Chapter 2: Review of literature..

2. Review of Literature

2.1 Indian Aquaculture scenario

Aquaculture practices involve the farming of various aquatic animals and plants species. Indian aquaculture systems have a vast potential and a promising role in developing worldwide aquaculture production (de Jong, 2017). According to DADF 2016, India have 195.20 kilometer area of river and canals, 29.2 lakhs hectare (ha) of reservoirs, 4.3 lakhs ha of freshwater ponds, 11.5 lakhs ha brackishwater ponds and around 8 lakhs ha wetlands. In aquaculture production, India ranked second after china and it is having around 10% of the world fish diversity (Ayyappan, 2007). In the year 1950-51, India was having an annual aquaculture production of around 0.75 MT which had showed a 10 fold remarkable increase up to 9.6 MT by 2013-14.

Indian aquaculture system plays vital role in socioeconomic development as it provides income for fishermen and also supplies the cheapest source of nutritious food (Gopalakannan and Arul, 2006; Ayyappan and Krishanan, 2004). Indian aquaculture system has three types of water *viz.* freshwater, brackishwater and marine water aquaculture. However, 95% of total aquaculture of India is dominated by freshwater aquaculture (FAO, 2016). Freshwater aquaculture mainly comprises of 2.3 million ha area of tanks and ponds. Freshwater aquaculture practices mainly include the culture of carps, Catfishes, tilapines, pangasius and freshwater prawns. However, IMCs; *Labeo rohita*, *Labeo catla* and *Cirrhinus mrigala* controls 70- 75% of entire fisheries production. Rest 25- 30% of freshwater production is contributed by other fish species like common carps, catfishes, tilapines, grass carps, silver carps etc. The three major states involved in freshwater aquaculture are West Bengal (0.276mha), Karnataka (0.41 mha) and Andhra Pradesh (0.52 mha). These states contribute around 50% of country’s aquaculture area. Orissa, Bihar, Assam, Punjab, Jharkhand, Maharashtra and Haryana also contribute in freshwater aquaculture production (Mishra et al., 2017). As per Press Information Bureau, 2017, by 2020 India is planning to achieve the productivity mark of 15 MT and also to increase its productivity by 8% of annual growth rate. However, over stocking of fish, poor-farming, farmer with limited knowledge, over exploitation and over feeding is leading to poor water quality resulting in chronic stress in farmed fishes. All together, this is resulting is widespread of various new epizootic diseases (Sebastião et al., 2015; Mishra et al., 2017).

2.2 Diseases in aquaculture

Disease occurs when immune system of host is compromised due to alteration of body function. Diseases are the main barrier and a major constrains towards successful farming. In comparison with other live stocks, fishes are highly prone to diseases leading to set back of socio- economic development of the country (Sahoo et al., 2013). According to Sahoo et al., 2013 in India, the production cost increases by 5- 10 % due to incidences of diseases. In aquaculture farms, some infectious diseases had just not only damaged the livelihood of the fish farmers but also shattered the future development of the industry (Subasinghe et al., 2001; Bondad- Reantaso et al., 2005). According to Snieszko (1974), a complete interaction between pathogen, host and environment plays a vital role for the progress of diseases (Fig. 1). In present scenario of aquaculture system, intensification of the culture system by farmers is practiced without maintaining the balance between the host, pathogen and environment (Subasinghe et al., 2001; Bondad- Reantaso et al., 2005). Combination of various environmental stresses along with these factors will ultimately lead to development of diseases in fish species. Stressors are combination of various activity, stimulus, events or factors which will lead to development of tension or stress in an organism. Usually stressors for fishes are divided into four types as depicted in the schematic diagram (Fig. 2) (Datta and Kumar, 2010). Most of the diseases occurring in fishes are due to stress. According to selye, 1936, “stress” is explained as sum of total physiological reaction by an organism in response to any altered physical and chemical process so that it can keep its normal metabolism. Stress does not allow the host to maintain its normal state.

