Chapter 2

REVIEW OF LITERATURE

2.1. Discovery of Salmonella

The genus Salmonella, a member of the family Enterobacteriaceae, is named after Daniel Elmer Salmon, who with Theobald Smith is credited with the discovery of the organism that caused hog cholera (Salmon and Smith, 1886), now known as Salmonella enterica subspecies enterica serotype Choleraesuis.

2.2. Evolution of Salmonella nomenclature

Salmonella nomenclature is still a matter of controversy as researchers all over the world use different systems to communicate about this genus. The nomenclature system for Salmonella has evolved from the one serotype-one species concept proposed by Kauffmann (Kauffmann, 1966) on the basis of the serologic identification of the O (somatic) and H (flagellar) antigens. Each serotype was considered as a species. These “species” were named after the host or the place from which they were originally isolated. This resulted in large number of “species”. DNA-DNA hybridization works of Crosa et al. (1973) showed that all of them except Salmonella bongori, were related at the species level and therefore belonged to a single species. Even though Salmonella Choleraesuis, being the approved type species (Skerman et al., 1980) had priority as the species name, it did not gain much support. It was biochemically different from the major serotypes like Salmonella Typhi and Salmonella Enterica, being arabinose and trehalose negative (Kauffmann and Edwards, 1952).
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In 1986, the taxonomic subcommittee on Enterobacteriaceae of International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology, Manchester, U.K., (Penner, 1988) unanimously supported the suggestion of accepting *Salmonella enterica* as the type species. Le Minor and Popoff (1987) made a request for an opinion, designating *Salmonella enterica* as type and only species of its genus and LT2 as the type strain of that species, to the Judicial Commission of the International Committee of Systematic Bacteriology. The commission had denied the request on safety grounds. The commission was reluctant to reduce the highly pathogenic *Salmonella typhi* to only a serovar (*Salmonella enterica* subsp. *enterica* serovar Typhi) (Wayne, 1991) as this can lead to neglect by the clinicians. However, the commission decided to reopen the original request for an opinion with alternative proposals. Le Minor and Popoff (1987) pointed out that *Salmonella bongori*, previously known as subspecies V, is considered as a separate species (Reeves et al., 1989).

The antigenic formula of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (WHO Collaborating Centre). Based on somatic (O) and flagellar (H) antigen there are more than 2600 serotypes of *Salmonella* which are included under the two species.

The nomenclature system used at the Centers for Disease Control and Prevention (CDC) (Brenner et al., 2000) is now widely accepted for communicating research findings in scientific publications. The CDC system divides the genus *Salmonella* into two species; *Salmonella enterica*, the type species and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies which are referred to by a Roman numeral and a name.
(I, *Salmonella enterica* subsp. *enterica*; II, *Salmonella enterica* subsp. *salamae*; IIIa, *Salmonella enterica* subsp. *arizonae*; IIIb, *Salmonella enterica* subsp. *diarizonae*; IV, *Salmonella enterica* subsp. *houtenae*; and VI, *Salmonella enterica* subsp. *indica*). Subspecies include serotypes which are differentiated by antigenic properties. The name usually refers to the geographical area from which it was first isolated. To differentiate it from the species names, the serotype names begin with capital letters and are not italicized.

Even though a unified system has been approved by competent authorities in *Salmonella* nomenclature, it will take some more time for it filter down to reach the bottom and become the norm.

### 2.3. Serotyping

Serotyping is the common method of differentiating strains of *Salmonella*. Serotyping separates strains based on their somatic (O) and flagellar (H) antigens. The O antigen, designated by numbers, is a polysaccharide present on the cell surface lipopolysaccharide. Flagellar antigen is diphasic with different set of H antigens. Phase 1 antigens are represented by letters and the first discovered phase 2 antigens are represented by numbers. Only one of the H antigens will be expressed at a time. A slide agglutination test is commonly used in serotyping which normally requires over 250 antisera.

A protein microarray method for serotyping the 20 common serotypes has also been reported (Cai *et al.*, 2005).
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2.4. Epidemiology

There is a close relationship between *Salmonella* serotypes in food of animal origin and public health problems. Sakai and Chalermchaikit (1996) associated the increase in human infections by *Salmonella enterica* subspecies *enterica* serotype Enteritidis with the increased prevalence of the same serotype in chicken. Reports from Northern Thailand showed the presence of *Salmonella* in all levels of food production, from farm to market (Padungtod and Kaneene, 2006).

2.5. Reservoirs of *Salmonella*

*Salmonella* is a food borne pathogen. They are transmitted through the oral-fecal route. There are several host adapted species. Nevertheless, almost all of them enjoy a wide host range without producing any symptoms in the hosts.

