports of the role of proteus retheri a representative of the family Enterobacteriaccae as a fish pathogens, specially in silver carp (Hypopthth and michthys molitrix) in many countries in Europe [31,37]. The members of proteus have been associated with fish and responsible for ulceration of fins and head of many fish [22,38]. Proteus may be isolated using NA, BHIA or any blood-enriched basal medium[31] Following culture of internal organs (kidney, liver, spleen) or external lesions, proteus will usually appears as large colonies with irregularedges or as large areas of pacthy growth covering the agar surface at 20-37°C [22].

Proteus may be differentated from the other enteric bacteria by using phenylalanine

Edwardsella

The microorganism has a world-wide distributions and is responsible for great economic loss to the eel culture industry fin South East Asia. In the United States E. torda has been responsible for shut down of catfish processing plant due to contamination of processing machinery by E. torda [39]. torda [39].

There were a number of fish diseases including potential haemorrhages on the skins around the throat and mouth, pale gills, exophthalmia and open lesions on the head and body surface caused by E. torda [22]. These organisms are isolated using HBIA, NA or TSA with or without 5% (v/v) of blood supplemented media incubated at 22-26° and recovered in f24-48 hr. Cultures are found to be fermentative rods which were weakly motile by means of perritichous flagella. Catalase, lysine and ornithine decarboxylase, but not exidase were produced. Nitrites were reduced to nitrates, methyl red test were positive but vogesproskaurer reaction was negative [22].

Flexibacter

Flexibacter infections of fish was revealed by the pioneering work of a researcher.[40] who first described a disease (columnaris) in fish from Mississippi River. This bacteria is associated with a number of fish diseases from fresh and marine environment. Flexibacter infection have been characterized by eroded mouth and tail rot of red sea bream and black sea bream in Japan [41].

The organism could possible be considered as cytophaga or flexibacter and are isolated using cytophaga agar medium at 20° and 25°C [42] . These organisms are strictly aerobic and rods, producing yellow-orange colonies, decomposing cellulose or chitin or utilizing carbohydrates.

B. Gram Positive Organism :

There have been relatively few grampositive bacterial organisms of disease importance, Gram-positive rods are subdivided into two groups on the basis of their endospore production and are further differentiated by their oxygen requirements. Howerevr, the organisms of for importance are described here.

Streptococcus spp.

An early study reported that streptococcus faecalis caused mortalities in juvenile rainblow [43]. Since then, this organisms has drawn special interest in Japan due to its spreading to marine cultural species like yelow tail. In addition, large scale epizootes have been reported in tilapia, and eels [44]. Steptococcicosis, 'pop eye' has also been reported in rainbow, trout and eel in South Africa [45,46]. It is unclear, however, that all the outbreaks of streptococcicosis were cause by a single or multiple bacterial species. There are many sharp characteristics, namely the presence of non-motile and fermentative cocci, which do not produces catalase, H_2S, indole, lysine or ornithine decarbosylases or oxidase and do not degrade gelation or reduce nitrates[47].

A variety of media has been used for isolation, including bovine blood tryptone agar, brain,heart infusion agar

and nutrient agar supplimented with rabbit blood with incubation at 37°C for 48 hr [48, 49]. Since incubation temperature was highr than the normal growth temperature of fish, it is presumed that the streptococci may have orginated from warm-blood animals and would suggest that they may be of public health importance.

Staphylococcus epidermidis

There is a report from Japan regarding outbreaks of disease in cultured red sea bream (chrysaph and phrys major) and yellow tail (seriola quinqueradiata) in the year 1976 and 1977 due to staphylococcus epidermidis [50]. The symptoms were exophthalmia and swellings on the caudal peduncle. The media used for the isolates was brain heart infusion agar (BHIA). This nonselective media would support the growth of genus at temperatures 25-35°C for 24-48 hr. Selective isolation of these organisms could be effective by fuse of manitol salt medium [51].