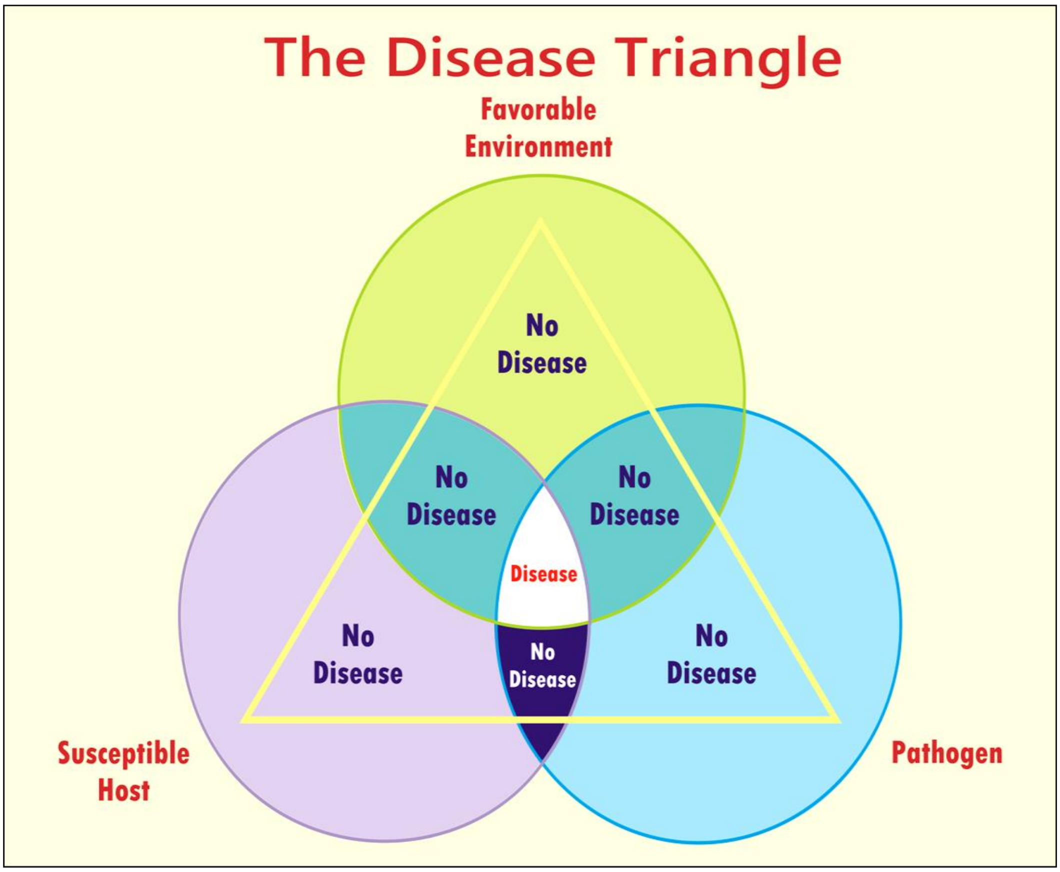

Fig. 1 Conceptual diagram showing relationship between hosts, environment and the pathogens for development of diseases.