2.5.1. *Salmonella* and humans

Along with a very few host adapted serotypes, human beings proved to be successful hosts for several *Salmonella* serotypes. Even though typhoidal and non-typhoidal salmonellosis of humans owes its origin to food-borne *Salmonella*, transfer among humans is not uncommon.

2.5.2. *Salmonella* and pork

Contaminated pork is an important source of *Salmonella* infections (Baggesen *et al.*, 1996; Mead *et al.*, 1999). Reports regarding pork as a reservoir for *Salmonella* were present since the first isolation of *Salmonella* (Salmon and Smith, 1886). Along with the host adapted serotypes like *Salmonella enterica* subspecies *enterica* ser Typhisuis and *Salmonella enterica* subspecies *enterica* serotype Choleraesuis, several other serotypes with broad
host range are reported from swine. Many of the hosts behave as asymptomatic carriers of these dreaded pathogens in their tonsils, intestine and the gut-associated lymphoid tissue (Wood et al., 1991; Fedorka-Cray et al., 1995). They can excrete about $10^5$ CFU/g Salmonella serotypes over a period and there are certain factors that may increase their potential to excrete the bacteria (Henry et al., 1983). The survival potential of Salmonella in feces is also very high (Plym and Ekesbo, 1993). Salmonella Typhimurium is the most common serotype associated with pigs (Letellier et al., 1999; Vieira-Pinto et al., 2006; Rostagno et al., 2007).

2.5.3. Salmonella and poultry

Poultry and poultry products, including meat and eggs have long been recognized as an important source of food-borne infections caused by Salmonella. High prevalence of Salmonella has been reported from chicken slaughterhouses (Carraminana et al., 2004). These slaughterhouses serve as potential sources of cross contamination as well. Salmonella Typhimurium and Salmonella Enteritidis are among the most frequently isolated serotypes from poultry (Henzler et al., 1998; Krishnamoorthy et al., 2003; Suresh et al., 2006; Messens et al., 2007). The global increase in human infections with Salmonella Enteritidis observed in the late 1980’s and early 1990’s was due to the presence of this organism within the poultry production industry worldwide (Rodrigue et al., 1990).

Salmonella Enteritidis associated with egg and egg products is cause of significant public health concern in many countries (Cowden et al., 1989; Stevens et al., 1989; Luby and Jones, 1993). Salmonella mainly colonizes the intestinal tract of poultry. Egg contamination occurs either by horizontal transfer through egg shell penetration, which takes place at the cloacal region during or after laying. Several serotypes of Salmonella are capable of
penetrating the egg shells (Schoeni et al., 1995). Secondly, through transovarian infection, *Salmonella* colonizes the pre ovulatory follicles of an infected bird, thereby contaminating the eggs and the next progeny (Snoeyenbos et al., 1969; Perales and Audicana, 1989; Barnhart et al., 1991; Thiagarajan et al., 1994). *Salmonella* can grow in eggs even at 4°C (Kim et al., 1989; Schoeni et al., 1995). Outbreaks of salmonellosis by *Salmonella* Enteritidis have been reported from several parts of the world (Perales and Audicana, 1989; Rodrigue et al., 1990; Wong et al., 1994; Sakai and Chalermchaikit, 1996).

*Salmonella enterica* subspecies *enterica* serotype Infantis has also been reported as a prevalent serotype in the poultry industry (Raevuori et al., 1978; Poppe et al., 1991; Hinz et al., 1996; Shahada et al., 2010) and has been responsible for several outbreaks in humans (Barrell, 1987; Hatakka, 1992; Meehan et al., 1992).

Other reports have shown that human salmonellosis caused by *Salmonella* serotypes like, Berta, Typhimurium and Livingstone were acquired from poultry (Olsen et al., 1992; Millemann et al., 1995; Crichton et al., 1996).

### 2.5.4. Salmonella and fish

Even though *Salmonella* serotypes do not produce any disease in fish and other aquatic organisms, they are potential carriers of these pathogens. Aquatic environments act as a major reservoir of *Salmonella*, aiding its transmission between hosts (Cherry et al., 1972). *Salmonella* contamination is a serious problem in seafood exporting industry. Heinitz et al. (2000) reported an overall prevalence of 7.4% and 1.3% for imported and domestic seafood respectively from 1990 to 1998 in United States. Contamination in raw seafood was 10% and 2.8% respectively for imported and domestic. In India
several studies have indicated high prevalence of *Salmonella* in seafood. Hatha and Lakshmanaperumalsamy (1997) reported that 14.25% of fish samples and 17.39% of crustacean samples collected from Coimbatore, South India were contaminated with *Salmonella*. Another report (Iyer and Shrivastava, 1989) showed that 12% of peeled and deveined shrimp, 10% of headless shell of shrimp, 14% of peeled undeveined shrimp, 25% of catfish and 20% of seer fish harbored *Salmonella*. Studies have demonstrated the ubiquitous presence of *Salmonella* in fishes and a close relationship between multidrug resistant *Salmonella Paratyphi* isolated from patients with gastroenteritis and those isolated from their home aquarium (Levings et al., 2006).

