Chapter 2

Review of Literature

2. REVIEW OF LITERATURE

2.1. Taxonomy and Classification of *Aeromonas*

Bacterial classification does not follow the rules that are followed for the naming of bacteria. The process of classifying bacteria keeps on changing depending upon studies based on their genomic analysis and the tools and process of investigation applied for such phylogenetic studies. The validity of any new classification scheme depends on the application of the scheme and approval of it by the scientists around the world.

The name *Aeromonas* is derived from Greek words *aer, aeros* which means air, gas and *monas* which means unit, monad; *Aeromonas* refers to gas (-producing) monad. Bacteria belonging to the genus *Aeromonas* may be grouped under aeromonads. The scientific classification of *Aeromonas* is as follows:

Kingdom – Bacteria (Cavalier-Smith, 2002)
Phylum – Proteobacteria (Garrity *et al*., 2005)
Class – Gammaproteobacteria (Garrity *et al*., 2005)
Order – Aeromonadales (Martin-Carnahan and Joseph, 2005)
Family – Aeromonadaceae (Colwell *et al*., 1986)
Genus – *Aeromonas* (Stanier, 1943)

Classification of the genus *Aeromonas* is replete with controversy. According to Bergey's Manual of Systematic Bacteriology (Popoff, 1984), the genus *Aeromonas* was divided into three motile, mesophilic species (*A. hydrophila, A. caviae* and *A. sobria*) and the non-motile, psychrophilic species (*A. salmonicida*). DNA–DNA hybridization studies (Popoff *et al*., 1981; Hickman-Brenner *et al*., 1987, 1988; Kuijper *et al.*, 1989; Carnahan *et al*., 1991) have revealed the presence of 14 so-called DNA hybridization groups (HGs): *Aeromonas hydrophila* (HG1), *Aeromonas sp*. (unnamed; HG2), *Aeromonas salmonicida* (HG3), *Aeromonas caviae* (HG4), *Aeromonas media* (HG5), *Aeromonas eucrenophila* (HG6), *Aeromonas sobria* (HG7), *Aeromonas veronii* biogroup *sobria* (HG8), *Aeromonas jandaei* (HG9), *Aeromonas veronii* biogroup veronii (HG10), *Aeromonas sp*. (unnamed; HG11), *Aeromonas schubertii* (HG12), *Aeromonas* group 501 (HG13; previously known as Enteric group 501) and *Aeromonas trota* (HG14). The name *Aeromonas bestiarum* has been

proposed for strains included in HG2 (Ali *et al*., 1996). During the past decades, many novel species have been described: *Aeromonas trota* (Carnahan *et al*., 1991), *Aeromonas allosaccharophila* (Martinez-Murcia *et al*., 1992b), *Aeromonas encheleia* (Esteve *et al*., 1995b), *Aeromonas bestiarum* (Ali *et al*., 1996), *Aeromonas popoffii* (Huys *et al*., 1997), *Aeromonas molluscorum* (Minana-Galbis *et al*., 2004), *Aeromonas simiae* (HarfMonteil *et al*., 2004), *Aeromonas bivalvium* (Minana-Galbis *et al*., 2007), *Aeromonas tecta* (Demarta *et al*., 2008), *Aeromonas piscicola* (Beaz Hidalgo *et al*., 2009), *Aeromonas diversa* (Minana-Galbis *et al*., 2010), *Aeromonas fluvialis* (Alperi *et al*., 2010b), *Aeromonas taiwanensis* and *Aeromonas sanarellii* (Alperi *et al*., 2010a), *Aeromonas rivuli* (Figueras *et al*., 2011), *Aeromonas australiensis* (Aravena-Roman*et al*., 2013), *Aeromonas cavernicola* (Martinez Murcia *et al*., 2013), *Aeromonas aquatica*, *Aeromonas finlandiansis* and *Aeromonas lacus* (Beaz Hidalgo *et al*., 2015), *Aeromonas rivipollensis* (Marti *et al*., 2015), *Aeromonas lusitana* (Martinez-Murcia *et al*., 2016), *Aeromonas intestinalis*, *Aeromonas enterica*, *Aeromonas crassostreae* and *Aeromonas aquatilis* (Figueras *et al*., 2017). The species names *Aeromonas enteropelogenes* and *Aeromonas ichthiosmia* (Schubert *et al*., 1990a, b) are now considered to be synonyms of *A. trota* and *A. veronii*, respectively (Carnahan, 1993; Collins *et al*., 1993). Currently there are 36 described species in the genus *Aeromonas* (Salvat and Ashbolt, 2019). Phylogenetic analyses based on 16S rRNA genes indicated that aeromonads are a very tight group of species (Martinez-Murcia *et al*., 1992a). In almost all species of the genus rDNA-derived relationships correlated well with DNA–DNA hybridization. DNA probes and RFLP profiles designed from 16S rDNA diagnostic regions have served to identify *Aeromonas* at the species level (Ash *et al*., 1993a, b; Dorsch *et al*., 1994; Borrell *et al*., 1997; Khan and Cerniglia, 1997; Figueras *et al*., 2000). However, there are reported discrepancies between different sets of DNA–DNA hybridization data (Hickman-Brenner *et al*., 1987; Schubert and Hegazi, 1988; Esteve *et al*., 1995a, b; Huys *et al*., 1996, 2001), and the fact that 16S rRNA is highly conserved (Martinez-Murcia, 1999) brings the latest descriptions of some species into question (Nair and Holmes, 1999).

It has been reported that gyrB (which encodes the B-subunit of DNA gyrase, a type-II DNA topoisomerase) could be a suitable phylogenetic marker for bacterial systematics (Yamamoto and Harayama, 1996; Stackebrandt *et al*., 2002). This protein plays a crucial role in the DNA replication process, and its gene sequence has a mean

synonymous substitution rate that is almost four times that of 16S rDNA (Yamamoto and Harayama, 1996).

2.2. Isolation of *Aeromonas* from different habitats and conditions

Aeromonads are essentially ubiquitous in the microbial biosphere. They are found in every possible bacterial ecosystem such as water, aquatic organisms, edible products, soil, domestic animals and several other vertebrates and invertebrates. The vast landscape of environmental habitats from which aeromonads can be isolated is responsible for constant interactions between *Aeromonas* and humans. According to research done so far three *Aeromonas* genomospecies (*A. hydrophila*, *A. caviae*, and *A. veronii* bv. sobria) are responsible for most of the (85%) human infections caused by this genus (Janda and Abbott., 1998). The *Aeromonas* species recovered from patients are also commonly found in various ecosystems among which *A. salmonicida* is the predominant species in fish and water samples. Some studies have reported the prevalence of species that are rarely encountered in environmental samples, like *A. schubertii* in organic vegetables (McMahon and Wilson, 2001). Information regarding the relative environmental distribution of the novel species (*A. aquariorum* and *A. tecta*) is rare, and very little is known about various species discovered in the last few years. The procedures applied for the identification of *Aeromonas* species vary considerably from one study to the next.

A brief account of the different *Aeromonas* strains isolated from various samples is represented in **Table 2.1**. Information regarding the genomospecies, phenospecies and hybrization groups to which the strains belong is also provided in the **Table 2.1**.

Table 2.1. Genomospecies and phenospecies of the Genus *Aeromonas*.

DNA Hybridization group	Type Strain/ Reference	Genomospecies	Phenospecies	Remarks	Reference
1	ATCC 7966	*A. hydrophila*	*A. hydrophila*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
1	BCCM/ LMG 19562	*A. hydrophila* subsp. *dhakensis*	*A. hydrophila* subsp. *dhakensis*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
1	BCCM/ LMG 19707	*A. hydrophila* subsp. r*anae*	*A. hydrophila* subsp. *ranae*	Pathogenic for frogs	Martin-Carnahan and Joseph, 2005
2	ATCC 14715	*A.bestiarum*	*A. hydrophila- like*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
3	ATCC 33658	*A. salmonicida*	*A. salmonicida* subsp. *Salmonicida*	Nonmotile fish pathogen	Martin-Carnahan and Joseph, 2005
3	ATCC 33659	*A. salmonicida*	*A. salmonicida* subsp. *Achromogenes*	Nonmotile fish pathogen	Martin-Carnahan and Joseph, 2005
3	ATCC 27013	*A. salmonicida*	*A. salmonicida* subsp. *Masoucida*	Nonmotile fish pathogen	Martin-Carnahan and Joseph, 2005
3	ATCC 49393	*A. salmonicida*	*A. salmonicida* subsp. *Smithia*	Nonmotile fish pathogen	Martin-Carnahan and Joseph, 2005
3	CDC 0434-84, Popoff C316	Unnamed	*A. hydrophila*- like	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
4	ATCC 15468	*A. caviae*	*A. caviae*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
5A	CDC 0862-83	*A. media*	*A. caviae*-like	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
5B	CDC 0435-84	*A. media*	*A. media*	--	Martin-Carnahan and Joseph, 2005
6	ATCC 23309	*A. eucrenophila*	*A. eucrenophila*	--	Martin-Carnahan

