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Antibiotic Resistant Bacteria Isolated from Fish Died on Exposure to Chromium

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Abstract: Exposure of fish to toxic heavy metals causes immunosuppression leading to microbial infections, morbidity and mortality. In this study, all the Common Carp fish (*Cyprinus carpio*) exposed to Chromium at 5 mg L^{-1} died suddenly after 30 days of exposure while 75% mortality was observed in the group exposed to 3 mg L^{-1} Chromium. Hemorrhages, generalized congestion and protrusion and redness of eyes were observed in the dead fish. Staphylococcus and Streptococcus organisms were isolated from the gut, gills, heart and liver of the dead fish. The Staphylococci were $Gram^{+ve}$, non-motile cocci, catalase and urease positive, negative for oxidase and showed hemolytic growth on blood agar but no growth on McConkey's Lactose Agar (MLA). The Streptococci were $Gram^{+ve}$, non-motile cocci, catalase and oxidase negative and showed hemolytic growth on blood agar but no growth on MLA. The Staphylococci were found to be resistant to Oxytetracycline, Streptomycin, Ampicillin and Erythromycin but were sensitive to Ciprofloxacin, Gentamycin, Cephotoxime, Neomycin, Chloramphenicol and Enrofloxacin. The Streptococci were found to be resistant to Chloramphenicol, Erythromycin, Enrofloxacin, Streptomycin, Oxytetracyclin, Neomycin and Cephotoxime but were sensitive to Ciprofloxacin, Ampicillin and Gentamycin. The findings have serious implication for public health as well as for fisheries and aquaculture in the region.

Key words: Antibiotic resistance, Streptococci, Staphylococci, fish, chromium, carp

Introduction

Pollution of water with heavy metals affects the fish immune system and leads to a higher incidence of infectious diseases. Even exposure to very low (sublethal) concentrations of certain heavy metals can have profound effects upon the immune system. Chromium is often found in the effluents of many industries, especially chrome-tanning industries which are a major source of pollution of surface and groundwater (Kudesia, 1985). The effects of Chromium on the fish immune system have been reported earlier (Strik *et al*., 1975; Sugatt, 1978). Depression of humoral immunity may lead to increased susceptibility of fish to infections. The present study was undertaken to explore the effects of immunosuppression of Common Carp fish by exposure to Chromium.

Materials and Methods

Exposure to Chromium

Common Carp fish (*Cyprinus carpio*) maintained in aquaria in two groups of 10 fish each were exposed to Chromium at 5 and 3 mg L^{-1} concentrations, respectively.

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Bacterial Analysis

Organs were collected aseptically from the dead fish for bacteriological analysis. Identification of bacteria was done as described by Cowan and Steel (1975).

Antibiotic Sensitivity of Bacteria

The sensitivity of bacteria to antibiotics was tested against 10 common antibiotics by disc diffusion method (Bauer *et al*., 1966) using available antibiotic discs (Himedia Labs, Mumbai). Agar plates were swabbed with the bacterial cultures using sterile cotton swabs. The plates were allowed to dry for a few minutes and the antibiotic discs were placed using sterile forceps. The seeded plates with antibiotic discs were incubated for 24 h.

Results

After 30 days of exposure, all the fish in the group exposed to 5 mg L^{-1} died suddenly while 75% mortality was observed in the group exposed to 3 mg L^{-1}. The postmortem changes of the dead fish, hemorrhages, generalized congestion and protrusion and redness of eyes, were suggestive of toxicity and infection. The organs of the dead fish were subjected to bacteriological investigation.

Staphylococcus and Streptococcus organisms were isolated from the gut, gills, heart and liver of the dead fish (Table 1). The Staphylococci were Gram +ve, non-motile cocci, catalase and urease positive, oxidase negative and showed hemolytic growth on blood agar but no growth on McConkey's Lactose Agar (MLA). *Staphylococcus cohnii urealyticum* is known to exhibit such characteristics but we have not found any report of its occurrence in fish.

The Streptococci were Gram +ve, non-motile cocci, catalase and oxidase negative, with hemolytic growth on blood agar but no growth on MLA. *Streptococcus iniae*, *Streptococcus milleri* and *Streptococcus phocae* are the pathogens of aquatic animals known to exhibit these characteristics.

The bacteria isolated from the fish were found to be resistant to several common antibiotics (Table 2). The Staphylococci were found to be resistant to Oxytetracycline, Streptomycin, Ampicillin and Erythromycin but were sensitive to Ciprofloxacin, Gentamycin, Cephotoxime, Neomycin, Chloramphenicol and Enrofloxacin. The Streptococci were found to be resistant to Chloramphenicol, Erythromycin, Enrofloxacin, Streptomycin, Oxytetracyclin, Neomycin and Cephotoxime but were sensitive to Ciprofloxacin, Ampicillin and Gentamycin.