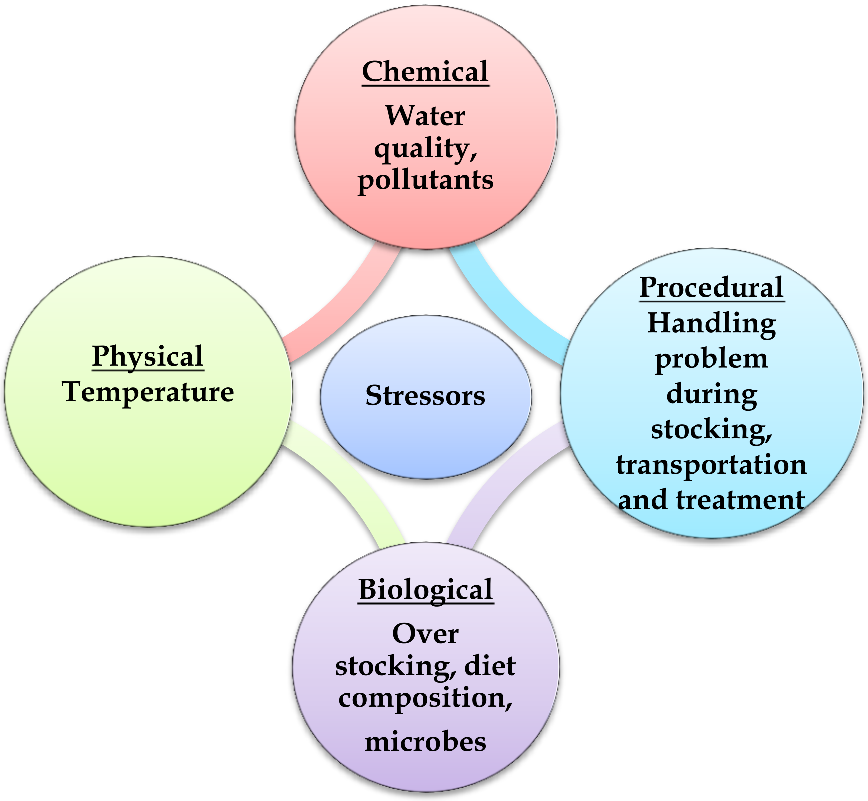

Fig. 2 Different types of stressors in aquaculture farms

2.3 Dynamics of Fish Diseases

The process of infection and transmission of diseases in fishes are familiar with humans and other terrestrial organisms. Environment always plays critical role in the development and spreading of any kind of infection. In case of fishes, aquatic environment facilitates the transmission of various kinds of diseases. The method of transmission of diseases in fish is represented in Fig. 3.

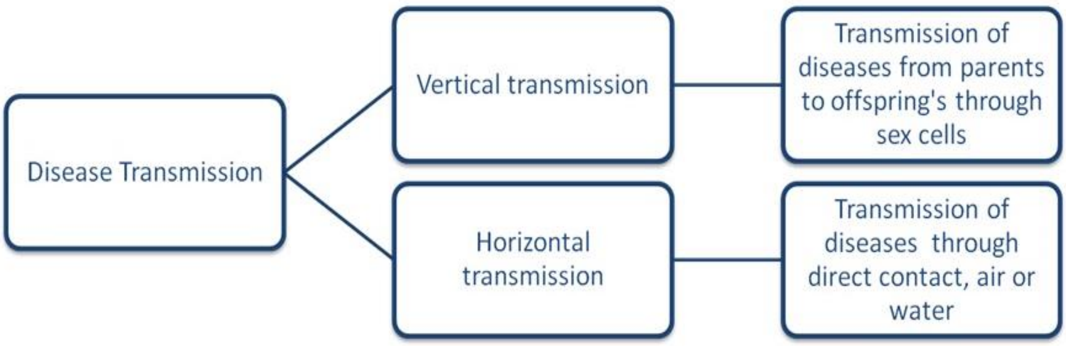

Fig. 3 Types of disease transmission in fish

A complex series of events lies behind the exposure and development of any diseases. The most important parameters responsible for development of any kind of diseases includes the virulence of invading pathogen, host immune system, environmental stress and population density (Nicolson, 2006).

2.4 Types of diseases

Broadly fish diseases can be grouped into two types-

- Non infectious diseases
- Infectious diseases

2.4.1 Non infectious diseases

Non living components are the etiological agents for causing non infectious diseases. The non infectious diseases are not contagious in nature. It usually occurs due to adverse environmental condition, genetic disorder or nutritional disorder (Erazo- Pagador, 2001).

2.4.1.1 Environmental conditions

Alterations in physiochemical condition of aquaculture farm water leads to development of environmental diseases. Environmental problems like depletion of DO (Dissolved oxygen), extreme alkaline or acidic pH, high nitrite, high ammonia, toxicity etc. will lead to development of stress will ultimately results in diseases (Martins et al., 2015).

2.4.1.2 Nutritional diseases

Presence of excess or low nutrient in fishes causes nutritional disorder in fish. Nutrients like protein, vitamins, and lipids are most crucial for the proper growth of organism. Symptoms of nutritional diseases include slow growth, lower fecundity, abnormal body development, susceptibility to pathogens and morbidity (Idowu et al., 2017). Examples of nutritional deficiency include scurvy, broken back syndrome, hepaticlipidosis, white fat disease, hyponatremia etc (Amar et al., 2004; Shefat and Karim, 2018). However, the disease condition can be reverted to normal by providing adequate amount of nutrients (Francis-Floyd and Wellborn, 1991).