					and Joseph, 2005
7	CIP 7433, NCMB 12065	*A. sobria*	*A. sobria*	--	Martin-Carnahan and Joseph, 2005
8X	CDC 0437-84	*A. veronii*	*A. sobria*	--	Martin-Carnahan and Joseph, 2005
8Y	ATCC 9071	*A. veronii*	*A. veronii* biovar sobria	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
9	ATCC 49568	*A. jandaei*	*A. jandaei*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
10	ATCC 35624	*A. veronii* biovar veronii	*A. veronii* biovar veronii	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
11	ATCC 35941	Unnamed	*Aeromonas* spp. (ornithine positive)	--	Martin-Carnahan and Joseph, 2005
12	ATCC 43700	*A. schubertii*	*A. schubertii*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
13	ATCC 43946	*Aeromonas* Group 501	*A. schubertii*-like	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
14	ATCC 49657	*A. trota*	*A. trota*	Isolated from clinical specimens	Martin-Carnahan and Joseph, 2005
15	ATCC 51208, CECT 4199	*A. allosaccharophila*	*A. allosaccharophila*	--	Martin-Carnahan and Joseph, 2005
16	ATCC 51020	*A. encheleia*	*A. encheleia*	Pathogenic for eels	Martin-Carnahan and Joseph, 2005
17	BCCM/ LMG 1754	*A. popoffii*	*A. popoffii*	--	Martin-Carnahan and Joseph, 2005
Unassigned	MTCC 3249, NCIM 5147	*A. culicicola*	*A. culicicola*	Isolated from mosquitoes	Martin-Carnahan and Joseph, 2005
Unassigned	--	*A. eucrenophila*	*A. tecta*	Isolated from clinical and environmental sources	Demarta *et al.*, 2008
Unassigned	--	*A. trota*	*A. aquariorum*	Isolated from monkey faeces	Harf-Monteil *et*

					al., 2004
Unassigned	--	*A. popoffii*	*A. bivalvium*	Isolated from aquaria of ornamental fish	Martinez-Murcia *et al*., 2008
Unassigned	--	Unnamed	*A. sharmana*	Isolated from bivalve molluscs	Minana-Galbis *et al*., 2004
Unassigned	868E^T(=CECT 7113^T=LMG 23376^T).	*A. bivalvium* sp. nov.	--	Isolated from bivalve molluscs	Minana-Galbis *et al*., 2007
Unassigned	--	*A. schubertii*	*A. simiae*	Isolated from midgut of Mosquitoes	Pidiyar *et al*., 2002
Unassigned	--	*A. sharmana* sp. nov.	*A. sobria*	Isolated from a warm spring	Saha and Chakrabarti, 2006
Unassigned	266^T(5CECT 8023^T 5LMG 26707^T)	*Aeromonas australiensis sp. nov.*	*Aeromonas fluvialis, Aeromonas veronii and Aeromonas allosaccharophila*	Isolated from irrigation water system	Aravena-Roman *et al*., 2013
Unassigned	A.11/6T (=DSMZ 24095T, =CECT 7828T)	*Aeromonas lusitana sp. nov.*	--	Isolated frm untreated water and vegetables (lettuce and celery)	Martinez-Murcia *et al*., 2016
--	ATCC 49803	*Aeromonas enteropelogenes*	*Aeromonas trota*	Isolated from human stool	Schubert *et al*., 1990a
--	CECT 4254T	*Aeromonas diversa*	*Aeromonas schubertii*	Isolated from leg wound of a patient	Farfan *et al*., 2013
--	717^T (=CECT 7401^T =LMG 24681^T)	*Aeromonas fluvialis*	*Aeromonas veronii*	Isolated from river water	Alperi *et al*., 2010b
--	848T^T (=CECT 5864^T =LMG 22214^T)	*Aeromonas molluscorum* sp. nov.	--	Isolated from Wedge- shells	Minana-Galbis *et al*., 2004
--	WB4.1-19^T (CECT	*Aeromonas rivuli* sp. nov	--	Isolated from a karst hard water creek	Figueras *et al*., 2011

	7518^T DSM22 539^T MDC 2511^T)				
--	S1.2^T (=CEC T 7443^T= LMG 24783^T)	*Aeromonas piscicola* sp. nov.	--	Isolated from wild diseased salmon	Beaz-Hidalgo *et al.*, 2009
--	A2-50^T (=CEC T 7403^T= LMG 24683^T)	*Aeromonas taiwanensis* sp. nov.	--	Isolated from wound infection of a patient	Alperi *et al.*, 2010a
--	A2-67^T (=CEC T 7402^T =LMG 24682^T)	*Aeromonas sanarellii* sp. nov.,	--	Isolated from a wound culture from a patient	Alperi and Figueras, 2010

-- Information not available

2.2.1. *Aeromonas* from aquatic environments

Aeromonas counts are greater in running water ecosystems than in stagnant water and are higher in temperatures spanning between 25°C and 35°C (Hazen *et al.*, 1978; Hazen *et al*., 1979). Although *A. hydrophila* grows over a wide range of conductivities, turbidities, salinity, temperature and pH, a very high fluctuation in these parameters inhibits the growth of this species of *Aeromonas*. Principally, species of *Aeromonas* are freshwater inhabitants. But they are also found to inhabit the marine waters, most frequent being the epipelagic layer. Most frequently they exist as free-living bacteria in estuaries. Sometimes they are found to inhabit the bodies of the crustaceans. The epipelagic layers of the estuaries are best suited for the growth of *Aeromonas*, since salt content is considerably low as compared to the marine benthic zone. A survey done on the coastal waters of Italy has revealed the density of *Aeromonas* isolates to vary from 102 to 106 CFU per 100 ml round the year (Fiorentini *et al*., 1998).

2.2.2. *Aeromonas* from diseased fishes

Species of *Aeromonas* are known to cause diseases in fishes since long ago, much longer than the discovery of their roles in affecting the human beings. Among them, the two most frequently occuring fish diseases are found to be caused in fishes by *Aeromonas salmonicida.* Furunculosis is caused by *A. salmonicida sensu stricto* in fishes, particularly in salmonids. The disease has several manifestations. It may be an acute form of the disease where fishes suffer from septicemia, loss of appetite and melanosis. Symptoms may also include clotting of blood due to rupture of blood vessels at the fin bases. It may also lead to chronic disorder if the age of the fishes is higher. The affected fishes may turn lethargic and also show slight exorbitism in extreme cases. Rupture of muscular blood vessels and within internal organs may also occur (Austin, 1997). Similar disorders are caused in fishes by mesophilic aeromonads like *Aeromonas veronii* and *Aeromonas hydrophila.* These species of *Aeromonas* cause septicemia and ulcerative syndromes in fishes like carps, catfishes and tilapia. Red sore disease is another infectious disorder caused by these bacterial species in carp and bass (Joseph and Carnahan, 1994). Massive economic loss in aquaculture industry is due to infections caused by mesophilic aeromonads, *Aeromons hydrophila* being the most significant among them (Monette *et al.,* 2006). In many of these instances, *Aeromonas* species were sole or co-pathogens causing invasive secondary infections in fishes whose immune system was suppressed which may be due to several reasons such as spawning, rising temperature or decreasing levels of water.

2.2.3. *Aeromonas* from drinking water

Various concentrations of *Aeromonas* species have been isolated from drinking water. Although the relevance of *Aeromonas* as the cusative agent of gastroenteritis through such drinking water samples is not evident, the long term interaction of immunosuppresed patients with aeromonads through waters contaminated with aeromonads can potentially lead to invasive diseases, such as septicemia (Leclerc *et al*., 2002). The World Health Organization (WHO) has enlisted *Aeromonas* as a potential hazard in the *Guidelines for Drinking-Water Quality* (third edition). In 1998, *A. hydrophila was enlisted* on the "Drinking Water Contaminant Candidate List" of the Environmental Protection Agency. According to the rule of the Consumer Confidence Report, detection of uncontrolled pollutants, such as

aeromonads in public water supplies needs to be reported immediately (Edberg *et al*., 2007).

2.2.4. *Aeromonas* from animals

Different species of invertebrates and vertebrates are found to harbor aeromonads. Reports of insects being the host to *Aeromonas* are known. The vertebrates being inhabited by the aeromonads are not studied as extensively as the aquatic bodies. There are several direct and indirect methods of identifying aeromonads from the vertebrates, some of which are:

- Regular screening of the fecal matter of animals for microbes like *Aeromonas*.
- Microbial analysis of foods available in the market at regular time intervals.
- Infections in humans caused by animal bites such as snakes and other vertebrates.

Species of *Aeromonas* can also be the causative agent of several infectious disorders in both poikilothermic and homeothermic animals like ulcerative stomatitis of reptiles like snakes and lizards, "red leg" disease of amphibians, infection in blood of dogs and infection in bone joints in calves (Gosling, 1996). Aeromonads also cause several types of diseases in seals (Thorton *et al*., 1998) and infection in the seminal vesicles of bulls (Moro *et al*., 1999). *Aeromonas* species have also been isolated from the midgut of *Culex quinquefasciatus* mosquitoes (Pidiyar *et al*., 2002; Chandel *et al*., 2013) and *Anopheles* mosquitoes (Djadid *et al*., 2011). These reports are evidences in support of the fact that animals serve as a pool for the origin and interchange of species of *Aeromonas* in the various ecosystems.

2.2.5. *Aeromonas* from various types of food

Transitory settlement of the gastrointestinal tract of humans by *Aeromonas* is mostly an incidental consequence of the ingestion of products contaminated with aeromonads. A number of studies have been done with an aim to determine the prevalence of different species of *Aeromonas* in food items available in the markets (Isonhood and Drake, 2002). Irrespective of the differences in the procedures and media used for detection, and varieties of retail items examined, the overall conclusion from these reports suggest that *Aeromonas* is commonly found in most types of food, irrespective of their geographic origin. *Aeromonas* isolates have been invariably isolated from all types of consumable products such as milk, seafish and all

types of edible meat available in the market (Palumbo *et al*., 1985). When these consumable products were stored at 5°C the concentration of aeromonads was found to vary from 102 to 105 CFU/g in them. Their concentration escalated after refrigerating the foods for a period of 7 days in most of the cases. Other researchers have reported the presence of *Aeromonas* in milk and milk products, raw vegetables, chicken and meats, with the highest incidence of aeromonads being reported from shellfish and fish (Borrell *et al*., 1998; McMahon and Wilson, 2001; Neyts *et al*., 2000).