The actual picture of the prevalence of *Salmonella* in seafood is not furnished by these reports as detection was based on conventional culture method, which is less sensitive than the molecular methods. Nucleic acid-based methods have estimated that the real prevalence is significantly higher. PCR assay detected *Salmonella* in 70% fish, 59% of shrimps and 30% of oysters (Sanath Kumar et al., 2003).

### 2.5.5. Other sources

Human salmonellosis associated with pets is a major public health concern (Woodward *et al.*, 1997; Austin and Wilkins, 1998; Jafari *et al.*, 2002). A study involving the analysis of the fecal samples of several reptiles showed dominance of several exotic *Salmonella* serotypes (Ebani *et al.*, 2005). However, reptiles usually do not show any sign or symptoms of *Salmonella* infection.

Reptiles are a major reservoir of *Salmonella* (Greenberg and Sechter, 1992; Monzon Moreno *et al.*, 1995; Geue and Loschner, 2002) with reptile-associated salmonellosis becoming a major public health issue. A high
prevalence of *Salmonella* with forty four different serotypes has been reported from samples collected from captive lizards (Pasmans *et al.*, 2005). However, *Salmonella* strains isolated from lizards are not usually found in mammals and birds (Baulmer *et al.*, 1998). The increased popularity of these pets is correlated with the increase in reptile-associated salmonellosis in humans (Woodward *et al.*, 1997; Mermin *et al.*, 2004).

Turtles are also considered a potential source of human salmonellosis (Williams and Helsdon, 1965; Cohen *et al.*, 1980) as they carry *Salmonella* as part of their normal intestinal flora and shed the bacteria in their feces. Transmission of *Salmonella* occurs mainly through reptilian eggs. One hour exposure of turtle eggs to contaminated internal contents can result in effective penetration (Feeley and Treger, 1969). Transovarian infection is also reported in reptiles (Austin and Wilkins, 1998).

Wild birds also proved to be a suitable reservoir for *Salmonella*. (Wilson and MacDonald, 1967; Refsum *et al.*, 2002; Pennycott *et al.*, 2006).

Fruits and vegetables too are sources for *Salmonella*. Contaminated alfalfa sprouts were responsible for outbreaks of *Salmonella Enterica* in Finland and Sweden (Ponka *et al.*, 1995). A study from the slums of Bangladesh showed that contaminated papaya was associated with enteric fever (Ram *et al.*, 2007). Another study reported that eating lettuce salad and cig kofte (a traditional raw food) was significantly associated with the development of typhoid fever in Turkey (Hosoglu *et al.*, 2006). Outbreak of infection by *Salmonella enterica* subspecies *enterica* serotype Muenchen has been reported to be caused by consumption of orange juice (Center for Disease Control and Prevention, 1999).
2.6. Host adapted serotypes of *Salmonella*

Majority of the *Salmonella* serotypes are ubiquitous, with a few host adapted ones. *Salmonella enterica* subspecies *enterica* serotypes Dublin, Choleraesuis and Pullorum are host-adapted serovars that cause disease primarily in cattle, swine and poultry, respectively. *Salmonella* Typhi and *Salmonella* Paratyphi are human-adapted causing enteric fever. Some of the host adapted serotypes infrequently cause diseases in other hosts.

2.7. Important serotypes

*Salmonella* Typhi is a host adapted serotype responsible for enteric fever in humans, most frequently isolated from humans (Kumar *et al.*, 2009). Infections with *Salmonella* Typhi continue to be a major health problem in many developing countries as this serotype naturally infects only humans, but is well adapted to establish a chronic carrier state with persistent excretion which can last for months or years (White and Parry, 1996). Multilocus sequence typing of housekeeping genes has showed that this serotype has evolved 50000 years ago (Kidgell *et al.*, 2002).

