CHAPTER VI

SUMMARY AND CONCLUSION
Environmental microbiological research programmes are increasingly gaining importance due to their significant contributions to the global phenomenon of ecoprotection and ecorestoration. The microbial flora of aquatic and terrestrial environment are in constant interaction with human and other animal microflora and are in constant circulation among the fauna through environmental sources. Environmentalists are becoming more and more aware of the importance of ecoprotection not only at its macrolevel, but also at the microbial levels as they are directly linked with the well being of humanity.

The objective of the present project of investigation "Studies on the Intestinal Bacterial Flora of Some Common Bird Species of Kerala" was to analyse the extent of influence of Gram negative intestinal bacterial flora of avian fauna on the environment. A comparative study has been conducted on human and avian bacterial isolates for a better critical analysis.

Eight different aspects of the problem were examined. The first part of the investigation revealed that the Gram negative intestinal bacterial flora of avian fauna consisted of a variety of bacterial genera namely, *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Aeromonas*, *Proteus*, *Acinetobacter*, *Arizona*, *Providencia* and *Alcaligenes*. The *E. coli* and *Klebsiella* sp. were the most predominant group among coliforms and have shown very high percentage of prevalence (*E. coli* 88% and *Klebsiella* sp. 48%) in avian droppings.
The bird species-wise analysis made, showed higher percentage prevalence of *Pseudomonas* sp. in Teals and Cormorants; *Proteus* sp. in Egrets. and a relatively higher incidence of Gram positive cocci in Cormorants in addition to the higher prevalence of *E. coli* and *Klebsiella* sp. Pigeons and Teals revealed poor diversity of bacterial genera. The variation in the bacterial species among the various avian species could be attributed to their feeding habits, and the extent of their association with man and his habitations. All these birds were found to harbour a very high percentage of coliforms mainly *E. coli* and *Klebsiella* sp., which are constantly voided along with droppings. Since these birds maintain very high population density in the area under investigation, the part played by them in contaminating the aquatic environment directly, as by Ducks, Cormorants, Teals, Egrets, or indirectly, as by Crow, Pigeon and Fowl, by faecal coliform bacteria, cannot be ignored.

The outbreaks of waterborne diseases were often associated with human faecal contamination of water, while the higher coliform content in water samples by extra human faecal contamination showed no such association. The next part of the investigation was concentrated on the comparative study and characterisation of human faecal and avian faecal coliforms and other Gram negative bacterial genera. Biochemical characterisation did not show any significant difference between isolates from human and avian sources. Antibiograms and drug resistance patterns when examined revealed striking differences in human and avian isolates. The incidence of strains showing multiple drug resistance (to five or more drugs) was significantly high among human strains. Isolates from avian sources on the other hand were more sensitive to drugs and exhibited multiple drug resistance to relatively low degrees, showing resistance to three or less than three drugs. The avians of nature are not in access with drugs directly, but the interaction with human surroundings and the exchange of microflora from avian to human and back to avian may furnish
a rational explanation for such a genetic set up in avian bacterial strains also. The drug resistance can be a plasmid mediated genetic character that can be transferred from strain to strain irrespective of the source.

The haemolysis and haemagglutination properties showed higher percentage of agglutinability of strains from avian intestine to fowl blood cells than human and rabbit cells possibly due to their adaptability to avian intestine. Bacterial isolates other than coliforms from birds showed lesser avidity in agglutinating fowl RBCs showing their poor adaptability to the intestinal tract of birds and poor adhesive property to bird tissues.

Higher percentage of haemagglutinating strains among human isolates possibly explain their adaptation to parasitic existence and their pathogenic potential in man and animals. Only a slight variation has been noticed in the haemolytic activity among human and avian faecal isolates. However *Pseudomonas pyocyanea* strains from clinical specimens showed high haemolytic and haemagglutination activity, which is often associated with their pathogenicity. The low haemagglutination and haemolytic activity among *Pseudomonas* isolates from avian faecal samples can be related with their low pathogenic potential.