2.3. Identification of *Aeromonas*

2.3.1. Biochemical identification of *Aeromonas*

A major difficulty in identifying the members of the genus *Aeromonas* is the current number of discovered species being 36 (Salvat and Ashbolt, 2019) and the unavailability of clearly distinguishable phenotypic characters among them (Janda, 2001). Researchers often report only selected biochemical properties of newly discovered species and compare the data with phenotypic characteristics of previously reported genetically related taxa. Most of the biochemical tests performed in different studies are identical but the procedures, growth and incubation conditions, and media compositions vary considerably which may affect the results to a great extent (Esteve *et al*., 1995b; Huys *et al*., 1997). Although all the *Aeromonas* strains possess cytochrome C oxidase, a considerable amount of variation is observed in many other biochemical characteristics of *Aeromonas*. Some of these properties include the production of enzymes like gelatinase, lipase, citrate, permease and catalase, gas and acetoin from glucose, production of indole (Altwegg *et al*., 1990b; Abbott *et al* 2003). Test for cytochrome oxidase and acetoin are the most important biochemical tests for identification of *Aeromonas* (Bullock, 1961). Studies have shown the occurrence of few oxidase-negative strains of *Aeromonas* too (Overman *et al*., 1979). Due to lack of uniformity in the data it can be concluded that biochemical characterization is alone not enough for identification of *Aeromonas* upto species level. Molecular characterization is necessary for confirmation of the results.

2.3.2. Molecular identification of *Aeromonas*

Phenotypic characterization cannot always lead to the correct identification of *Aeromonas* upto species level (Sujita *et al*., 1994). Therefore, molecular methods are employed for correct identification of *Aeromonas* species. Different molecular biology techniques like microplate hybridization method (Sujita *et al*., 1994), DNA-DNA hybridization technique (Cascon *et al*., 1996), PCR of 16S rDNA using specific primers (Chen *et al*., 2019), RFLP of the amplified DNA fragments (Borrell *et al*., 1997; Lee *et al*., 2002; Nawaz *et al*., 2006; Puthucheary *et al*., 2012) and also sequencing of the amplified DNA (Dorsh *et al*., 1994; Hossain, 2008) are currently in use. Amplification of housekeeping genes like *gyr*B gene, it's DGGE analysis (Tacao *et al*., 2005) and sequencing of the amplified DNA (Yi *et al*., 2013; Chen *et al*., 2019), *rpoD* gene, and sequencing of the amplified DNA (Puthucheary *et al*., 2012; Yi *et al*., 2013) are widely used for the identification of the different species of *Aeromonas*. Phenotypic and biochemical methods of identification of bacteria may give varying results due to variability in expressions. However, sequencing of housekeeping genes like 16S rRNA (Hossain, 2008), *gyr*B (Yi *et al*., 2013; Chen *et al*., 2019) and *rpo*D (Yi *et al*., 2013) provide univocal data that can be reproduced in and between laboratories. This holds true even for the rare species. Some other housekeeping genes such as *gyr*A, *atp*D, *rec*D, *dna*J and *dna*X are also sequenced for identification of *Aeromonas* (Martinez-Murcia *et al*., 2011).

2.3.3. Other methods of identification of *Aeromonas*

Among the methods used for the identification of *Aeromonas* other than the biochemical and molecular methods are VITEK systems and DuPont QualiconRiboPrinter® microbial characterization system (Kivanc *et al*., 2011). A comparative study on the efficiency of three commercially available kits API 20E, API 20NE and Microbact 24E showed kits API 20NE and Microbact 24E to be more accurate in the identification of *Aeromonas* (Ogden *et al*., 1994). MALDI-TOF MS is also a powerful tool used to detect pathogenic *Aeromonas* species from clinical samples (Vavrova *et al*., 2015). Investigation of fatty acids present in the cells also serves as a powerful marker for identification of different species of *Aeromonas* by using the method FAME (Fatty Acid Methyl Ester) analysis (Huys *et al*., 1994; Kuhn *et al*., 1997; Rahman *et al*., *2002*).

2.4. Epidemiology

There is an element of regularity related to the detection of aeromonads from the human gut. *Aeromonas* is not the natural resident of the human gut. Most of the researchers have reported a rise in the recovery of *Aeromonas species* from fecal samples during the summer season. This increase in their counts can be correlated to the fact that mesophilic aeromonads grow well at higher environmental temperatures, thus leading to a rise in bacterial concentration in freshwater habitats as well as in domestic water supplies (Edberg *et al*., 2007; Khardori *et al*., 1988). Similar seasonality has been observed with respect to *Aeromonas* isolated from the human gastrointestinal tract as well as the extraintestinal locations of the body. 42% to 67% of the cases of *Aeromonas* septicemia also occur during the summer season (Tsai *et al*., 2006). While the prevalence of less frequently occurring extra-intestinal infections caused by aeromonads is more difficult to track due to their less frequent incidence, it is justifiable to correlate the increased concentrations of *Aeromonas* in aquatic habitats during the warmer months of the year with the elevated chances of exposure to these microbes and thus an increased probability of being infected by them. The close relationship between *Aeromonas* and aquatic environments has compelled many scientists to nearly contemplate the name "*Aeromonas*" to be used interchangeably with "water." However, with respect to the infection/colonization status of human beings with *Aeromonas*, some of these hydrophilic relationships may not always be that obvious. Most of the reports which are available propose that most of the mesophilic aeromonads are obtained by drinking water contaminated with aeromonads or via the uptake of foods which normally come into close proximity with *Aeromonas* either by being irrigated by *Aeromonas* contaminated waters or through other means. In India, *Aeromonas sp*. was isolated from 33 (13.4%) out of 246 food samples of animal origin examined (Kumar *et al.*, 2000). 16.7% of poultry meat samples, 12% of goat meat samples and 7.7% of buffalo meat samples were found to harbour *Aeromonas*. They also reported the predominance of *A. hydrophila* (51.5%), followed by *A. veronii* biovar sobria (39.4%) and *A. caviae* (9.1%). Along with these consumable products, molluscs such as mussels and oysters are naturally immersed in estuary waters contaminated with these bacteria, and due to their filter-feeding process, these bacteria get concentrated within their meats. Aeromonads have been isolated and identified in India from varied sources like stool samples from

patients with diarrhea (Sinha *et al*., 2004), sulphur spring (Patra *et al*., 2007). Although in most of the surveys undertaken in human diarrhea *Aeromonas hydrophila* appeared to be the dominant species, few reports in India indicated that *A. caviae* can be carried in asymptomatic human subjects showing septicemia, but without any history of gastroenteritis (Dwivedi *et al*., 2008).

Apart from these major routes, *Aeromonas* can also invade the humans through some other, less significant pathways. Amusement water ventures can cause infectious diseases due to coming in contact with *Aeromonas* contaminated water or ingestion of water due to any accident such as in the survivors of cases who were nearly being drowned (Bossi-Kupfer *et al*., 2007). As urban civilization continues to intrude rural environments, the events of transfer of *Aeromonas* from wild animals to human beings will rise. While humans getting infected by *Aeromonas* through bites of reptiles such as snakes are known since long ago, *Aeromonas* infection in humans as a result of confrontation with other wild animals such as bears are reported in the last two decades (Angel *et al*., 2002; Kunimoto *et al.,* 2004). The true picture of the prevalence of infections caused due to *Aeromonas* round the world is not known yet. *Aeromonas* infection is less apparent in the developed countries like the United States of America.

2.5. Infections caused by *Aeromonas* and their symptoms

Few species of Gram-negative bacteria compete with the aeromonads in opportunity and diversity of infectious diseases caused by them in human beings. Aeromonads are causative agents of a variety of intestinal and extra-intestinal diseases and syndromes. These may extend from comparatively mild diseases like acute gastroenteritis to lethal conditions including septicemia, myonecrosis and necrotizing fasciitis (Janda *et al.,* 1994). The types of infectious diseases, in addition to those mentioned above, that *Aeromonas* can cause in humans are problems associated with the intra-abdominal region, infections in the eyes, bones and joints. Other infrequently occuring disorders caused by *Aeromonas* in humans are infection of the respiratory and urogenital tracts. With regard to their prevalence, disorders in humans caused by *Aeromonas* infections can be categorized into-

- Gastroenteritis.
- Skin and soft tissue infections.
- Blood-borne infections.
- A mixed group consisting of infrequently occuring infections.

2.5.1. Gastroenteritis

Gastrointestinal tract is a familiar area of human body which shows the highest incidence of infection by aeromonads. Supporting affirmation for *Aeromonas* as gastrointestinal pathogen arises from the detailed case studies and epidemiologic case-controlled research on *Aeromonas*-associated diarrhea (Janda and Abbott, 1998). There is a consensus among different investigators that certain strains of *Aeromonas* that carry required virulence factors are likely to be human enteric pathogens while others are not (Kelly *et al.,* 1993). Diarrhea caused by *Aeromonas* presents with diverse clinical manifestations. Watery and self-limited diarrhea is common. But some patients may suffer from fever, abdominal pain, and bloody diarrhea. Dehydration may accompany the above-mentioned symptoms in severe cases. More than 25% of children pass mucus and blood with stools during diarrhea caused by *Aeromonas* and nearly 35% of patients show signs of fever and vomiting (Ghenghesh *et al.*, 1999). Passage of blood along with stool is a sign of dysentery. Children with diarrhea caused by *Aeromonas* may pass up to ten episodes of stool per day which may exist for 2-10 days (Ghenghesh *et al.*, 1999). Some exceptionally scarce cases of *Aeromonas* causing a disease similar to cholera are known (Janda and Duffey, 1988). It has been reported to occur in several developing countries. A Thai woman from Bangkok traveling to France was admitted to a hospital in Paris for a cholera-like diarrhea illness (Champsaur *et al.*, 1982). *A. veroni* biovar sobria (reported as *A. sobria*) was isolated from the patient's “rice water" stool but no *Vibrio cholerae* and enterotoxigenic *Escherichia coli* was found. The bacterial isolate showed the presence of hemolysin, enterotoxin, proteolysin, cytolysin, and a cellrounding factor. Sera from acute- and recovering patients showed a rise in neutralizing antibodies to enterotoxin, hemolysin and cytolysin. Immunocompromised patients may have severe symptoms. A study reported severe acute diarrhea with cholera-like clinical symptoms in a 26-year-old male from Cuba caused by *A. veroni* biovar sobria (Earle *et al.*, 1997). The patient was suffering from Crohn's disease and had been previously colectomized. Isolation of *Aeromonas sp*. from patients with cholera-like diarrhea strongly

reinforces the fact that some strains of *Aeromonas sp*. are enteropathogenic to humans. Diarrhea caused by *Aeromonas sp*. may also be chronic and persist for months, particularly in immunocompromised patients. The isolation of *Aeromonas sp*. from 8 (13.3%) of 60 HIV patients with chronic diarrhea in rural communities of the Limpopo Province, South Africa was reported (Obi and Bessong, 2002). Immunocompetent individuals may also suffer from chronic diarrhea caused by *Aeromonas*. Two immunocompetent patients from Saudi Arabia, suffering from chronic inflammation of their colon were found to be infected by *Aeromonas hydrophila* (Ibrahim *et al.*, 1996).

