

Chapter 5

Comprehensive Discussion

5. COMPREHENSIVE DISCUSSION

Aeromonads are an important group of bacteria being both human and animal pathogen. There is a huge loss in aquaculture round the world every year due to infection caused by *Aeromonas* spp. Therefore there is a need to study this clinically significant group of bacteria in detail. *Aeromonas* is ubiquitous and autochthonous in aquatic environments. They have been isolated from various types of habitats in both India and abroad. *Aeromonas* is Gram negative bacillus showing variable result in biochemical reactions. Therefore, molecular characterization is necessary to confirm their identity. In the current study after performing a set of 10 biochemical tests, attempt was made to amplify a 953 bp fragment of 16S rRNA gene using *Aeromonas* specific primers (Lee *et al.*, 2002) for screening the bacterial isolates to isolate *Aeromonas*.

For studying the species diversity of the *Aeromonas* in the aquatic environment of the study area RFLP was performed using the restriction endonucleases AluI and MboI. Since the fragments smaller than 100 bp were not clearly distinguished by agarose gel electrophoresis it was difficult to discriminate between the closely related species of *Aeromonas*. Therefore, the 953 bp fragment of the 16S rDNA was sequenced. Four species of *Aeromonas* were found i.e. *A. hydrophila*, *A. caviae*, *A. sobria* and *A. popoffii* among which *A. hydrophila* and *A. caviae* was reported earlier from the aquatic environment of North Bengal (Das *et al.*, 2009). Our study is the first report of *A. sobria* and *A. popoffii* from the aquatic environment of North Bengal. Both *Aeromonas sobria* and *Aeromonas popoffii* are clinically very important as they cause infections in humans. *Aeromonas sobria* can cause necrotising fasciitis and sepsis in immunocompromised patients (Spadaro *et al.*, 2014). Association of *Aeromonas sobria* with human infections is known since long ago in patients who undergo immunosuppressive treatments (Daily *et al.*, 1981). *Aeromonas popoffii* has been reported to cause urinary tract infections in humans (Hua *et al.*, 2004). *Aeromonas popoffii* harbour most of the virulence genes such as lipase, DNase, serine protease and aerolysin/hemolysin known to be present in *Aeromonas* (Soler *et al.*, 2002). This makes them a potent pathogen. Isolation of *A. sobria* and *A. popoffii* from the aquatic environment of North Bengal indicate chances of enhanced gastrointestinal and extraintestinal *Aeromonas* infections in humans of the study area.

Rising antibiotic-resistance among the clinically important bacterial species is a serious problem for the human society. Therefore, regular screening of the antibiotic resistance status of *Aeromonas* in the North Bengal area is of utmost importance. In this study resistance status of the commonly used antibiotics was checked and compared with the data available since the last 26 years in North Bengal. Resistance to some antibiotics had decreased, probably due to the decrease in their usage. Resistance to some antibiotics have been seen to increase dramatically in this study, which can be attributed to their indiscriminate use. Multidrug resistant aeromonads have been isolated from India and abroad. All the twenty-one (21) *Aeromonas* isolates found in our study were resistant to more than one antibiotic which indicates the presence of multidrug resistant *Aeromonas* in the aquatic environment of North Bengal.

Plasmids are mobile genetic elements which can easily get transferred to bacteria of the same as well as different genus. Plasmids of small size are more freely transferable to other bacteria in the environment (Ohse *et al.*, 1995; Bennett *et al.*, 2008). In this study, we have found few plasmids of quite small size which got experimentally transferred to *E. coli* laboratory strain through DNA transformation. Antibiotic resistance genes present in the plasmids can spread antibiotic resistance in the environment which can be even more alarming for the entire human society. In the current study, resistance to a number of antibiotics have been shown to be transferred from *Aeromonas* natural isolates to *E. coli* through plasmids using the procedure of transformation. Spreading of the antibiotic resistance genes among the clinically important bacteria can make it more difficult for the bacterial infections to fight with. We have found a low transferability of few antibiotic-resistance from the *Aeromonas* natural isolates to naïve *E. coli* JM109 via plasmid transformation. This can be attributed to the events of integration of the antibiotic-resistance genes from the plasmids to the bacterial genome (Vries and Wackernagel, 2002; Heap *et al.*, 2012; Stratev and Odeyemi, 2016; Goswami *et al.*, 2020). This can further enhance the problem of spread of the antibiotic resistance genes in the environment through bacterial cell division as genes integrated in the chromosomal DNA are more stable. Antibiotic resistance is a worldwide problem of today. It can only be solved through judicious use of antibiotics and alternate ways of treating infectious diseases.