Clostridium botulinum

The first report of fish disease caused by the anaerobic spore forming bacteria was found from Denmark [52]. As symptoms of the disease the fish swam sluggishly and erractically, with the mouth directed upwards until death caused [53]. The majority of outbreak have been caused by type E fstrains [52, 53]. This disease was subsequently reported in Great Britain and in USA [54] in Salmonid fish. Diagnosis could be done by demonstration of circulating toxin in the blood of moribund fish and by culture of the organism. It is possible to isolates C botulinum uithin 6 days at 37°C from moribund rainbowtrout using Robertson's meat broth [53].

Renibacterum salmoninarum

This orgainism was first reported as a cuase of fish disease during 1930 in Scotland [55]. It was later reported in the United States during 1933 [56]. Although they were well documented as the cause of bacterial kidney disease (BKD), the disease in more prevalent in cultured rather than in wild fish. And the symptoms are physically oticeable exophthalmia, eye lesions, as well as ulcers, abcesses or blood filed blisters and internal lesions of the liver, kidney, spleen and heart [57]. These Renibacteria are identified being acrobic, catalase positive but oxidase negative, asporogenons, non-motile, slow-wrowing, fastidious, and cysteine requiring rods [58]. Isolation and cultivation of Renibacterium Salmoninarum has been difficult conventional media are not suitable for the isolation of the organism. The method was successfully improved [59,4]. L-cysteine recognized as an essential ingredient. More recently, and effective selective medium (SKIM) was devised which has proved useful for the isolation of Renibacterium from environmental samples [60]. Staining technique [61] and serological techniques [62,63,64] have been used in diagnosis.

BKD is serious problem for increasing mortality levels in the fish stock [65]. And the control measures are wholly inadequate [43]. vaccines are ineffective[66] and the intracellular nature of the pathogen [67] makes the applicatin of chemotherapy as a difficult one [68]. The presence of this gram positive bacilli in kidney smear is considered positive diagnostic to R Salmoninorum infection. This need, whenever possible, to be confirmed by isolation on a propriate enriched media. Incubation for at least a week, sometime considerable longer time, is required before visible growth of white to creamy yellow colonies.

Lactobacillus

The first reported mortality associated with Lactobacillus spp. was in rainbow trotrout in a California hatchery [69,70]. Cone documented a similar condition in New Foundland. The infected fish developed distended abdomens, full of ascitic fluid in which short gram-positive bacili were observed. The term pseudokidney diseases was adopted to contrast the condition with bacterial kidney diseases [69] and the isolates of this organisms finally named as Lactobacillus pisicicola.

There are very little work in the area of selective media development for Lactobacillus. Most investigators utilize either tryptone soya agar (TSA, Oxid) or brain-heart infusion on agar (BHIA) for recovery at 37°C during two to three days. By comparing the phenotypic characters of these organisms and the diagonostic scheme [22], identificatin could be interpreted as chains of cocci rather than short rods.

Summary

Fish an excellent source of nutrition are often infected by various pathogenic bacteria, viruses, fungus and parasites. Most of the causative agents associated with fish diseases are bacterial pathogens. This paper have elaborated the major disease descriptions like red spot, haemorrhage, red mouth and exopthhalmia and the developments of bcterial identification procedures that are rapid and utilizable for quick sampling techniques. Further this paper have provided information on different and major gram negative bacterial pathogens e. g. Pseudomonas, Vibrio. Aeromonas Cytophaga. Yarsinia Flavobactcrium Proteus Edwardsiella and Flaxibacter. Gram positive bacterial pathogen are Streptococcus. Lactobacillus Pisicicola etc. and the different type of media e. g. BHBA (Bovine Horse blood Agar), TSA (Tryptone soya Agar) BHIA (Brain Heart fInfusion Agar), NA (Nutrient Agar) etc, are used in this identification procedure. Many of these methods may be used in studying accurate determination of fish population with minimal time. effort and equipments and thus will be useful in preventing nutritional losses due to spoilage factors of fish and fishery products.

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