Diarrhea caused by *Aeromonas* is known to occur in all parts of the world and affecting people of all ages. Although it is mostly seen in healthy people, people already suffering from some disease as well as immunocompromised people such as AIDS patients may also have gastrointestinal infections due to *Aeromonas* (Figueras, 2005). *Aeromonas* gastrointestinal disease is a less severe infection from which the patients may recover without any medications. Numerous extensive and probable studies on diarrhea caused by bacteria concluded that *Aeromonas* was identified from the microbial culture of 0.5% to 16.9% of patients' stool samples whereas 0% to 10% of those of healthy people (Holmbergand Farmer, 1984). These wide and converging frequencies with which aeromonads are found in both diseased and healthy persons are still true and this is why gastroenteritis caused by *Aeromonas* is still a debatable issue.

Aeromonas gastroenteritis can medically be found in five different forms such as non-descript inflammation of the small intestine, an acute form characterized by the passage of blood in stools, as a long term gastroenteric syndrome, in rare cases a disease showing symptoms similar to cholera, or as a short-lived diarrhea in returned travellers. The most frequently appearing symptom of gastrointestinal infection caused by *Aeromonas* is acute onset secretory diarrhea which is the most common symptom of any bacterial infection of the human gut (Figueras, 2005). The watery form of diarrhea accounts for 75% to 89% of all *Aeromonas* gastrointestinal infections reported where *Aeromonas* is the only pathogenic bacterium recovered from the stool samples of patients (Chan *et al.,* 2003). Most frequently observed symptoms of this type of diarrhea are mild rise in body temperature with stomach ache; frequent vomiting in more than 50% of an affected infant population has been reported in a study (Essers *et al.,* 2000; Vila *et al.,* 2003). Dehydration is typically mild to

moderate. Severe diarrhea caused due to *Aeromonas* infection is quite unusual (Chan *et al*., 2003). The usual manifestations of *Aeromonas* gastroenteritis are painful cramps in the abdomen and passage of mucus and blood with stool (Chan *et al*., 2003; Essers *et al*., 2000; Janda and Duffey, 1988). When such symptoms of diarrhea are observed patient often needs to be hospitalized. Blood cancer patients or those who have tumors in their gut or those suffering from some other disease of the gastrointestinal tract may get predisposed to *Aeromonas* and are likely to develop *Aeromonas* gastroenteritis (Sherlock *et al.,* 1987).

There are many uncommon manifestations and problems that can occur from gastroenteritis caused by *Aeromonas*. Most of these traumas pave the way for extreme episodes of inflammation of the intestine specially the colon due to *Aeromonas* infection. Rarely in some patients of *Aeromonas* colitis chronic diseases, like ulcer of the colon or entire large intestine may develop, which may last for months to years. Surgery may be required in extreme cases along with anti-inflammatory medicines for the recovery of the patients (Willoughby *et al*., 1989). Aeromonads are not isolated from blood, stool or biopsy samples of most chronic patients. Another unusual case sometimes correlated with *Aeromonas* infection in the human gastrointestinal tract is segmental colitis. Segmental colitis caused by aeromonads can sometimes be confused with ischemic colitis or Crohn's disease because of their similar symptoms (Bayerdorffer *et al*., 1986). While the infection can affect any portion of the large intestine, mostly the ascending or transverse colon is affected. Other conditions found to be associated with *Aeromonas* enteritis/colitis are ileal ulceration (Yamamoto *et al.,* 2004), intramural intestinal hemorrhage with small bowel obstruction (Block *et al*., 1994), and refractory inflammatory bowel disease (Doman *et al*., 1989).

2.5.2. Skin and Soft Tissue Infections

After gut the second most frequent site of the human body infested by *Aeromonas* is the skin and the tissues present under the epidermal region. The range of severity of skin and soft tissue infection caused by aeromonads may vary from meek superficial issues like pus-filled pimples to fatal conditions. When infection is severe, symptoms may extend from life threatening cellulitis of tissues lying below the skin to flesh eating disease of the dermis and subcutaneous tissues and even serious destruction of muscles (myonecrosis). Some other consequences of severe *Aeromonas* infections may be infectious arthritis involving bones and joints or even

spread of infection throughout the body (Lai *et al*., 2007). The wounded body sites most commonly infected by *Aeromonas* are the upper and lower arms. Many treatment methods like hirudotherapy can also enhance the probability of *Aeromonas* infestations in the site of injury (Moawad *et al*., 2002). Infections caused by aeromonads in surgical sites are an exceptionally scarce situation, but may sometimes occur after undergoing treatment methods like surgical removal of appendix, gall bladder and colon (Tena *et al*., 2009). Practically all reported cases of surgical site infections have been found to develop in patients with medical history of gastroenteritis or any bile related disorder. Over 75% of these diseases are caused by multiple microbial infections.

Obscure or evident injurious events can lead to numerous types of *Aeromonas* wound infections. Superficial wear and tear of the skin or incisions can give rise to remarkable disease if the wound is immersed in water contaminated with *Aeromonas* (Lai *et al*., 2007). More conspicuous damage to body parts can occur from pervasive wounds, such as bites of *Aeromonas* infected animals or the insertion of foreign particles like mud, wooden or metallic items infected with *Aeromonas* into deeper tissues through road accidents (Lamy and Kodjo, 2009). Severe injuries caused by road accidents that lead to deep wounds and fractures, enhance the chances of *Aeromonas* infections in those wonded sites (Monaghan *et al*., 2008). The deeper the wound, the more is the chance of developing a fatal disease due to infection by *Aeromonas*.

Aeromonads may cause infections in survivors of natural disasters like cyclones and floods. Electrical or inflamed burns are frequently managed or quenched by immersing burnt areas of body in water contaminated with *Aeromonas*. Such incidents may lead to *Aeromonas* colonization in devitalized tissues, causing cellulitis to septicemia (Kienzle *et al*., 2000). *Aeromonas* is frequently found to be a part of the oropharyngeal bacterial population of reptiles among which snakes are the most common reservoirs. Wound infections ranging from skin degradation to damage of the flesh underlying the skin in more severe cases have been reported to result from bites of water moccasin, cobra, and viper snake (Angel *et al*., 2002).

2.5.3. Blood-Borne Infections

Septicemia is the typical invasive disease associated with the genus *Aeromonas*. A pioneer report on *Aeromonas* infections was published, which

mentioned two cases of septicemia in adults with preexisting hepatic cirrhosis. These reports obtained long back than four decades have suggested that patient populations are at a greater risk of developing *Aeromonas* sepsis. While some differences are reported in the types of *Aeromonas* sepsis based upon the geographic location or populations studied over the years it has been noted that the major criteria describing septicemia caused by *Aeromonas* have been well defined for over two decades. Three species (*A. hydrophila sensu stricto*, *A. caviae*, and *A. veronii* bv. sobria) are responsible for 95% of all blood-borne infections caused by *Aeromonas*. Besides this, three other species of *Aeromonas* (*A. jandaei*, *A. veronii* bv. veronii and *A. schubertii*) are less often associated with septicemia (Janda *et al.,* 1994). While previously the word bacteremia was used to define the detection of bacteria in the blood of asymptomatic human subjects, and septicemia was known to be established when symptoms of infection in blood appeared, at present, there is no difference between the two when related to *Aeromonas*. The two names are now used interchangeably.

The huge majority (80%) of *Aeromonas* septicemia cases occur in persons who are substantially immunocompromised. Disease in this group most often occurs in middle-aged men (approximately 53 to 62 years of age). The male/female ratio being affected by *Aeromonas* is 1.6 to 4:1 and 71% to 79% of it is community acquired. Septicemia is caused by *Aeromonas* round the year, with a greater chance of occuring when the environmental temperature is higher. Patients with uncontrolled blood cell growth or liver cirrhosis are the immunocompromised ones who are at the greatest risk of developing *Aeromonas* septicemia. The main preexisting diseases which are related to systemic infection are hepatic cirrhosis (54%) and malignancy (21%). Other studies have suggested similar findings concerning to susceptibilities for sepsis, with long term liver disease being 26% to 36%, neoplasia being 33% and biliary disease 24% as the three most vulnerable conditions. Among hematologic disorders, acute myelogenous leukemia is the dominating one, followed by myelodysplastic syndromes, non-Hodgkin's lymphoma, and acute lymphocytic leukemia (Ko *et al*., 2000; Tsai *et al*., 2006). Many other preexisting diseases or complications like diabetes mellitus, renal problems, cardiac anomalies, and numerous other hematologic conditions, such as aplastic anemia, thalassemia, multiple myeloma, and Waldenstrom's macroglobulinemia have been linked with *Aeromonas* septicemia (Janda and Abbott, 1996,). Unfortunately, there are no clinical symptoms that can differentiate *Aeromonas* septicemia from those whose causative

agents are other gram-negative bacteria. The most obvious symptoms that appear during *Aeromonas* infection are high body temperature, jaundice, stomach ache, septic shock, and shortness of breath. Diarrhea immediately preceding or coincident with the onset of *Aeromonas* infection in blood is seen infrequently. *Aeromonas* bacteremia may reoccur in a patient on rare occasions after two or more months with a percentage of reccurance spanning between 1.4% to 9.8% (Ko *et al*., 2000; Tsai *et al*., 2006). In these cases, it is not always understandable whether relapse of infection has occurred from a secured nest or a clonally different strain of bacteria resulted in the reccurrence of bacteremia. The percentage of chief *Aeromonas* bacteremia in this patient population approximately varies from 40% to 57% (Ko *et al*., 2000), with secondary cases often occurring from internal conditions, including peritonitis, soft tissue infections, or biliary disease. A study done in South-East Asia has suggested that a common staple food, seafood of that place contaminated with *Aeromonas* may be the vector for transmission of aeromonads into the human gut. Patients with hematologic malignancies or under anti-neoplastic medications may suffer degradation of the gastrointestinal mucosa which brings about transmission of seafood-derived *Aeromonas* isolates from the gut to the blood vascular system (Tsai *et al*., 2006). *Aeromonas* can also enter the human blood stream through contaminated medical devices used to pass out urine or clear any other biliary obstruction in patients (Doudier *et al.,* 2006).

