

IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL PATHOGENS IN RAINBOW TROUT AT JUGLOTE, GILGIT, BALTISTAN.

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Abstract: The aim of study was to diagnose and identify the bacterial pathogens in rainbow trout (*Oncorhynchus mykiss*) at Trout Research and Multiplication Centre (TRMC), Juglote and to determine effective antibiotic treatment. Diseased fish samples were collected from TRMC Juglote and preserved in ice boxes which further were transferred through by air to Disease Diagnostic Laboratory, Aquaculture and Fisheries Program, NARC, Islamabad where the bacteria had been two isolates I and II identified as *Staphylococcus sp.* These isolated from trout fish belly. The isolates were found sensitive to oxacillin, cefoxitin, tobramycin, amikacin, ciprofloxacin, chloramphenicol, azithromycin, penicillin, cefazolin, and tetracycline. The ciprofloxacin was applied for the treatment of the infections. Recovery (90%) at initial stages indicated the suitability of the antibiotic for infection.

Key words: *Staphylococcus*, rainbow trout, *Oncorhynchus mykiss*.

INTRODUCTION

Staphylococci are gram-positive cocci (about 0.5 to 1.5µm in diameter) occurring singly, in pairs, in tetrads, short chains of three or four cells, or most often in irregular "grape-like" clusters. Their name is derived from their latter frequent formation stemming from the Greek "Staphylococci" meaning grape (Alves *et al.*, 2000). They are non motile, nonperforming and usually catalyse positive and unencapsulated. Although most species are facultative anaerobes, growth is more rapid and abundant under aerobic conditions. Most strains grow well on common no inhibitory media. The cell wall contains peptidoglycan and teichoic acid. The amino acid present in the peptidoglycan is L-lysine. Staphylococci are susceptible to lysis by the endopeptidase lysostaphin. They are generally resistant to

lysis by the muramidase lysozyme (Bercovier and Ghittino, 1997). Staphylococci are currently included together with micrococci and planococci in the family Micrococcaceae as a matter of convenience, since these genera are not closely related phylogenetically and should not be combined. All three genera represented in the family Micrococcaceae are catalase-positive and gram-positive cocci with cell wall peptidoglycan containing L lysine as the diamino acid. Micrococci are generally resistant to lysostaphin. Among others, staphylococci and micrococci may be differentiated on the basis of anaerobic acid production from glucose, which is positive for most *Staphylococcus* species and negative for most *Micrococcus* species. Colonies of most staphylococci, which have been isolated from fish on non-selective media, such as tryptic soy agar (TSA), are circular, smooth and raised with a butyrous consistency and a bright white, sometimes glistening, or pale white colour. Their diameter may be 0, 2-5mm subsequent to 36-48 hours of incubation at room temperature of around 25°C. Such colonies may become sticky with age. Micrococci produce on TSA yellow colonies of similar shape (Dalsgaard, 2001).

Staphylococcus species are not possible to be distinguished on the basis of colony morphology. Colonies conforming to the description above should be Gram stained, sub-cultured and tested for genus and species biochemical properties (*e.g.*, by means of the API STAPH commercial identification system). In human and veterinary medicine a few additional key characteristics must be examined for the identification of the most clinically significant species. Most *Staphylococcus* species isolated from fish are coagulase negative (Taylor *et al.*, 1975).

The aim of present study was to identify the infectious pathogens and evaluate the potential of antibiotics against these infectious agents.

MATERIALS AND METHODS

Infected fish samples were collected from Trout Research and Multiplication Centre (TRMC), Gilgit. These samples were preserved in ice boxes which further were transferred through by air to "Disease Diagnostic Laboratory", Aquaculture and Fisheries Program, NARC Islamabad during the year 2009-10. The following methodology was adopted for diagnosis of fish disease occurrence.

Samples from inside trout belly were collected. These were cultured on the selected media. The cultured plates were incubated at 37°C. Various tests were performed for identification of bacteria.

Antibiotic susceptibility

An antibiotic test was performed using the disk diffusion method on Mueller-Hinton agar (Oxoid), a minimum of three colonies from the Columbia blood agar medium, incubated at 25°C for 24 hours were suspended in 2ml of sterile saline to a density approximately equal to the McFarland Opacity standard No.5. Dry sterile cotton wool was placed in the suspension and excess liquid was expressed into the tube. The bacterial suspension was inoculated onto Mueller-Hinton agar with the swab in such a way that the whole surface of the agar was covered.

Disks containing the following antibiotics for use oxacillin, cefoxitin, tobramycin, amikacin, ciprofloxacin, chloramphenicol, azithromycin, penicillin, cefazolin, and tetracycline. These disks were dispensed on the surface of the medium and incubated aerobically at 25°C for 24 hours. The results were recorded as resistant or susceptible by measurement of the inhibition of the zone diameter according to the interpretive standards.

RESULTS AND DISCUSSION

Gram positive bacterial colonies were found Gram-stained smears from belly. The bacteria were identified as, *Staphylococcus sp.* On the basis of results of antibiotic susceptibility test (Table I). Ciprofloxacin was chosen and applied for the treatment of the infections at the rate of 2–3% /body weight administrated with food. Recovery was almost 90% at initial stages of these infections.

The *Staphylococcus spp.* are the most common infectious diseases with significant economic and sanitary repercussions for trout farms in Mediterranean countries during the summer months. The staphylococcal infections which have been diagnosed so far on cultured marine fish in Greece, comprise infectious systemic diseases characterised by septicaemia. Similar symptoms and lesions prevail on all fish species infected. Despite the increasing daily mortality and the number of dark coloured, lethargic, moribund fish. Swimming erratically close to the

surface and the corners of the net cage, the infected population as a whole does not seem to lose its appetite and behaves rather normally. Apart from the dark colouration and ataxia, the sick fish show rather large external skin lesions covered by whitish mucus.

Table I: Sensitivity profile of different antibodies

Antibodies	Type I	Type II
Oxacillin	S	R
Cefoxitin	S	R
Tobramycin	S	R
Amikacin	S	S
Ciprofloxacin	S	S
Chloramphenical	S	R
Azithromycin	R	R
Penicillin	R	R
Cefazolin	S	R
Tetracycline	R	S

R- Resistant, S – Sensitive

By removing the mucus surface necrosis in the form of shallow skin ulcers are uncovered (Shraer and Ratgauz, 1975). Gallbladder is distended and full of light yellow or dark green bile, especially in sparrids (breams). The gills are usually anaemic with areas of necrosis. The liver is usually pale and may show inflamed, darkened areas. Congestion and haemorrhage of the brain is evident in small fry, mainly of sea bass. Apparently, the staphylococci proliferate in the intestinal tract and cause enteritis with diarrhoea, which may spread the cocci in the water flushing and infecting other fish. Distension of the gall bladder may be a result of cholecystitis and accumulation of haemolysis products (Taylor *et al.*, 1975). The staphylococci have been isolated almost in pure culture by plating material taken from the anterior kidney or the brain of the fish or from external necrotic lesions usually onto TSA agar, Blood agar and Chapman agar. Small about 1mm, white, round and regular colonies appear subsequent to incubation for 18 to 20 hours at 30-37°C. Most of the species of staphylococci that have been isolated from fish are coagulase negative, namely, *Staphylococcus epidermidis*, *S. xylosus*, *S. lentus*, *S. capitis*, *S. lugdunensis*, *S. hominis*, *S. warneri*, *S. cohnii*, *S. chromogenes*. Only *S. aureus* has been found to be coagulase positive.

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