2.4.1.3 Genetic Disorder and Neo plastic abnormalities

According to Francis-Floyd and Wellborn, 1991, genetical disorders comprise of conformational changes i.e. the absence and presence of an extra tail. Usually genetic disorders have very minimal effect, however using a broodstock having a genetic abnormality will lead to production of defective spawns in later generations. Neo plastic diseases occur due to abnormal generation of any organ, leading to malfunctioning of functional ability of the affected organ. The abnormal growth of tumor can be fatal or mild pathogenic (Abowei et al., 2011). In case of malignancy, the tumor usually spreads from affected organ to different body part through circulatory system.

2.4.2 Infectious diseases

Infectious or contagious diseases are defined as diseases occurred due to living pathogenic organism like bacteria, fungus, parasites and viruses. Various infectious agents are commonly present in environment. However, they can infect the fishes under suitable environmental conditions. Infection can be either externally or internally. Though internal infection cannot be diagnosed visually however, externally diseases can be diagnosed by observing the different signs like haemorrhages, red spots, ulcer etc. on the body surface. Infectious diseases are highly contagious in nature and are hard to control (Idowu et al., 2017; Sharma et al., 2012).

2.4.2.1 Bacterial diseases

In the aquaculture ponds bacteria are most commonly present in the aquaculture ponds, on the fish skin and also in the internal organs of the fish. However, when the fish is under stress they became a serious threat (Omojowo and Sogbesan, 2003). Bacteria may cause infection as a primary agent or it may act as secondary agent for causing infection. They can multiply and proliferate within a short period of time and may lead to mass mortalities in the pond. So bacteria are considered as the major reason behind the economic loss to the fish farmers (Govind et al., 2012; Madhuri et al., 2012). Bacterial pathogen behind infection & disease outbreak varies within different species. Bacterial infections usually occur when the fish immune system is compromised due to early infections by parasites, adverse quality of water, rough netting and stress (de Guzman et al., 1986). The common clinical signs and symptoms of bacteria borne diseases include reddish lesions, red spot on skin, ulcers, haemorrhages, tail rot, fin rot and colour changes. Common bacterial infections reported in fin fishes in Indian aquaculture farms have been shown in Table 1.

2.4.2.2 Parasitic diseases

Parasites are considered as an important critical problem in aquaculture farms. They aren't strict pathogens however; they are accompanied by bacteria and fungus as secondary pathogens. Fish parasites are of two types i) endoparasites (present within body) ii) exoparasites (present outside on the body surface). Parasites can be found attached to gills, muscle, body surface which causes weight loss, irritation, impaired functioning and sometimes kills the fish. Large scale parasite infection in farms causes overwhelming socio-economic loss. Commonly reported parasites in fin fishes from India have been reported in Table 1.

2.4.2.3 Fungal diseases

On comparison with the parasitic and bacterial diseases in fish, there are very few species of fungus which causes infection (Francis Floyd, 2005; Govind et al., 2012). The fungal oomycetes are commonly present in aqueous environment and under favourable condition they cause infection (Mukherjee, 2002). Fungus is opportunistic secondary pathogen. They are saprophytic in nature. They colonize on the wound already caused by bacteria or parasite. Fungal growth usually appears as white cotton patches on body surface of fishes. The cottony appearances of fungus are due to filamentous network of the hyphae. Most of the fungus causes infections on the external tissues however few funguses can infect the internal tissues (Verma, 2008). In both brackish & freshwater aquaculture, water molds were considered as most frequent fungal pathogens (Yanong, 2003). Common fungal infections reported in fin fishes of Indian aquaculture system have been reported in Table 1.

2.4.2.4 Viral diseases

Viruses are considered as obligate pathogens and they occur as particles. For replication, they depends on the synthesis of the hosts cells. Diagnosis of the viral diseases is difficult and no medicines are available for the treatment of the viral diseases (Francis Floyd, 2005). So once there is an outbreak of viral diseases in a pond, it is very hard to control the mortality of the fishes. Different fish viruses were reported for causing high mortality and morbidity within a very short period of time. However, viruses have few general characters including i) host specificity ii) temperature and climate dependent pathogenecity iii) usually younger fishes are more prone to viral infections than the older fishes. Globally more than 125 viruses found responsible for infection in fishes (Mishra et al., 2017). However, in India there are few viral infections reports in fin fishes (Table 1).

