Chapter 7: Summary..

7. Summary

Fish health is dependent of environmental factors as well as the maintenance skill of farmers. Consequently, feeding intensity, water standard and production are most important factors for determining the actual health condition of fish. In modern aquaculture system, fish farmers are intensifying their aquaculture practices. They are stocking fishes in high quantity, irrespective of the species. They are also providing excess quantity of artificial feed. However, the unutilized feeds will deteriorate the water quality leading to different kind of stress in fishes. Fishes under stressed condition are easily prone to wide range of pathogens. However, in aquatic animals, bacterial diseases are mostly common and dominant. In fresh water fishes, infection due to *Aeromonas* species or by *Edwardisella* species was commonly reported. However, earlier there were no reports of *Klebsiella pneumoniae* infections in fresh water fishes. Presently, fish samples exhibiting clinical signs of hemmorhages and lesions were collected from three potential fish producing districts of West Bengal.

Primarily, the bacteria were isolated from the blood and different internal organs of the diseased moribound fishes. *Klebsiella pneumoniae* specific media was used for the preliminary identification of the pathogen. Further, the isolates were confirmed as *K. pneumoniae* through biochemical and molecular identification. Phylogenetic analysis of the isolated strains revealed the species diversity. The phylogenetic analysis revealed, transfer of strains from one location to other despite of different area. From the antibiotic disc assay it was found that all the isolates were resistant against various antibiotics and from the MAR value it can also be hypothesized that in those aquaculture farms antibiotics are commonly used. The

present study had helped to determine the concentration of *K. pneumoniae* required for the 50% mortality of the fish population. Furthermore, after challenging the fishes with the target pathogen ultra structural changes were observed histopathologically in the internal tissues.

Various genes are responsible for the development of any kind of infection. They work in a sequential manner for development of pathogenesis of the organism. Presently all the strains were found to be positive for different virulent genes. This is the first report of virulent genes in *K. pneumoniae* causing infection in fishes. In the present study, protein structures were also designed for each of the amplified virulent genes and the structures were validated using various bioinformatics tools. Different drug pockets were found for each protein. In future, these data will help in development of drugs or therapeutic agents against *K. pneumoniae.*

In the present research objective, different immune related parameters and various immune genes of the challenged fishes were studied at various time interval to study the pattern of immune system modulation of the host against this particular pathogen. After injecting the isolate, elevated superoxide production, lysozyme and myloperioxidase activity were observed. Whereas the antiprotease activities were found to be downregulated.C3, IL-1β and Il- 6 gene expression were found to be gradually induced from 12 hours to 24 hours in the *L. rohita* liver following bacterial challenge; and there expression had decreased with the progression of the incubation period. In kidney tissue C3, IL-1β and Il- 6 expression were lowered at the early stages of infection and it had upregulated with the progression of infection. In the

muscle tissue C3 and IL-1β expressions were found to be unaltered in all the treated groups whereas IL-6 was not showing a significant pattern.

From the present research study it could be concluded that *Klebsiella pneumoniae* is b an emerging pathogen for developing and sustainable aquaculture. A stringent surveillance is required to identify the abundance of *Klebsiella pneumoniae* in aquaculture farms. Though the *K. penumoniae* is known to be a human pathogen, it is the first report on causing diseases in variety of farmed fishes which have been also confirmed through challenge studies. The findings would help in management of diseases in aquaculture farms.