Infrequently another type of *Aeromonas* sepsis occurs which affects people who have suffered a vital distressing cicumstance shortly before contracting septicemia. However, this group of people may not have been exposed to *Aeromonas* in the past. These traumas are most frequently community based and can occur as a result of various insults. In many cases, the high mortality rate associated with this type of infection is interconnected to the shock as well as the pathogen. Medicinal leech therapy is often applied to relieve venous congestion. Since leeches have a symbiotic relationship with *Aeromonas*, there is a high chance of the patients getting infected while undergoing such therapies. Normally such diseases are restricted to a small area of the body. However, spread of the infection is seen in some cases. *Aeromonas* septicaemia has been reported due to leech therapy being carried out in patients suffering from, accidental amputations or those who have undergone plastic surgery related to malignancies (Fenollar *et al.*, 1999) and has also resulted in subsidiary infections like inflammation of the meninges (Ouderkirk *et al.,* 2004). The

most frequent *Aeromonas* species responsible for such diseases is *A. veronii* bv. sobria ("*A. sobria*").

2.5.4. Intra-Abdominal Infections

"Intra-abdominal infection" is a wide term including a number of infections but it is frequently used as a synonym for peritonitis. In reality, intra-abdominal infections refer to the infections that originate in the visceral space and spread into the peritoneal space. Types of intra-abdominal infections include pancreatitis, acute cholangitis, hepatic abscesses and peritonitis. Such types of infections are significant medical problems of South-East Asia as compared to the developed countries of the United States or Europe. Similar to *Aeromonas* septicemia, most of the intra-abdominal infections caused by *Aeromonas* are acquired from the community and mostly middle-aged men are found to be affected who have one or more underlying illnesses.

Some severe infectious complications may develop in cirrhotic patients (Brann, 2001). Peritonitis is a swelling of the peritoneum, the serous membrane forming the inner lining of the abdomen. It may occur in three medical conditions, one of which is bacterial infection in the peritoneum even in the absence of a common pathogen, another being developed when a patient undergoes continuous ambulatory peritoneal dialysis (CAPD) or invasion of aeromonads from the gut into the peritoneum through perforations of the intestine (Wu *et al.,* 2009). When CAPD patients develop *Aeromonas* peritonitis, many of them are also suffering from problems of liver which may or may not be detected during peritonitis (Yang *et al.,* 2008). Peritonitis can be of two types, primary and secondary. The less common form is primary peritonitis, which occurs as a result of the spreading of *Aeromonas* from the blood vascular or lymphatic system into the peritoneum. Secondary peritonitis occurs more frequently and is caused from the spread of infections from the biliary or gastrointestinal tract. Most of the cases (95% or more) of bacterial peritonitis in South-East Asia are caused by *Aeromonas hydrophila*. Some other species of the genus such as *A. veronii* bv. sobria may also sometimes serve as the causative agent of peritonitis (Choi *et al.,* 2008). In most of the patients, the route of infection is not pronounced.

Acute suppurative cholangitis is a very common medical complication of the hepatobiliary tree caused by aeromonads. Research works done in Hong Kong and

Michigan suggests that the percentage of occurance of *Aeromonas* cholangitis varies from 1.3% to 2.9%. Cholangitis caused by *Aeromonas* are generally polymicrobial with *Enterobacteriaceae*, *Enterococcus* and *Pseudomonas aeruginosa* being the other three common pathogens involved with the disease. These findings indicate that these illnesses originate from the gastrointestinal tract. Many patients developing cholangitis have had previous episodes of *Aeromonas* infections. Most of the patients diagnosed with *Aeromonas* cholangitis have one of the underlying illnesses like cholelithiasis or choledocholithiasis, non-malignant biliary strictures, cholangiocarcinoma or pancreatic carcinoma (Chan *et al*., 2000).

2.5.5. Respiratory Tract Infections

Aeromonas species are at times isolated from sputum or other secretions from the respiratory tract of hospitalized patients. Previously, in most of the cases, these isolates were regarded to represent transitory colonization only (Janda and Abbott, 1998). But now the concepts regarding *Aeromonas* and respiratory diseases associated with them have changed. Previously, Aeromonas was responsible for infection of the epiglottitis, purulent pleuritis, lung tissue necrosis, and alveolar inflammation in patients with no chronic disorders or in people with impaired immune system. Nowadays, serious respiratory tract infection cases caused due to *Aeromonas* are increasing in number. Such diseases are often difficult to diagnose and pose a greater diagnostic challenge to the doctors and microbiologists. As of now, the most common respiratory illness associated with *Aeromonas* is pneumonia. Bacterial pneumonia is found to affect two types of people. The first type arises from severe trauma, the most frequent of which is near-drowning cases, whose estimated cases of a year in the United States are 16,000 to 160,000 (Ender *et al*., 1996). Accidents leading to near drowning in the sea, a shallow irrigation ditch, and other massive aquatic exposures may lead to pneumonia along with septicemia caused by aeromonads in the victims (Mukhopadhyay *et al*., 2008). Many adults with pneumonia caused by *Aeromonas* have preexisting underlying diseases which may be liver cirrhosis, renal disease, or multiple sclerosis but children who are diagnosed with *Aeromonas* pneumonia may not have any of such preexisting conditions. Some of these cases may be aspiration pneumonia (Mukhopadhyay *et al*., 2003), while contaminated water may also serve as the source of infection in others (Rodriguez *et al*., 2005). Blood is the most common site from where aeromonads are isolated, others being secretions fron the inner lining

of the respiratory tract, and postmortem specimens, such as pleural discharge. The patients whose blood has been infected may often collapse very fast. Two strains of *A.hydrophila* were detected from one dead patient. One of the strains was multi drug resistant, which enhanced the difficulty in treatment (Murata *et al.,* 2001). 50% of the patients getting affected by pneumonia due to *Aeromonas* infections succumb to the disease.

2.5.6. Urogenital Tract Infections

Aeromonads are frequently involved in the urogenital tract infections, although this disease has received less attention from the medical and scientific communities. The frequency of occurrence of these urogenital tract infections (UTIs) is not clear as they are often mentioned with less significance in published literature (Huang *et al***.,** 2006). A 69-year-old male diabetic patient, also suffering from long term hepatitis was reported to develop UTI caused by *A. veronii* bv. sobria (Hsueh *et al*., 1998). He was cured by the application of ceftriaxone but again developed necrotizing fasciitis due to infection from the same organism. A 13-year-old myelomeningocele patient was reported to have developed UTI due to *A. popoffii* which is generally considered to be an infrequent human pathogen (Hua *et al*., 2004). An infected urinary catheter was identified as the route of infection. A 39-year-old man who complained of increased urination, dysuria, and hematuria for the past two months was diagnosed with cystitis due to *A. caviae* (Al-Benwan *et al***.,** 2007). A 39-year-old alcoholic male was reported to have developed prostitis due to *Aeromonas* infection (Huang *et al***.,** 2007). The patient had developed fatty liver. Microbial examination of his blood and urine samples revealed the presence of *A. veronii* bv. sobria ("*A. sobria*"). No origin of his infection could be identified, but the researchers hypothesized that his lower socioeconomic condition may have enhanced the chances of his encounter with environmental aeromonads.

2.5.7. Eye Infections

Eye infections due to *Aeromonas* species can cause inflammation in the ocular chambers, cornea and ulcer in the cornea (Sohn *et al.***,** 2007). In many cases, previously occuring trauma or encounter with the physical environment possibly contaminated with aeromonads is unknown. However, sometimes soft contact lenses

may become contaminated with *Aeromonas*, among other microbes (Hondur *et al*.**,** 2008).

2.6. Pathogenicity

Only two types of infections caused by *Aeromonas* in humans (gastroenteritis and wound infections) are dominant in healthy people, in opposition to those with underlying illnesses. Only 3 of the recognized *Aeromonas* species (*A. hydrophila sensu stricto*, *A. caviae*, and *A. veronii* bv. sobria) are responsible for causing most of the *Aeromonas* infections in human beings (Janda and Abbott, 1998). Screening of environmental samples indicates that while these virulent species may be frequently recovered from some habitats, they are not primarily found in water and food used for human consumption and surface of freshwater and marine water ecosystems. This suggests that the process of establishment of disease in a person likely to get infected prioritizes those strains which have certain features that enhancs the chances of pathogenicity (Borrell *et al.,* 1998). Some other species of the genus *Aeromonas* who are emerging pathogens are *Aeromonas salmonicida* and *Aeromonas dhakensis*.