Table. 1. List of Common diseases recorded from cultivable fish species in India.

Sl. No.	Pathogen	Symptoms	References
Bacterial diseases			
1.	*Aeromonas hydrophilla*	Dropsy, haemorrhagic skin lesions, Darkness in color with hyperaemia, brown or red spot on the skin and lysis of the fins, ulcer, swimming closer to the surface, lethergy, fin haemorrhages and slower movement	Sangeetha et al., 2018, Lini, et al., 2013; Sahoo et al., 2011; Manoj et al., 2010; Jeeva et al., 2013, Harikrishnan et al., 2009; Kumar et al., 2016; Karunasagar et al., 1986
2.	*Edwardsiella tarda*	Ulcer, Darkness in colour red spotted skin, fin haemorrhages and red patches on the body, bulging of eyes	Kumari et al., 2018; Zaki et al., 2015; Basu et al., 2012; Swain et al., 2012; Mohanty and Sahoo, 2010; Swain et al., 2007
3	*Flexibacter columnaris*	Gill necrosis, skin lesions	Tripathi et al., 2003; Nayak et al., 2014; Dash et al., 2009, Sarker et al., 2017
4	*Renibacterium salmoninarum*	Darkness of the skin, gills are pale in colour, swelling of the kidney	Mastan et al., 2013; Fryer and Lannan, 1993; Eissa and Faisal, 2014; Bryníldsrud et al., 2014; Gahlawat et al., 2009
5	*Proteus spp.*	Tail rot, haemorrhages on the body along with red patches over the operculum and lower abdomen	Chauhan et al., 2015; Pattanayak et al., 2018; Takyi et al., 2012; Kumar et al., 2015; EL-Deen, 2013
6	*Pseudomonas spp.*	Red Skin Disease	Saikia et al., 2017; Tripathy et al., 2007; Mastan, 2013; Younes et al., 2015; El-Hady and Samy, 2011
7.	*Staphylococcus aureus*	Gills and fins are affected by haemorrhages.	Bujjamma and Padmavathi, 2015; Kubilay et al., 2004; Murugadas et al., 2016; Khatri et al., 2009
Parasitic diseases			
1.	*Argulus spp.*	Parasite can be seen moving on the body surface. After detachment it can cause skin lesion leading to bacterial or fungal infections.	Das et al., 2016; Patra et al., 2016; Radkhah, 2017; Petchimuthu et al., 2018; Ramudu et al., 2018; Dash, 2007

2.	*Learnea spp.*	Minute thread like appearance attached anywhere on the body. Fish become lethargic, loss of weight	Ramudu et al., 2018; Hemaprasanth et al., 2017; Dash et al., 2015; Hossain, et al., 2013
3.	*Myxobolus spp.*	Whirling diseases, White spores on the gills	Sarkar and Ghosh, 1990; Kaur and Singh, 2011; Kaur and Singh, 2011; Harpreet and Ranjeet, 2009; Kaur et al., 2014
4.	*Dactylogyrus and gyrodactylus*	Inflamed gills, excessive mucus secretion, debilitation, dyspnea and ulceration on the skin.	Ghosh et al., 1987; Dash et al., 2015; Ramudu and Dash, 2013
5.	*Trichodiniasis* spp.	Whitish cysts in skin, fins and gills; numerous infections usually identifiable as sphere-shaped organisms.	Tantry et al., 2016; Saha et al., 2017
Fungal infections			
1.	*Saprolegnia parasitica*	Cotton wool like growth on the body. Weakening of fish, ulceration and haemorrhages on the skin	Salgotra et al., 2005; Mastan and Ahmad, 2018; Al-Niaeem et al., 2015;Kumari and Kumar, 2015
2.	*Aphanomyces invadans*	Darker body then anorexia, floating on surface, large red colour shallow ulcers on the body, necrosis	Kar et al., 2000; Pagrut et al., 2017; Das and Das, 1993; Vishwanath et al., 1998
3.	*Branchiomyees sanguinis,* *B. demigram*	Redness in gills, greyish white when attack is severe, dropping of affected gill filaments.	Ibrahim, 2011; Khalil et al., 2015; Ravichandran et al., 2013; Chidambaram, 2009; Ramaiah, 2016.
Viral diseases			
1.	Cyprinid herpesvirus-2	Haemorrhages on body surface, gills and fins, necrosed gills, lepidorthosis, emaciated eyes and protruded anus	Sahoo et al., 2016
2	Koi ranavirus	Erythemic lesions on the dorsal head region and caudal peduncle	Sivasankar et al., 2017
3	Carp edema virus	Anorexia, lethargy, skin erosions, enophthalmos and gill necrosis	Swaminathan et al., 2016
4	Tilapia Lake virus	Lethargy, inappetance and skin erosions	Behera et al., 2018; Thangaraj et al., 2018