Biological characterisation of isolates from the two different sources thus revealed that avian strains are indistinguishable from human strains by routine identification methods. Bird species in intimacy with the human habitation can easily acquire organisms of human origin which possibly can be disseminated to the environment; hence a definite demarcation as ‘human strains’ and ‘avian strains’ cannot be made. Majority of the isolates from avian sources exhibit relatively low pathogenic potential and drug resistance. High degree of multiple drug resistance is believed to be a character associated with bacteria from human sources. Occurrence
of such bacteria among the isolates from bird faecal samples provides evidence in support of the suggestion that the birds acquire microbial flora of man and animals and disseminate them to the environment, the intensity and frequency of which is related to the intimacy of the bird species to human habitations. The role of several bird species in the dissemination of microorganisms from human sources to various parts of the ecosystem, including the water bodies providing drinking water and those used as recreational waters thus cannot be ignored as insignificant.

Bacteriocin production profile of avian and human isolates has been studied to understand the influence of these strains on the microbial flora of the environment. Broad spectrum bacteriocinogenicity has been exhibited by both avian and human strains, the incidence being higher among human strains. The resistance to antibiotics and other antimicrobials and the broad spectrum bacteriocinogenicity are the two important characters contributing to the ability of the strains in competitive survival. Increased proliferation of such bacterial species in the environment can lead to substantial alteration in the microbial flora of the ecosystems. Alterations in the composition of the innate microflora can lead to selection of certain microbial species in the long run which can adversely influence the involvement of the soil microflora in several natural processes viz., biological nitrogen fixation, biodegradation, bioconversions, etc.

Microbial environmental study will not be complete, if the terrestrial habitats are ignored. Modern agricultural methods and the widespread use of fertilizers and pesticides pollute the environment and upset the ecological balance. As revealed by the earlier investigations, the present study also supports the fact that the crop protection chemicals, selectively inhibit the growth of Gram positive useful bacteria of soil and aquatic environment, which may be called as susceptible species to
pesticides. This phenomenon possibly is one of the causes of enormous increase in the count of Gram negative bacteria including the potential pathogens which can lead to massive alterations in the microbial, chemical and biochemical constitution of the ecosystems. Such changes in the water bodies can lead to slow or rapid destruction of various forms of aquatic lives. Epizootics among the fishes, to the belief of several scientists, is one such hazard.

The prevalence of coliform bacteria in various fresh water habitats has been investigated, taking seasonal variations into consideration. Well water samples (untreated) used for human consumption showed comparatively very low coliform count (165 CFU/ml) but still a much higher count than the permissible limits. The paddy fields and ponds showed much higher coliform counts. The river water samples from the Meenachil river and its tributaries showed still higher counts, and the small streams of the river in close proximity to agricultural lands, poultry farms, duck farms, Kumarakom Tourist Complex, and the human dwelling areas showed very high coliform counts, i.e. above 5000 CFU/ml of coliforms. Irrespective of the seasons, and sources of water bodies high coliform counts were noted in all aquatic environments, except that of wells. Not only human beings but also other animals including the avian fauna do play a major role in contaminating the water bodies with coliform bacteria. Contrary to the belief of the epidemiologists, high coliform content of the water samples from wells in the area is not associated with epidemics of waterborne diseases. Presumptive coliform count and Eijkman test are among the standard tests for the quality control of potable water. The present study showed that the above mentioned tests provide dependable results for treated water samples only, and fail to provide dependable information regarding the suitability of water for consumption as far as non-treated water is concerned. Faecal coliform (E. coli) from both human and extrahuman (avian) sources showed positive in
Eijkman tests. Dependability of Eijkman test as a procedure for the detection of
*E. coli* due to human faecal pollution appears to be questionable. Faecal indicators
like *Streptococcus faecalis* are not frequently isolated from avian droppings. Tests
for the detection of such indicators can possibly be more dependable substitutes for
coliform tests.

The last section of the investigation helped to reveal the survival ability of
coliforms in various aquatic environments. The various physical parameters like
pH, salinity and organic content of the various water samples have been taken into
consideration. Longer period of survival ability of coliforms and other Gram
negative bacteria of human and avian sources in various aquatic ecosystems
irrespective of varied physical parameters, like pH, salinity and organic content has
been proved to be true in the present study. This shows the higher adaptive ability
of these microbes to the varied environments, which are important in natural
selection and evolution of the species.

Survival ability in water of the coliforms and enteric pathogens account for
their role in waterborne diseases. The longevity or survival of these organisms for
weeks and months in aquatic environments, makes the picture much complicated as
their presence in water may not indicate recent pollution and therefore suggests the
need for a constant surveillance on the quality control of water resources and
reservoirs.

In short, the avian human interaction in the ecosystem leads to the interaction
in the microbial levels which has several implications on various aspects of
environment.