2.6.1. Gastroenteritis

To establish itself as a successful enteropathogen, a bacterium has to enter the body of the host, surpass normal physiologic barriers, escape defense mechanisms of the host, and give rise to disease. The presumed route through which *Aeromonas* enters the gastrointestinal tract and produces gastroenteritis is through contaminated foods or water. The bacteria after entering the alimentary canal bypass the lethal effects of gastric acidity, attaches itself to the small or large intestine competing successfully against native microbes of the human gut. Although numerous genes and virulence factors are involved to make this complicated process a success, only a few of them have been studied extensively. One possible way by which aeromonads can escape the corrosive effects of acidic pH in the stomach is by having an acid tolerance mechanism. A strain of *A.hydrophila* has been adapted to tolerate pH 3.5 through a mechanism close to that applied by *Salmonella* (Karem *et al*.**,** 1994). If such an adaptation is found in many other *Aeromonas* species this will facilitate them to subsequently invade and colonize the human gastrointestinal tract.

Once aeromonads enter the human alimentary canal, a sequence of events occur which help them to compete against the normal gut flora by producing some by-products of metabolism and bacteriocin-like compounds which help them to attach themselves to the gut wall. This mechanism includes a sequence of interdependent steps, including movement toward a particular direction, adherence to the lumen of the gut, formation of biofilm, colonization, production of virulence factors and infection. Two types of flagella (polar and lateral) and pili are the two important structures that play significant roles in the establishment of the pathogenesis of *Aeromonas*. The gene for the polar flagellum (*Pof*) is constitutive and that for lateral flagella (*Laf*) is inducible (Martin-Carnahan and Joseph, 2005). Polar flagella help aeromonads to swim and the lateral ones help in forming colonies on colonization sites (Kirov *et al.,* 2002). It has been thought that Pof has a significant role in the adherance of aeromonads to the lumen of the gut and Laf has a significant role in successive stages of infection, including enhanced cell attachment, biofilm formation, and chronic colonization. A research showed that the lost pathogenicity of *laf*-negative *Aeromonas* isolates was gained back after reintroduction of *laf* genes in them (Gavin *et al*., 2003). Similar to this, structurally distinct two forms of pili are found in aeromonads, one is small and stiff similar to type I and Pap pili present in *E. coli.* This is more commonly found. The other one is long and curved type IV pili. The type IV pili can be of two subtypes, those linked with bundle forming pili (Bfp), which seem to help in attachment to intestinal lining, and another form, known as type IV *Aeromonas* pilus (Tap), formed by the expression of a cluster of genes known as *tapABCD* (Martin-Carnahan and Joseph, 2005). While there is considerable research data that suggests bundle forming pili to be important factors in intestinal colonization, there presently is no sufficient evidence to suggest a similar function of Tap (Kirov *et al.,* 2000).

Formation of biofilm in aeromonads seems to be enhanced by their quorum sensing abilities (Lynch *et al*., 2002). Many, if not all species of *Aeromonas,* carry *luxRI* homologs which encodes an acyl-homoserine lactone (acyl-HSL)-dependent transcriptional activator (Jangid *et al.,* 2007). Mutation done in the *luxS* gene of an *A. hydrophila* clinical isolate, strain SSU, changed formation of biofilm to a great extent and increased virulence potential of a septicemic mouse model but did not significantly alter the production of cytotoxin or hemolysin or the function of Type three secretion system (TTSS) (Kozlova *et al.,* 2008). In the diarrheal isolate, SSU,

the production of one or more enterotoxin is controlled by quorum sensing and production of lactone along with TTSS. This could be concluded from increased enterotoxin production with increased bacterial cell density increased (Sha *et al.,* 2005). Once able to colonize the lumen of the human gut, *Aeromonas* can evidently cause diarrhea by producing toxins in the intestine and inflammation of the intestinal lumen, or by invading the epithelial cells of the intestinal lining leading to more severe forms of the disease. It is found that simultaneous production of both enterotoxins and invasins is also feasible (Janda and Abbott, 2010). These molecules can be categorized into toxins with cytolytic activity on erythrocytes and cytotonic toxins targeting the intestinal cells. Hemolysin (also called Bernheimer's aerolysin) produced by *Aeromonas hydrophila* is the most effective and widely studied of these toxins. Two/third of *A. hydrophila* strains possesses this toxin which has pore forming property. It is also present in many other species of *Aeromonas* like *A. veronii, A. sobria*, *A. caviae* and *A. trota.* Another group of hemolysins (AHH1) show amino acid sequence similar to the HlyA hemolysin expressed by *Vibrio cholerae* (Janda, 2001). Most of the species of *Aeromonas* (*A. veronii*, *A. jandaei* and *A. trota*) express HlyA in different frequencies and it is invariably found in *A. hydrophila* (Heuzenroeder *et al.,* 1999). Another cytotoxic enterotoxin in *Aeromonas* is Act. It is a type II pore-forming toxin showing hemolytic activity. Many other toxins or virulence factors have been found to play roles in gastrointestinal disease pathogenesis induced by *Aeromonas*. Species of *Aeromonas* express two different cytotonic enterotoxins one of which is heat-labile known as Alt and a heat-stable cytotonic enterotoxin designated as Ast (Sha *et al.,* 2002). Another toxin isolated and purified from certain strains of *A.veronii* bv. sobria was approximately a 60-kDa non-hemolytic enterotoxin whose mode of action is like a serine-protease which causes apoptosis in Vero cells (Martins *et al*., 2007). Antibodies produced against aerolysin can partially neutralize this toxin (Janda and Abbott, 2010).

2.6.2. Wound infections

Data available regarding the *Aeromonas* pathogenicity with respect to wound infections is insufficient. Although data regarding *Aeromonas* is not enough, it is probable that the virulence factors and their application in establishing the pathogenicity of *Aeromonas* is similar to another Gram-negative wound pathogen, *P. aeruginosa*. The process by which *Aeromonas* is likely to cause superficial or deep-

seated wound infections with probable systemic extension includes three major steps which are as follows:

- Attachment of aeromonads to the site of a wound and their initial colonization.
- Production and secretion of protease enzymes by the aeromonads and breakdown of proteinaceous material of the host which is then used as a source of energy by the bacteria for multiplication.
- Migration of *Aeromonas* into deeper tissues of the host towards areas that have a higher protein concentration through chemotactic movement.

Numerous factors are involved in this process. Along with adhesive factors required in the first step, aeromonads express a wide range of microbial proteases showing proteolytic activity on complex connective tissue and serum proteins (Janda, 2001). Breakdown of these tissues and proteins provide energy to the bacteria for further multiplication. With the depletion of nutrient sources, a chemotactic gradient is developed, with higher to lower protein concentrations from deeper to superficial tissues that are already colonized by *Aeromonas*. Most *Aeromonas* species (80% to 95%) show chemotactic motility in response to proteins, amino acids or mucins (Janda, 1985). Such directional chemotactic movements would lead to rapid migration of aeromonads into subcutaneous tissues, leading to their migration to the tissues which are rich in nutrients. Apart from this many other factors like quorum sensing and TTSS also probably play significant roles in wound infections.

2.6.3. Septicemia

Most commonly, primary *Aeromonas* septicaemia occurs when aeromonads from the gastrointestinal tract invade the circulatory system. Secondary cases occur due to the transfer of aeromonads from infected wounds, peritonitis, or biliary disease into the bloodstream. Normal and immunocompromised mice models are inoculated intraperitoneally which showed an association of secondary *Aeromonas* bacteremia with peritonitis.

It is not likely that proteins such as toxins affecting the intestine or worldwide regulatory systems (TTSS or quorum sensing) are the only observable virulence factors explicitly connected with bacteremia. The host applies several defense mechanisms that pathogens have to overcome to survive and proliferate in

extraintestinal spaces. It is observed that all *Aeromonas* species are not associated with septicemia, and ~90% of the infections are caused by a small number of genomospecies. Among the species responsible for septicemia, specific strains with certain lipopolysaccharide (LPS) antigens which act as specific markers are likely to be responsible for most of the blood-borne diseases. Studies show that *Aeromonas* of the serogroups O:11, O:16, O:18, and O:34 (Sakazaki and Shimada scheme, 1984) are the causes for most of the cases of systemic pathogenesis. Thus, LPS antigens and their composition are significant in determining the virulence potential of *Aeromonas* (Janda *et al*., 1994). Due to the presence of LPS or the Surface (S) layers, the virulent *Aeromonas* species can escape the lytic effects applied by the classical complement pathway (Janda *et al.,* 1994; Merino *et al*., 1996). There is rapid degeneration of C3b and the final components of the classical complement pathway fail to come together. Thus, the lytic membrane complex fails to form. It has been observed in a study that *Aeromonas* isolates are poorly phagocytized by J774 macrophage cell line irrespective of their species designation. However, it was observed in this experiment that the scavenging of bacterial isolates by the machophages was much better in case of *A. caviae* than that of *A. veronii* bv. veronii and *A. hydrophila*. The bacteria could continue with their multiplication for three hours even after being uptaken by the J774 cells in approximately 1/3rd of the bacterial isolates. Thus it can be concluded that *Aeromonas* strains have a mechanism of their own to resist the intracellular fatal consequences which help them to survive in the host body (Krzyminska *et al*., 2008).

2.7. Genes and proteins involved in virulence

The mechanism of *Aeromonas* pathogenesis is quite complex and not well understood yet. Multiple factors are responsible for the virulence caused by *Aeromonas.* The virulence factors which have been recognized in *Aeromonas* are enterotoxins, haemolysins, cytotoxins, proteases [serine protease (AspA), elastase (AhpB)], lipases (Pla and Plc, Sat), DNAses and adhesins [type IV pili, polar flagella (FlaA and FlaB)] (Agarwal *et al.,* 1998; Cascon *et al*., 2000; Rabaan *et al*., 2001). The *Aeromonas* strains isolated from water have been found to carry many of these virulence factors (Handfield *et al*., 1996; Kuhn *et al.,* 1997b; Janda and Abbott 1998; Kingombe *et al.,* 1999; Schubert, 2000; Sechi *et al*., 2003). Among these virulence factors, five of them are directly involved in the pathogenesis of the bacteria. This has

been proved by gene disruption techniques in animal models or cell lines. The five factors directly involved in the pathogenesis of *Aeromonas* are the enterotoxin (Act), aerolysin (AerA) (Chopra *et al*., 1994; Chopra and Houston, 1999; Xu *et al.,* 1998; Sha *et al.,* 2002) enterotoxin (Ast) (Chakraborty *et al*., 1986; Sha *et al*., 2002), elastase (Cascon *et al.,* 2000), and flagellin (Rabaan *et al.,*2001).