2.5 The Genus *Klebsiella*

Klebsiella belongs to Enterobacteriaceae family and the organisms were named by Trevisan (1885) in honour of Edwin Klebs (Brisse et al., 2006). Klebsiella species are generally gram negative, non- motile, aerobic or facultative anaerobic, rod shaped bacterium having width of 0.3 to 1.0 μm and 0.6 to 6.0 μm in length along with a prominent capsule (Paterson et al., 2014; Brisse et al., 2006). Members of *Klebsiella* genus consist of acidic polysaccharides on their cellular surface. The capsule guards the bacteria from different host defence mechanisms. On the cell surface of *Klebsiella,* they have two different antigens namely lipopolysaccharides (O antigens) and capsular polysaccharides (A antigens). Genus Klebsiella gives a positive reaction for Voges-Proskauer, Catalase test, urease and citrate test and shows negative reaction for Oxidase, Indole and Methyl red tests. *Klebsiella* genus comprises of many species *viz. Klebsiella pneumoniae, K. granulomatosis, K. mobilis, K. ornithinolytica, K. oxytoca, K. planticola, K. singaporensis, K. terrigena, K. trevisanii* and *K.variicola* (Euzéby, 1997; Drancourt et al., 2001). However, *K. pneumoniae, Klebsiella oxytoca and Klebsiella granulomatis* are the three species which causes serious illness in different organisms (Mandell, 2009).

Among the known *Klebsiella species, K.* pneumoniae accounts for 70- 80% of the infections (Hansen et al., 2004; García et al., 1985; Westbrook et al., 2000).

2.6 *Klebsiella pneumoniae*

In 1882, Carl Friedlander had first time narrated *Klebsiella pneumoniae*. He had isolated the bacterium from patient lungs, suffering from pneumoniae and described it as encapsulated bacillus. Earlier it was known as Friedlander's *Bacillus,* however from 1886 it was named as *Klebsiella pneumoniae* (Martin and Bachman, 2018). *K. pneumoniae* belongs to *Enterobacteriaceae* family and is gram negative, encapsulated, non motile and dome shaped (3- 4 mm diameter) bacterium (Ashurst and Dawson, 2018). It grows at a most favourable temperature of around 30- 37^{o} C, having mucoid and sticky properties depending upon the strain and composition of the media (Brisse et al., 2006). Standard biochemical tests for the characterization of *K. pneumoniae* have been found to be positive for citrate, Voges- Proskauer, lysine whereas indole, H_2S, and ornithine are standard negative test (Hansen et al., 2004; Brisse et al., 2009; Trivedi et al., 2015; Diana and Manjulatha, 2012). *K. pneumoniae* utilizes arabinose, alanine, arabitol, aspartate, cellobiose, citrate, fructose, fumarate, xylose, Omethyl-β-glucoside, galactose, gentiobiose, gluconate, glucosamine, glucose, glycerate, glycerol, myo-inositol, keto-gluconate, lactate, lactose, malate, ribose, maltose, mannitol, mannose, melibiose, proline, raffinose, serine, trehalose as sole carbon source and utilizes these carbohydrates for their growth (Brisse et al., 2009; Trivedi et al., 2015).

