

Chapter 6

Summary and Conclusion

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6.1. Summary

- Aeromonads have been detected, isolated and characterized from varied sources such as brackish, fresh, estuarine, marine waters, chlorinated and un-chlorinated water supplies, heavily polluted waters, cold and warm-blooded animals worldwide and humans alike. *Aeromonas* infections in fishes cause a great economic loss every year all over the world. It also causes many gastrointestinal and extraintestinal infections in humans. Therefore, it caught my interest to investigate the presence of new species of this genus or species with novel disease potential(s). A meticulous survey was undertaken to find new *Aeromonas* species from the aquatic bodies (rivers and lakes), domestic sewage, hospital sewage and municipality drinking water supplies of the Himalayan foothill region. One hundred and twenty (120) water samples collected from 45 rivers, 6 hospital and 3 town-sewage sites were screened to find twenty-one (21) *Aeromonas* natural isolates. *A. hydrophila* and *A. caviae* were found from the rivers, hospital and domestic sewage of this region which have been reported earlier from this region by other researchers. Two species of *Aeromonas*, *A. sobria* and *A. popoffii* were revealed by this study which, to the best of our knowledge, was not reported earlier from the water bodies of this region.
- Aeromonads are phenotypically and genotypically heterogenous bacterial group. In contrast to traditional morphological and biochemical differentiation, identification of aeromonads from clinical and environmental sources are presently based on ribotyping and analysis of *gyrB* (gene encoding the B-subunit of DNA gyrase) (Yanez *et al.*, 2003), sequencing of housekeeping genes like 16S rRNA (Hossain, 2008), *gyrB* (Yi *et al.*, 2013; Chen *et al.*, 2019), *rpoD* (Yi *et al.*, 2013) *gyrA*, *atpD*, *recD*, *dnaJ* and *dnaX* (Martinez-Murcia *et al.*, 2011) in order to differentiate natural bacterial isolates. In the present study, Gram negative bacillus was chosen among all the bacterial colonies grown from the different dilutions of the collected water samples, for further study. These Gram-negative bacilli were subjected to conventional biochemical characterization recommended for *Aeromonas* spp. The biochemical tests performed were catalase, indole, lipase, glucose fermentation, gelatinase, diastase, citrate utilization, methyl red, Voges

Proskauer and oxidase tests. The bacterial isolates which showed positive result for 40% of the biochemical tests and above were chosen for further analysis.

A PCR-based genotyping approach was undertaken in order to identify endemic *Aeromonas* isolates. PCR-based RFLP of the 16S rRNA gene was done and bacterial isolates were confirmed through sequencing of this gene and comparing the sequences from the data available in the NCBI database. This study has generated information regarding existing diversity of this medically important bacteria group in the sub-Himalayan North Bengal, a region which falls within one of the biodiversity hotspots of the country. The region being catchment area of several rivers flowing down the Himalaya, cold-adapted (usually more virulent) *Aeromonas* strains may be discovered. Therefore, a periodic screening of this area is of utmost importance.

- In the post-World War II period, rapid development and extensive use (or abuse) of a variety of antibiotics have given rise to resistant varieties of bacteria, owing much to the success and speed of bacterial adaptation. Bacteria apply a number of mechanisms to show antibiotic resistance. These resistance genes get accumulated in plasmids and are horizontally spread among other bacteria through them (Bennett, 2008). In order to find solution to this problem many researchers have undertaken plasmid profiling and molecular characterization of aeromonad plasmids (Toranzo *et al.*, 1983; Majumdar *et al.*, 2006; Rhodes *et al.*, 2000).

Therefore, antibiotic resistance evaluation and the detection of probable association of bacterial plasmids in antimicrobial resistance in this agriculturally and clinically important group of bacteria isolated from the Himalayan foothill region were undertaken in the present study. This study has generated information regarding the present status of antibiotic drug-resistance in the clinically important *Aeromonas*: *Aeromonas hydrophila*, *A. caviae*, *A. sobria* and *A. popoffii*, isolated from the freshwater bodies of this region. All the twenty-one (21) *Aeromonas* isolates were found to be resistant to ampicillin, amoxyclav and ampicillin/sulbactam with varying degrees of resistance shown against rest of the twenty (20) antibiotics among all the twenty-nine (29) antibiotics tested. Quite high resistance was obtained against cefazolin, cefuroxime, ceftazidime, clindamycin, cephalothin, erythromycin, nalidixic acid, oxacillin, nitrofurantoin, ticarcillin and vancomycin. Some isolates were also resistant to tetracycline, streptomycin,

norfloxacin, co-trimoxazole, ciprofloxacin, chloramphenicol, cefotaxime, azithromycin and amikacin. The incidence of antibiotic resistance is found to be more intense in the *Aeromonas* isolates obtained from the densely-populated downstream regions of the rivers, than that from sparsely populated upstream areas of the same rivers. This finding indicates escalated uses of antibiotic in densely populated part of the river hinterlands. The data obtained has also been compared with that of the drug-resistance status of *Aeromonas* isolated from this region by previous researchers. It has been found that resistance against some drugs such as ampicillin, chloramphenicol, tetracycline, nalidixic acid and norfloxacin has risen significantly over the years in this bacterial group which may make the treatment of the infections caused by this bacteria group difficult. The information obtained in this study will be helpful in the treatment of the gastrointestinal and extraintestinal diseases caused due to *Aeromonas* infection in humans.