Table 1: Characteristics of bacteria isolated from the dead fish

	Characteristics							
Bacteria	Gram's stain	Shape	Motility	Oxidase	Catalase	Urease	Growth on blood agar	Growth on MLA
Staphylococcus	+	cocci	-	-	+	+	+	-
Streptococcus	+	cocci	-	-	-	-	+	-

Table 2: Antibiotic sensitivity of bacteria isolated from the dead fish

	Antibiotics	
Bacteria	Susceptible to	Resistant to
Staphylococcus	Ciprofloxacin, Gentamycin, Cephotoxime, Neomycin, Chloramphenicol and Enrofloxacin	Oxytetracycline, Streptomycin, Ampicillin and Erythromycin
Streptococcus	Ciprofloxacin, Ampicillin and Gentamycin.	Chloramphenicol, Erythromycin, Enrofloxacin, Streptomycin, Oxytetracyclin, Neomycin and Cephotoxime

Discussion

In a healthy host, the organisms considered to be opportunistic pathogens may be a part of the normal flora. In a stressed animal, these same bacteria may overcome host defence mechanisms and cause morbidity or infection in the animal.

In the present study, fish immunosuppressed by exposure to Chromium-polluted water were found to be infected with Streptococci and Staphylococci which led to the mortality of the exposed fish. Chronic exposure of Trout and Carp to Chromium has been reported to suppress the primary humoral response (O'Neill, 1981).

Staphylococci occur both as commensals on the skin and mucous membranes and as environmental contaminants. Many infections are opportunistic and may be associated with immunosuppression (Quinn *et al*., 2002). These organisms have been found to be pathogenic in several species of fish, sea mammals and other aquatic organisms. *Staphylococcus aureus* has been reported earlier to cause eye disease with degeneration of eye tissue and red cornea becoming opaque in Silver Carp *Hypophthalmichthys molitrix valenciennes* (Gunn and Colwell, 1983; Shah and Tyagi, 1986).

Staphylococcus epidermidis causes ulceration and hemorrhages on the fins in Red Sea Bream and Yellow Tail. *Staphylococcus warneri* causes ulcerated lesions on fins, exophthalmia, ascietic fluid and death in Rainbow Trout. *Staphylococcus warneri* is an opportunistic infection in Rainbow Trout (Buller, 2004). The organisms have been found in kidney and liver. It is also associated with septicemia, endocarditis, conjunctivitis and urinary tract and wound infections in humans (Gill *et al*., 2000). *Staphylococcus dolphini* has been found to cause purulent skin lesions in the common dolphin. *Staphylococcus pyogenes* has been isolated from fish products. It is probable that such infection is derived from fish handlers who are carriers of the organism (Buxton and Fraser, 1977).

Streptococci usually live as commensals on the mucosae of the respiratory tract and urogenital tract. The enteric Streptococci found in the intestinal tract are opportunistic pathogens (Quinn *et al*., 2002). Streptococci have been found to be pathogenic leading to mortality in many species of fish, sea mammals and other aquatic animals like Plecoglossus, Sea Bass, Striped Bass, Epinephelus, Sea Bream etc. Streptococci cause exophthalmia, hemorrhages and mortality in Bull Minnows (Buller, 2004). *Streptococcus milleri* causes ulcers on flank and tail in Koi Carp (Austin and Austin, 1999). *Streptococcus phocae* has been found to cause pneumonia in common seal.

Streptococcus iniae causes meningoencephalitis and deaths in fish, systemic infections in bull frogs and subcutaneous abscesses in freshwater dolphin. These organisms have been isolated from the heart and spleen in European Sea Bass and were found to cause splenomegaly, exudative meningitis and panophthalmitis in Sea Bream. It causes mortality in the ornamental fish Japanese Flounder. These bacteria are pathogenic in humans. The infection spreads through wounds following handling the fish causing cellulitis and ulcers (Morris *et al*., 1982).

Streptococcus iniae and *Streptococcus agalactiae* have also been reported to be a part of the normal flora in Common Carp (Eldar *et al*., 1995). In another study, these two organisms were found to cause disease and mortality in Tilapia (St. Peter's fish). Both the bacteria have been found in brain and eye of Rainbow Trout causing meningoencephalitis and septicemia. *Streptococcus agalactiae* causes exophthalmia, opacity of eyes and hemorrhages on body, particularly around the mouth, snout, operculum and fins in Sea Bream. It has been reported to be epizootic with 100% mortality (Toranzo *et al*, 1991). *Streptococcus agalactiae* causes epizootics in wild mullet. It causes exophthalmia, hemorrhage and mortality in Blue fish, Sea Bream, Striped Bass, Sea Trout, Bull Minnows and aquarium fish. This organism also causes infections in many other animal species, mastitis in cattle and neonatal meningitis in humans.

Streptococcus dysgalactiae causes septicemia, bronchopneumonia, myocarditis and pyelonephritis in porpoise. *Streptococcus parauberis* causes hemorrhages in anal and pectoral fins and eyes in cultured Turbot (Buller, 2004).

In our study, the bacteria isolated from the fish were found to be resistant to a number of common antibiotics like Oxytetracycline, Streptomycin and Erythromycin. This finding raises the question regarding the source of infection of fish with antibiotic resistant bacteria. Pollution of water with human waste and sewage seems to be a possible source of these organisms in fish. Staphylococci frequently yield drug-resistant mutants. Fish infected with human bacterial pathogens may constitute a potential hazard to human health. Fish may act as carriers for enteric pathogens which are a risk to public health, especially when eaten raw or semi-cooked. The bacteria isolated in our study were sensitive to a few antibiotics like Gentamycin and Ciprofloxacin. However, more studies are needed before recommending the use or discontinuation of use of these antibiotics.

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