Unlike most of the other serotypes, *Salmonella* Typhi strains show a high degree of homogeneity among themselves, a single profile in multilocus enzyme electrophoresis indicating the clonal relatedness of *Salmonella* Typhi strains from different sources (Reeves *et al.*, 1989). A similar study also showed high degree of relatedness among the strains even though they showed two electrophoretic profiles with multilocus enzyme electrophoresis (Selander *et al.*, 1990)

*Salmonella enterica* subspecies *enterica* serotype Paratyphi is another host adapted serotype of humans, and is the second causative agent of enteric fever prevalent in many regions of Asia (Hafiz *et al.*, 1993; Sood *et al.*, 1999).
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*Salmonella enterica* subspecies *enterica* serotype Typhimurium, a ubiquitous serotype responsible for the majority of non-typhoidal salmonellosis in humans, has been isolated from a wide range of sources which includes poultry (Singh et al., 2010), seafood (Hatha and Lakshmanaperumalsamy, 1997) pork (Wondwosen et al., 2000) calves (Ahmed et al., 2009) and wild birds (Refsum et al., 2002).

*Salmonella* Enteritidis is considered the main serotype infecting humans and poultry worldwide (Rodrigue et al., 1990; Altekruse et al., 1993; Roberts and Sockett, 1994) and its association with egg and egg products plays an important role in human salmonellosis (Luby and Jones, 1993; Suresh et al., 2006). Even though most *Salmonella* Enteritidis infections in humans are sporadic, few outbreaks (Levine et al., 1991; Taylor et al., 1993) stress the importance of *Salmonella* Enteritidis in public health.

*Salmonella enterica* subspecies *enterica* serotype Weltevreden is another prevalent serotypes isolated from fishes and crustaceans (Hatha and Lakshmanaperumalsamy, 1997; Shabarinath et al., 2007), reportedly associated with human infections in Malasia and Thailand (Thong et al., 2002; Bangtrakulnonth et al., 2004; Padungtod and Kaneene, 2006). An outbreak of salmonellosis in Singapore has been attributed to vegetables and fruits contaminated with *Salmonella* Weltevreden (Ooi et al., 1997). It is a prominent serotype in the South East Asia and Western pacific region causing non-typhoidal salmonellosis (World Health Organisation, 2005).

*Salmonella enterica* subspecies *enterica* serotype Gallinarum has two biotypes; Gallinarum and Pullorum. They are non-motile pathogens which are host restricted to fowls causing distinct diseases. *Salmonella* Gallinarum causes fowl typhoid and *Salmonella* Pullorum causes dysentery (fowl typhoid).
Even though, considered as host adapted, infections in primates are also reported (Ocholi et al., 1987).

Salmonella enterica subspecies enterica serotype Infantis is another serotype which is prevalent world-wide in the poultry industry (Poppe et al., 1991; Crichton et al., 1996). This serotype was involved in a large broiler chicken-associated epidemic in Finland (Raevuori et al., 1978). It is a pathogen of both animals and humans, and frequently isolated from swine also (Wondwossen et al., 2000).

2.8. Salmonellosis

Salmonellosis is one among the major food-borne diseases the world over. The clinical manifestations of salmonellosis can vary from life threatening enteric fever to self limiting gastroenteritis.

2.8.1. Enteric fever

Enteric fever, which includes typhoid fever and paratyphoid fever, is a systemic disease caused by Salmonella Typhi and Salmonella Paratyphi respectively. Sarnighausen et al. (1999) reported that Salmonella Kapemba is also capable of producing enteric fever. Typhoid fever is a serious threat to public health all over the world, especially in the developing countries (Crump et al., 2004). They are transmitted through the fecal-oral route and hence the infections are more prevalent where sanitary conditions remain poor and water supplies are untreated. Human beings are the only true reservoir and transmission occurs through contaminated food materials and water, and carriers.

Ingestion of Salmonella is followed by an asymptomatic period extending up to 60 days, followed by fever and malaise, signaling bacteraemia.
Chills, head ache and gastro intestinal disorders are the most common symptoms associated with enteric fever. Gastrointestinal bleeding and intestinal perforation are seen in severe cases of the disease. Hepatomegaly and splenomegaly is common. Rose spots usually occur on the abdomen and chest. Chronic biliary carriage may occur in a few cases.

2.8.2. Non-typhoidal salmonellosis

Salmonellosis caused by serotypes other than *Salmonella* Typhi and *Salmonella* Paratyphi is designated as non-typhoidal salmonellosis. Majority of them are non-host-specific serotypes. Human acquisition of these serotypes occurs mainly by consumption of contaminated food like poultry, pork, egg, seafood etc. Several factors like the serotype, host, inoculum to name a few, determine the severity of the disease produced by Salmonellae: the serotype determines the clinical syndrome produced.