2.7 Infection report of *Klebsiella pneumoniae*

Klebsiella pneumoniae are ubiquitous in nature. They are usually present in nature including soil, water, humans, plants and animals (Huntley et al., 1976; Niemelä and Väätänen, 1982; Podschun and Ullmann, 1998). Earlier, *K. pneumoniae* were considered as an opportunistic pathogen. However, by the time this organism has been found to be zoonotic and emerging as a significant pathogen. There have been several reports of *K. pneumoniae* in different organisms like pneumonia, liver abscess, septic metastatic complication, infections in urinary tract, blood infection, infections at the wound site and infected blood in humans (Jarvis et al., 1985; Jarvis and Martone, 1992;Yinnon et al., 1996; Edmond et al., 1999; Chang et al., 2000, Fang et al., 2004; Magill et al., 2014; Persichino et al., 2015;Holden et al., 2016; Vading et al., 2018); severe enteritis, septicemia, dysfunction of multiple organ in dogs (Cavana et al., 2009; Roberts et al., 2000); hemorrhagic lesion, suppurative meningitis, multi systemic abscesses, ventriculitis and clinical abscesses in monkeys (Twenhafel et al., 2008; Whitehouse et al., 2010; Soto et al., 2012; Kasuya et al., 2017); septicemia in pigs (Wilcock, 1979; Williamson, 2017); acute respiratory tract infection and septicemia in camels (Sharma et al., 2013;Younan et al., 2013; Wareth et al., 2014); clinical mastitis in cows (Munoz et al., 2006; Munoz et al., 2007; Langoni et al., 2015); pleuritis, suppurative pneumonia, septicemia, meningitis purulent, broncho pneumonia, fibrino necrotizing pleuritis with pyothorax in sea lions (Jang et al., 2010; Seguel et al., 2017; Roe et al., 2015); exopthalmia, ulcer and discoloration of skin in *Nemipterus japonicas* (Diana and Manjulatha., 2012); red spots and haemorrhage on body of *Cyprinus carpio* and *O. Niloticus* (Takyi et al., 2012; Oliveira et al., 2014; Al-Imarah., 2008); haemorrhages on the skin of *Amphiprion nigripes* (Gopi et al., 2016).

2.8 Virulence property of *Klebsiella pneumoniae*

According to Brisse et al., 2006, four components *viz.* adhesion (for entry of pathogen), iron scavenging property (development of siderophores), lipopolysaccharides and capsular polysaccharides (resistant to host serum), endotoxins and exotoxins (infect the host) are mainly responsible for infection process in the host. *K. pneumoniae* possess many virulence genes including *fimA* (major fimbrial subunit encoding type 1 fimbrial adhesion), *fimH* (minor fimbrial subunit encoding type 1 fimbrial adhesion), *mrkD* (subunit of major pilus of type 3 fimbriae adhesion), *uge* (encoding uridine diphosphate galacturonate 4-epimerase), *urea* (associated with urease operon), *wabG* (responsible for external core lipopolysaccharide biosynthesis), *magA* (mucoviscosity-associated gene A), *rpmA* (regulator of mucoid phenotype), *allS* (activator of the allantoin regulation) and *ent* (iron-uptake system). (Gao et al., 2014; Brisse et al., 2006; Lascols et al., 2013; Fang et al., 2004).

The essential step for development of any infection by a bacterium is by adhesion on the host mucosal surface and in case of gram -ve bacteria this property is achieved by filamentous organelles known as fimbriae. These organelles are available on the bacterial cell surface (Struve et al., 2008). Almost every *K. pneumoniae* have the capability to produce two type of fimbrial adhesion (Struve et al., 2008). The first type is known as mannose sensitive Type 1 pili. They belong to class of chaperone–usher fimbriae family and are encoded by the *fim*ABCDEFGHIK gene (Stahlhut et al., 2012). Type 1 pilus is mainly made up of major fimbrial subunit (*fim*A) and minor (*fim*H). The second type is known as type 3 pili. The Type 3 fimbriae system is encoded by five genes (*mrk*ABCDF) and they belongs to chaperone-usher class of fimbriae (Allen et al., 1991; Langstraat et al., 2001). In type 3, the major pilus subunit is (*Mrk*A) and a minor adhesion subunit is (*Mrk*D) (Alcántar-Curiel et al., 2013; Schroll et al., 2010; Stahlhut et al., 2009). The adhesive property of Type I Fimbrie is due to the minor fimbrial subunit (*fim* H) (Choudhury et al., 1999) and it plays vital role for identification of the mannose-containing glyco- proteins present on the host tissue. Thus it allows the bacterial species to target and attach on the host tissue (De Vries et al., 1996; Martinez et al., 2000).