2.7.1. Cytotoxic enterotoxin (act), haemolysin (hlyA)/ aerolysin (aerA)

The *act* gene of *Aeromonas hydrophila* encodes for cytotoxic enterotoxin which has cytotoxic, haemolytic as well as enterotoxic roles (Xu *et al.,* 1998; Chopra and Houston 1999). Other species of *Aeromonas* have haemolytic activity due to the expression of other genes, namely *hly*A and *aer*A and some strains of *Aeromonas* may have more than one of these genes (Howard *et al*., 1987; Kozaki *et al.,* 1989; Hirono and Aoki 1991; Heuzenroeder *et al*., 1999).

2.7.2. Cytotonic enterotoxins (ast, alt)

The crypts and villi of the small intestine are not degraded by cytotonic enterotoxins (Chopra and Houston 1999). But these factors play a role in causing diarrhea. Knockout mutations in either of the two genes expressing the cytotonic enterotoxins of *Aeromonas hydrophila*, and administration of these mutated strains into mice models showed significantly less collection of fluid in the ligated ileal loop of the mice models as compared to the ones administered with the wild type of *Aeromonas* strains (Sha *et al*., 2002).

2.7.3. Elastase (ahpB)

The elastase, a zinc metalloprotease, expressed by the *ahp*B gene is an important virulence factor in the establishment of the pathogenesis of the bacteria (Cascon *et al*., 2000). Three elastase genes have been recorded in the Genbank database. Two of them are from *Aeromonas caviae* (accession nos AB022174 and ABO24302) and one from *Aeromonas hydrophila* (accession no. AF193422).

2.7.4. Flagella

Most of *Aeromonas* species and all of the species of bacteria which are recognized as human pathogens are motile due to the presence of polar flagella. Motility is a very important virulence factor for *Aeromonas* (Rabaan *et al*., 2001).

Polar flagella are made up of two flagellin subunits Fla A and Fla B expressed by the genes *fla*A and *fla*B respectively. The two genes have been cloned and sequenced from *Aeromonas salmonicida* (Umelo and Trust, 1997; Rabaan *et al*., 2001). Mutations in these genes resulted in an absolute loss of motility of *Aeromonas* and its ability to adhere to human HEp-2 cells.

2.8. Antimicrobial susceptibility

The overall sensitivity profile for members of the genus *Aeromonas* shows that inducible chromosomal-lactamases are the dominant resistance mechanism for most aeromonads. Along with this expression of metallo-β-lactamases which are active against carbapenems is also a reason for concern (Janda, 2001; Zhiyong *et al.,* 2002). Pathogenic species of *Aeromonas* and *Plesiomonas* which are less abundant are identified following the rules given by Clinical and Laboratory Standards Institute (CLSI) (Jorgensen and Hindler*,* 2007). The susceptibility of the aeromonads to different antibiotics is not species-specific and it does not depend on their isolation sites (Kampfer *et al***.,** 1999). However, the sensitivity of anti-folates or few β-lactamase–inhibitor combinations, like amoxicillin-clavulanic acid may differ from this rule (Zhiyong *et al*., 2002). The effect of therapeutically active drugs against *Aeromonas* isolates does not seem to be dependent on species designation.

2.9. Antibiotics and their classification

The word antibiotic has come from two Greek words, ‘anti’ which means against and ‘bio’ which means life. Antibiotics are against life in regard to harmful microbes but pro-life with regard to human beings. The term antibiotic was used for the first time by Selman Waksman to describe a type of antimicrobial secretion from environmental microbes that has antagonistic effect on the growth of other microbes (Clardy *et al*., 2009). However, in the modern era this definition of antibiotic has been updated to include antibiotics produced by synthetic means. Although antibiotics or antimicrobials refer to antibacterial compounds which means those which have an antagonistic effect on the growth of other bacteria, antibiotics can be classified as antibacterials, anti-fungals and anti-virals (Brooks *et al*., 2004; Russell, 2004).

The period 1940 to 1960 is referred to as the golden era of antibiotics. But research has revealed traces of antibiotics from human skeletal remains long before this time. Traces of tetracycline have been found from the skeletal remains of the ancient Nubia in Sudan (Bassett

et al., 1980; Nelson *et al*., 2010). Another study revealed tetracycline remains along with fluorochrome labelling in histological samples of the late Roman period (Cook *et al*., 1989). There is a possibility of tetracycline intake in the diet during this period which may be the cause of less infectious diseases documented during that time and no trace of infection in the skeletal samples obtained from Dakhla Oasis (Armelagos, 1969; Cook *et al*., 1989).

In **Table 2.2**, antibiotics are classified based on their chemical structure. A brief description of how they work and the medical fields where they are applied are also highlighted.

Table 2.2. Classification of antibiotics.

Class of Antibiotic	Chemical Structure	Some members	Mode of action	Uses	References
Beta-lactam	3-carbon and 1-nitrogen ring	Penicillins (PenicillinG, oxacillin, ampicillin, etc) Cephalosporin (Cefalexin, cephapirim, cefovecin, ceftaroline, etc) Monobactam (Aztreonam) Carbapenems (Imipenem, meropenem, faropenem, etc)	Interferes with the synthesis of peptidoglycan layer of bacterial cell wall	Treatment of skin and soft tissue infections, streptococcal pharyngitis, bacteraemia, endocarditis, surgical prophylaxis, etc	Etebu and Arikekpar, 2016; Khan, 2018
Macrolide	14 to 16-membered macrocyclic lactose rings attached with deoxy sugars L-cladinose and D-desosamine	Erythromycin, azithromycin, clarithromycin, clindamycin, etc	Inhibit translation in bacteria	Treatment of respiratory, skin, soft tissue and some sexually transmitted infections, etc	Etebu and Arikekpar, 2016; Khan, 2018
Tetracycline	4 hydrocarbon rings	Chlorotetracycli-n, oxytetracycline, doxycycline, etc	Inhibit translation in bacteria	Treatment of malaria, elephantiasis, amoebic parasites and rickettisia, etc	Etebu and Arikekpar, 2016
Quinolone	2 to 3 hydrocarbon rings	Nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin,	Interferes with bacterial DNA	Treatment of UTI, respiratory tract	Etebu and Arikekpar, 2016

		etc	replication and transcriptio n-n	infections and systemic infections, etc	
Aminoglycoside	Compounds having 3 amino sugars bonded by glycosidic bonds	Streptomycin, kanamycin, gentamycin, netilmicin, amikacin, etc	Inhibit translation in bacteria	Treatment of tuberculosis, bubonic plague, tularaemia and pseudomonas infections in cyctic fibrosis patients, etc	Etebu and Arikekpar, 2016; Khan, 2018
Sulphonamide	Sulphur compound similar to p-Amino benzoic acid	Sulphadiazine, sulphamethoxaz -ole, trimethoprim, etc	Inhibit the production of nucleic acids	Treatment of septicemia, tonsillitis, bacillary dysentery, meningococc al meningitis, and some UTIs, etc	Etebu and Arikekpar, 2016
Glycopeptide	2 sugar molecules bound to a cyclic peptide consisting of 7 amino acids	Vancomycin, etc	Inhibit peptidoglyc an synthesis	Treatment of pseudomemb-ranous colitis and a number of life threatening infections caused by Gram positive bacteria, etc	Etebu and Arikekpar, 2016
Oxazolidinone	Organofluorine compound consisting of a fluorine, phenyl and acetamidomethy l group	Linezolid	Inhibit translation in bacteria	Treatment of respiratory tract, surgical and skin infections, etc	Etebu and Arikekpar, 2016

Antimicrobial substances can be broadly grouped under two categories i.e. bacteriostatic and bactericidal. Those antimicrobials that inhibit the growth of other bacteria are termed bacteriostatic and those that kill other bacteria are termed bactericidal (Walsh, 2003). Antibiotics can be classified in several ways the most

common way is based on their chemical structures, mode of action and activity spectrum (Calderon and Sabundayo, 2007). Based on the differences in their molecular structures, antibiotics can be classified as beta-lactams, tetracyclines, macrolides, aminoglycosides, quinolones, glycopeptides, sulphonamides, oxazolidinones (Van Hoek *et al*., 2011; Frank and Tacconelli, 2012; Adzitey, 2015).

2.10. Sources which add antibiotics to the environment

Antibiotics hold a significant position in the treatment of infectious diseases in agriculture, aquaculture, livestock and humans. The release of effluents from the pharmaceutical industries, animal farms, aquaculture units, agricultural lands, hospitals and cities are the main sources that add antibiotics and their metabolites to our soil and water. This happens due to incomplete metabolism, non-judicious use and improper disposal of antibiotics. Effluents from wastewater treatment plants (WWTP) and leakage of sewage also contribute to antimicrobial compounds in the terrestrial and aquatic ecosystems. Increased use of antibiotics for human, animal and agricultural benefits leads to their accelerated release into the environment (Nielsen *et al*., 2018). Antibiotic consumption by humans and the percentage of each antibiotic consumed varies in different parts of the world. Also, intake of antibiotics with or without prescription also varies from country to country (Molstad *et al*., 2002). Antibiotics are used to promote growth and feeding efficiency in animal husbandry (Cowieson and Kluenter, 2019). Antimicrobial compounds have been found in effluents from hospitals and WTTP (Barancheshme and Munir, 2018), veterinary and pharmaceutical industry discharges (Obayiuwana *et al*., 2018) soil, WWTP sludge, surface water and groundwater (Zhang *et al*., 2018). Discharge from dairy industries, poultry farms, municipal wastes and animal excreta (Pruden *et al*., 2013). Inappropriate ways of discarding unused or expired antibiotics directly into the sewage system or dumped into landfills or accidental spills from the pharmaceutical industries also contribute to the contamination of water and soil (Akici *et al*., 2018). The antimicrobial compounds persist in the environment and lead to the development of resistance against them among the environmental microbes (Kim *et al*., 2018). Soil and water pollution due to the addition of antimicrobials has turned out to be a global threat. The unparalleled advantage of antibiotics in the medical field cannot be denied (Berger *et al*., 2018) but antimicrobial residues have become widespread in the

aquatic environment which makes water security a matter of concern for the policymakers. Even trace amounts of antibiotics found in the surface waters pose a great challenge for assessment of water quality because of their noxious impact on non-target biota (Vasiliadou *et al*., 2018). The discovery of antibiotics has revolutionized the entire treatment process of infectious diseases but its overuse has posed a negative impact on both the terrestrial and aquatic ecosystems (Leung *et al*., 2012). The primary failure of antimicrobials is the development of antibiotic resistance in bacteria as well as other organisms (Tacconelli *et al*., 2018).