- In order to understand fully the virulence potential of any pathogen, it is imperative to understand pathogenic factors and/or mechanisms that are involved in their virulence. This is crucial since the expression of different virulence genes could contribute to infection depending upon the anatomical niche where the pathogenic organisms colonize and the micro-environment that dictates the differential expression of genes. Till date, numerous virulence factors expressing genes have been located and characterized from the aeromonads, especially from *Aeromonas hydrophila*, the pathogenic bacterium which causes wound infections, septicemia and diarrhea in humans and in animals. The production of enzymes like lipase, gelatinase, diastase, oxidase, catalase and production of acid by the fermentation of glucose have been observed in the *Aeromonas* isolates obtained during the study. The expression of these genes contributes to the virulence potential of the bacterial isolates.
- Transfer of genes horizontally via the plasmids are supposed to be one of the means of adaptation by which aeromonads obtain genes that may be helpful in fighting environmental pressures such as resisting potentially hazardous antimicrobial substances like antibiotics (Bennett, 2008). Nature probably selects the beneficial genes and keeps them persistent as they bestow upon their carriers better adaptability to microbes. Plasmid profiling in pathogenic isolates of *Aeromonas*

hydrophila from fishes with ulcers, had been done to investigate plasmid-mediated virulence potential of the bacterium by other researchers. Plasmid characterization, plasmid-mediated transfer of antibiotic resistance and virulence genes in *Aeromonas* has been explored by several research groups and also in the present study. The current study revealed the presence of 2000 bp to 10000 bp plasmids in this bacterial group which is alarming because small plasmids are easily transferable to other bacteria.

- The transfer of antibiotic-resistance from *Aeromonas* to *E. coli* through natural plasmids by using the process of transformation has also been studied. Novel presumptive virulence factors such as the multidrug resistant NDM-1 gene and mobile genetic elements which transfer these genes are being discovered frequently from this heterogeneous and ubiquitous bacteria group. In the present study, resistance to multiple antibiotics have been seen to be transferred to *E. coli* laboratory strain (JM109) from natural isolates of *Aeromonas* through transformation proving the presence of these antibiotic-resistance genes in the plasmids present in those *Aeromonas* isolates. Resistance to ampicillin, ampicillin/sulbactam, amoxycylav, cefuroxime, cephalothin, clindamycin, oxacilin, ticarcillin and vancomycin are found to be transferred to *E. coli* through transformation. This is quite hazardous as it can spread fast to other bacterial species of the environment through horizontal transfer of resistance, making it more difficult for us to fight any kind of bacterial infections. Further research in this field may be helpful in enhancing knowledge of involvement of plasmid in the transfer of antibiotic resistance and virulence in this bacterial group.

6.2. Conclusion

The development of resistance to multiple antibiotics in many bacterial species in our hospitals is posing a great challenge to medical science and agriculture. *Aeromonas* isolates found from the rivers of this region are found to be resistant to multiple antibiotics in the current research undertaken. The discovery of NDM-1 gene from this agriculturally and clinically important group of bacteria calls for a thorough inspection of anti-microbial resistance and also method of transferability of multi-drug gene in this group of bacteria. Accordingly,

understanding how antibiotic resistance develops and is spread by mobile genetic elements is necessary for designing and developing prevention strategies intended to minimize the threat of bacterial infections. Considering the highly adaptable nature of *Aeromonas* vis-a-vis the environmental pressures, and the rising abuse of antibiotics in combating pathogenesis caused by *Aeromonas*, newer genes responsible for virulence may be acquired by these bacteria. Research undertaken for mapping putative virulence genes present in *Aeromonas* species of this region may be rewarding. This survey of diversity of *Aeromonas* in this part of sub-Himalayan West Bengal and their drug resistance profiles may further help to design proper control measures against these pathogens in future. Considering the rise in antibiotic resistance, in not only these but also other microbes too, it is high time to resort to other means for combating microbial infections instead of using antimicrobials. Antibiotics that were once useful in fighting deadly infectious diseases seem to be no more rewarding and efficacious. Modern health science is trying to find out alternate ways of fighting infectious diseases. Application of predatory bacteria, competitive exclusion of bacteria, bacteriocins and bacteriophage therapy is some of the new rays of hope.