Along with different *Klebsiella* spp., the type 3 fimbriae have been reported in *Enterobacter*, *Citrobacter*, *Proteus*, *Serratia* and *Providencia* isolates (Stahlhut et al., 2012). They are generally 2- 4 nm in width and have 0.5-2 μm long surface organelles. They are identified by their capability of agglutination human erythrocytes (MR/ K agglutination) (Duguid, 1959; Clegg et al., 1994). Watnick and Kolter, 2000, have reported that biofilm formation by bacteria have played a promising role in development of diseases. The formation of biofilm is a complex process mediated by bacteria (O'toole and Kolter, 1998). Langstraat et al., 2001 have reported that the major type 3 fimbrial subunit (*Mrk*A) is mainly responsible for the biofilm formation. The *Mrk*A protein is hydrophobic in nature and its hydrophobicity helps in bacterial interaction thus facilitate the formation of biofilm. However the minor subunit, *Mrk*D does not have rapid and efficient biofilm formation capacity but, it is somehow essential for binding to extracellular matrix proteins (ECMPs). Thus, the function of type 3 fimbriae is specific receptor-ligand interaction with host tissues and cells and also involved in biofilm formation.

Iron is important for both hosts as well as for the microbes. Eukaryotes uses iron for transition towards S-phase of cell cycle whereas microbial species uses iron as an important nutritional factor for their growth and development. *Klebsiella pneumoniae* have low MW and higher affinity iron chelator known as siderophores specifically enetrobactin (*ent*) which helps the bacteria to grow also in iron restrictive environment. *K. pneumoniae* produces a mixture of siderophores, resulting in localization of the tissues, systemic dissemination and also have severe effect on the survivality of the host. In eukaryotic system, nitrogen is the major component in intestinal tract. The enzymes are basically associated with nitrogen assimilation (McLean et al., 1998). In nitrogen depleted environment, *K. Pneumoniae* can activate urease enzyme. The enzyme is activated through two membrane transduction mechanism consisting of NtrC (response regulator) and NtrB (modulator kinase). Maroncle et al., 2006 have shown in their experiment that mutant *K. pneumoniae* lacking urease gene (Δure) were not able to colonize in the mouse intestinal tract. Urease is also responsible for secretion of ammonia which damages the tissue and helps the bacteria to survive (Burne and Chen, 2000).

2.9 Diagnosis of *Klebsiella pneumonaie*

Primary identification of *Klebsiella pneumoniae* includes microbiological culture followed by biochemical tests. *Klebsiella pneumoniae* usually grows on any normal media like Brain Heart infusion Agar (BHI), nutrient agar (NA), tryptic soya agar (TSA), eosin methylene blue agar (EMB), Macconkey agar, blood agar, Drigalski agar, MacConkey-Inositol- Carbenicillin agar (MCIC) and Simons-Citrate agar supplemented with 1% inositol (Brisse et al., 2006; Maal et al., 2014; Ohtomo and Saito, 2003; Bagley and Seidler, 1978; Orellana and Lagos, 1996, Okonkwo et al., 2018). However, the molecular markers used for recognition of *K. pneumoniae* includes various housekeeping genes sequencing like 16S rRNA, *parC*, *gyrA*, and *rpoB* genes (Brisse and Verhoef, 2001; Drancourt et al., 2001, Das et al., 2018). PCR ribotyping, multilocus sequence typing (MLST), amplified fragment length polymorphism (AFLP)and restriction fragment length polymorphism (RFLP) were used for identification of the *Klebsiella* species (Kostman et al., 1992; Savelkoul et al., 1999; Jonas et al., 2004; Diancourt et al., 2005; Mahmudunnabi et al., 2018).