2.11. Mechanism of antibiotic resistance

The expression of one or more unrelated inducible β-lactamases which is active against a wide variety of β-lactam antibiotics including cephalosporins, penicillins, and extended-spectrum cephalosporins is a matter of grave concern in antimicrobial susceptibility testing of *Aeromonas.* The three main groups of β-lactamases expressed in *Aeromonas* species, are a class B metallo-β-lactamase (MBL), a class C cephalosporinase and a class D penicillinase (Libisch *et al*., 2008). *Aeromonas* strains producing β-lactamases can be categorized into five groups i.e. complicated *A. hydrophila* strains which produces class B, C, and D β-lactamases, *A. caviae* strains which produces class C and D β-lactamases, strains of *A. veronii* group which express class B and D lactamases, strains of *A. schubertii* which produce class D lactamases and strains of *A. trota* which express class C β-lactamases (Fosse *et al.,* 2003). Many *A. veronii* bv. sobria isolates have been reported to express a class C cephalosporinase.

A single strain of bacteria can carry up to three classes of β-lactamases which work together in a coordinated manner (Walsh *et al.,* 1997). Class C cephalosporinases of the AmpC family are mostly resistant to some cephamycins and extended-spectrum cephalosporins. They are also not inhibited by β-lactamase inhibitor compounds either (Fosse *et al.,* 2003). DNA sequence expressing Class D penicillinases are homologous to the oxacillinases (OXA) and can hydrolyse cloxacillin and carbenicillin better than benzylpenicillin (Rasmussen *et al*., 1994). Rarely, sporadic *Aeromonas* infection cases have been reported where the causative strain of bacteria had a class A β-lactamase of the TEM family of Extended-spectrum

β-lactamases (ESBLs), a trait typically associated with the family *Enterobacteriaceae*. TEM has been named after the name of an an Athenian patient (Temoneira), from whose feces culture *E. coli* and *Salmonella* isolates were derived encoding TEM-1 and TEM-2 in 1963 (Ruiz, 2018).

The most common metallo-beta-lactamase expressed by *Aeromonas* is of the "CphA" type, whose genes are commonly found in strains of *A. hydrophila* and *A. veronii* (Walsh *et al.,* 1997). Two other (matallo β-lactamases) MBLs known as (Verona Integron- encoded Metallo-β-lactamase) (VIM) and (imipinimase) (IMP) have been detected in strains of *A. hydrophila* and *A. caviae*. The gene expressing the VIM was found on an integron and the one encoding the IMP was detected on a plasmid (Libisch *et al*., 2008). In both the cases, these MBL-expressing strains were resistant to most β-lactam antibiotics, including cefepime, imipenem, ceftazidime, and piperacillin-tazobactam; both strains were sensitive to aztreonam when tested *in vitro*.

A study has reported NDM-1 (New Delhi Metallo-β-Lactamase) gene in aeromonads isolated from environmental samples of New Delhi (Walsh *et al*., 2011). The spread of mobile NDM-1, a carbapenemase, among bacterial pathogens is of great concern. These enzymes confer resistance to carbapenems and other β-lactam antibiotics. Pathogens possessing NDM-1 are resistant to multiple antibiotic classes, which renders a very few treatment options amenable (Walsh, 2010; Livermore, 2009). This concern is certainly warranted for Enterobacteriaceae that produce NDM-1 β-lactamase (Yong *et al.*, 2009). Plasmids having the sequence expressing for this carbapenemase, *bla*NDM-1, can carry up to 14 other antibiotic resistance genes which can transmit this drug resistance to other bacteria, giving rise to multidrug-resistant phenotypes (Kumarasamy *et al*, 2010). The resistance of this scale could have serious public health implications because so much of modern medicine is dependent on the ability to treat infection (Livermore, 2009; Bonomo, 2011; Moellering*,* 2010).

Aeromonas strains are almost universally susceptible to fluoroquinolones. In a study strong chromosomal resistance was detected in *Aeromonas caviae* strains against antibiotics like nalidixic acid, ciprofloxacin, and norfloxacin (Sinha *et al.,* 2004). In a study, two mutations caused in the *gyrA* gene and one mutation in the *parC* gene was observed to impart resistance to fluoroquinolones in *Aeromonas* isolates. Resistance to quinolone resistance was also reported to be associated with the 218-amino-acid QnrA protein expressed by genes present in a plasmid. Another

quinolone resistance gene *qnr*S has been reported in *A. caviae* and *A. media* isolated from natural water bodis and in *A. veronii* isolated from hospital (Sanchez-Cespedes *et al*., 2008). The *A. veronii* isolated from clinical source was resistant to multiple antibiotics such as nalidixic acid, ciprofloxacin and levofloxacin.

2.12. Role of plamids, integron systems and transposons in disease transmission

Aeromonas spp have been regarded as "emerging pathogens" that possess multi-factorial virulence genes and systems. Bacteria employ various mechanisms of antibiotic resistance. These include target substitution, target protection, antibiotic detoxification and blocking intracellular invasion of antibiotics. Bacterial conjugative plasmids, integron systems and transposable elements are the array of platforms on which they rely for their resistance to antibiotics. Plasmids specifically serve as a platform on which functional resistance genes are collected and subsequently dispersed (Dowson *et al*., 1989; Bennett, 2008).

Plasmid profiling and molecular characterization of aeromonad plasmids were undertaken by several research groups in order to address the problems of generation and transmission of antibiotic resistance genes (Toranzo *et al*., 1983; Rhodes *et al*., 2000; Majumdar *et al*., 2006). Toranzo *et al*. (1983) identified plasmids, with molecular weights ranging from $3.4x10^6$ to $30x10^6$, from *A. salmonicida* and *A. hydrophila* which were isolated from diseased fishes. Twenty-eight (28) out of the thirty-eight (38) bacterial isolates obtained during the study carried one or more plasmids. Plasmid curing showed loss of tetracycline resistance in *A. hydrophila* which proved the role of plasmid in tetracycline resistance. Rhodes *et al*. (2000) obtained oxytetracycline and tetracycline resistant *A. hydrophila* and *A. veronii* bv. sobria isolates from hospital effluents and fish farm samples. Plasmids obtained from the hospital derived isolates were much smaller (5.2 to 5.5 Kb) than those obtained from fish-farm isolates. The tetracycline resistant gene was located on a Eco RI restriction site of 5.5 Kb which could be transferred to *E. coli* (JM109) through conjugation. Majumdar *et al*. (2006) identified a 21 Kb plasmid in *Aeromonas hydrophila* isolates and found this plasmid to induce pathogenic potential in these bacterial isolates. Research in this area also dealt with the characterization of species of *Aeromonas* isolated from silurid and cyprinid fishes suffering from epizootic

ulcerative syndrome (EUS) and a plasmid having low molecular weight has been seen to be involved in the cause of EUS in fishes (Pradhan and Pal, 1990; Majumdar *et al*., 2006; Majumdar *et al*., 2007). Thorough research through the past 27 years has proved that there is a gradual rise in antibiotic resistance in the aeromonads (Pradhan and Pal, 1993; Saha and Pal, 2002; Das *et al*., 2009). High resistance to antibiotics like erythromycin, rifampicin, novobiocin, and sulphadiazine in aeromonads obtained from fishes affected with ulcerative disease in 2008 (Das *et al*., 2009) shows a similar scenario in environmental *Aeromonas* isolates (Pal and Bhattacharjee, 2011). Alkaline lysis is a very efficient method of plasmid isolation for such studies (Freitas *et al*., 2018). Plasmids have been detected in *Aeromonas* isolated from environmental samples such as wastewater treatment plant (Kim *et al*., 2017), river sediment samples (Hu *et al*., 2019), river water (Ngoci *et al*., 2012), diseased fishes (Han *et al*., 2012; Freitas *et al*., 2018), etc in different parts of the world.

Plasmids are reported to be involved in the transfer of antibiotic resistance genes to other bacterial isolates in many studies (Adams *et al*., 1998; Kim *et al*., 2017; Hu *et al*., 2019). Bacterial conjugation (Adams *et al*., 1998) and transformation (Kim *et al*., 2017; Hu *et al*., 2019) are the commonly used techniques for these experiments. Mega-plasmids have been isolated from *Aeromonas* isolates which are not involved with the transfer of antibiotic resistance or heavy-metal resistance transfer to other bacterial isolates (Freitas *et al*., 2018). In contrast to bacterial conjugative plasmids, which tend to be larger, mobilizable resistance plasmids tend to be relatively smaller (~10 to 20 kb) and encoding only a handful of genes including the resistance gene(s) (Bennet, 2008). Therefore, small-sized plasmids with multi-drug resistance genes present in environmental *Aeromonas* isolates may indicate a possible threat to human beings and aquaculture (Pal and Bhattacharjee, 2011).