

Abstract

Aeromonas is a group of Gram-negative bacilli which do not form spores. They are facultative anaerobes found ubiquitously and autochthonously in the aquatic environment. Currently there are 36 described species in the genus *Aeromonas*. Earlier, *Aeromonas* was known to cause diseases in only poikilothermic or ectothermic animals but now have been proved to cause diseases in humans and other homeothermic animals too. Aeromonads are isolated from various sources such as different types of water (from polluted ones to drinking water sources), various types of food (raw milk, chicken, and meats such as lamb, veal, pork, and ground beef), as well as cold- and warm-blooded animals. In the present study, *Aeromonas* was isolated from water samples collected from the rivers of the Himalayan foothill region, and from hospital and domestic sewage. Municipality drinking water samples were also screened for the presence of *Aeromonas* at Siliguri and Bagdogra towns, but no *Aeromonas* was found from the drinking water supplies. In total, one hundred and twenty (120) water samples collected from forty-nine (49) different sites of freshwater bodies including both lotic and lentic systems (45 river sites and 4 stagnant water bodies), drainage system of six (6) hospitals and drainage system of three (3) towns were screened for the presence of *Aeromonas spp.* After Gram stain and biochemical characterization ninety (90) isolates among them were chosen for molecular analysis and twenty-one (21) *Aeromonas* isolates were scored from them. Although they are a phenotypically and genotypically heterogenous group, they are successfully identified by ribotyping and analyzing housekeeping genes like 16S rRNA, *gyrB*, *rpoD*, *gyrA*, *atpD*, *recD*, *dnaJ* and *dnaX*. In the present study, a PCR-based genotyping approach was adopted in order to identify endemic *Aeromonas* isolates. PCR, followed by RFLP of the 16S rDNA amplicons were performed and analyzed. The results were further confirmed through sequencing of the 16S rRNA gene and comparing the sequences from the data available in the GenBank database of the National Centre for Biotechnology Information (NCBI), USA. This study has thrown light on the *Aeromonas* species diversity of this region. Two species of *Aeromonas* (*A. sobria* and *A. popoffii*) have been reported from North Bengal for the first time in this study; and the two other species, *A. caviae* and *A. hydrophila*, have also been reported from this region previously by other researchers.

Aeromonads cause great loss in the aquaculture industry round the world every year. They cause fin and tail rot, ulcer, septicaemia, gill and systemic diseases in aquatic organisms. In humans they cause gastroenteritis and extra-intestinal diseases such as septicaemia, skin, eye, wound, urogenital tract and respiratory tract infections. Aeromonads harbour virulence and antibiotic resistance genes which if present in their mobile genetic elements such as

plasmids, the integron system and transposons, may be horizontally transferred which may pose a major threat to the society. After the World War II which is known as the golden era of antibiotics, antibiotics have been isolated and developed and used sometimes non-judiciously in agriculture, aquaculture and treatment of infectious human diseases. The indiscriminate use of antibiotics has given rise to many resistant varieties of bacteria. NDM1 (a multidrug resistance gene) has also been identified in this group of bacteria which is of serious concern. As aeromonads are a group of bacteria which are significant both clinically and agriculturally, it is very important to detect its antibiotic resistance status from time to time. It is also very important to understand how antibiotic resistance develops and spreads in order to undertake preventive measures to reduce the threat of bacterial infections. In the present study, the antibiotic-resistance status of the *Aeromonas* isolates was analyzed. The common antibiotics used in medicine and aquaculture were chosen for our study. This study has detected *Aeromonas* isolates which are resistant to multiple antibiotics. This threatens the efficiency of antibiotics in combating the infectious diseases caused by *Aeromonas* in near future. The data obtained during the study was also compared with the data available on the antibiotic-resistance status of aeromonads isolated from the sub-Himalayan foothill region as reported from time to time by other researchers. This study revealed that while resistance to some antibiotics has decreased the resistance to some antibiotics has dramatically increased in this bacterial group, isolated from the aquatic bodies of this region. The differential use of antimicrobial substances may account for this finding. A comparative account of the antibiotic resistance status of aeromonads isolated from sparsely populated higher altitude areas with those isolated from densely populated low elevation areas of rivers was also worked out. The study revealed higher incidence of antibiotic resistant aeromonads in downstream areas of rivers than that in the upstream areas of the same rivers. The residues of the antibiotics are drained into the water bodies as surface run-offs which give rise to more resistant varieties of *Aeromonas* in this region.

Research must be undertaken to search and map presumed virulence genes present in *Aeromonas* for combating the pathogenesis caused by them. Plasmids have been isolated from the *Aeromonas* isolates obtained in our study. These plasmids were characterized and were used to transform *E. coli* to record resistance transferability. Genes responsible for resistance to multiple antibiotics were found being transferred to *E. coli*. This can lead to serious issues for the human society as the stock of antibiotics to treat *Aeromonas* infections may gradually run out. This may also lead to difficulties for the fish farmers in fighting diseases caused by *Aeromonas* in fishes which may be the reason of a great economic loss for them. Some other ways must be found for the treatment of infectious diseases other than antibiotics. Several studies are going on in this field currently. Bacteriophage therapy, predatory bacteria,

competitive exclusion of bacteria and bacteriocins may be the new rays of hope in combating infectious diseases caused by pathogenic bacteria.