The main symptoms are gastroenteritis with nausea, vomiting, and diarrhea with or without fever. A small proportion of these patients develop invasive infections which may result in extra gastrointestinal infections including bacteremia, and localized infections. Many serotypes are capable of producing invasive infections which begin by adhesion and penetration of epithelial cells (Groisman and Mouslim, 2000). Several bacterial fimbriae are involved in the adhesion process (Darwin and Miller, 1999). *Salmonella* is capable of gaining entry into non-phagocytic cells and producing gastroenteritis by multiplying in the Peyer’s patches. The ability of *Salmonella* to gain entry into the non-phagocytic cells makes them a successful pathogen.

2.9. Detection of *Salmonella*

*Salmonella* being one of the major bacterial contaminants in food and food products, rapid and sensitive detection is of prime importance in food-
industry and public health sector. Traditional method relies on isolation of bacteria and subsequent identification by biochemical and serological methods (Andrews et al., 1998) requiring a minimum of 6 days.

Several alternative methods were proposed for the easy detection of Salmonella. An immuno-magnetic monoclonal antibody-based assay was developed by Luk and Lindberg (1991) claiming rapidity and sensitivity. Enzyme linked immunosorbant assays were widely used for Salmonella detection and identification (Lee et al., 1990; Keller et al., 1993). Hybridization methods proved to be another effective method for Salmonella detection (Fitts et al., 1983; Fitts, 1985; Gopo et al., 1988). Hanes et al., (1995) reported an allele-specific DNA probe (SE-probe) that targets the spvA gene in the virulence plasmid of Salmonella Enteritidis that utilized a single base difference between Salmonella Enteritidis, Salmonella Dublin and Salmonella Typhimurium. Under stringent conditions this probe will hybridize with all Salmonella Enteritidis strains which harbor the virulence plasmid, regardless of the phage type and geographical location.

Nucleic acid based methods by polymerase chain reaction proved to be the best known, fast and sensitive assay, compared to the culture method (Nissen and Sloots, 2002; Shabarinath et al., 2007; Kumar et al., 2008). Detection by nucleic acid-based assays began when Widjojoatmodjo et al. (1991) reported the suitability of oriC as a molecular marker for Salmonella. Later Salmonella invasion gene, invA, became the first choice of researchers (1992). PCR assays targeting other Salmonella specific genes were also developed for specific detection of Salmonella. PCR amplification of agfA gene has been used for Salmonella detection by Doran et al. (1993). A nested PCR based on viaB gene was developed by Hashimoto et al. (1995) to detect Salmonella Typhi. A PCR method using a primer to amplify a 199bp
Salmonella-specific DNA fragment derived from a repetitive DNA of Salmonella Weltevreden was reported by Jitrapakdee et al. (1995). Cheng-Hsun and Ou (1996) reported a multiplex PCR based on invA and spvC genes which could detect the presence of Salmonella along with the virulence plasmid. Shabarinath et al., (2007) reported an increased efficiency for hns primer compared to invA and invE primers in detecting Salmonella from seafood.

PCR assay based on hilA was successfully used for the detection of Salmonella in tomatoes (Guo et al., 2000) and is suggested to be a suitable candidate for Salmonella detection in feces (Pathmanathan et al., 2003). PCR primers targeting tyv, prt, viaB, and fliC genes were used in combination for accurate detection and identification of Salmonella Typhi and Salmonella Paratyphi A (Hirose et al., 2002).

Even though rapid and sensitive detection of Salmonella is possible with molecular methods, the culture method is inevitable for the isolation and further research including epidemiological studies.

2.10. Loop Mediated Isothermal Amplification (LAMP)

LAMP is a novel nucleic acid amplification method performed under isothermal conditions (Notomi et al., 2000) thereby doing away with the precision equipments for thermal cycling. It can produce about $10^9$ copies of DNA in less than an hour from a few templates, requiring only a water bath as equipment.

LAMP is based on the auto-cycling strand displacement DNA synthesis property of Bst polymerase (Notomi et al., 2000). It relies on 4 oligonucleotide primers; two inner and two outer primers, that targets 6 specific regions in the template DNA. The inner primers are designated as
forward inner primer (FIP) and backward inner primer (BIP). Various steps of LAMP reaction is illustrated in Fig 2.1.

**Fig.2.1 Schematic representation of LAMP reaction mechanism**

Figure courtesy (Notomi et al., 2000)
The regions inside both ends of the template DNA to be amplified are designated as F2c and B2. F1c and B1 are two sequences present internal to F2c and B2 respectively. Outside the F2c and B2 are two sequences designated as F3c and B3 respectively. The FIP primer consists of 3 distinct sequences: F1c, a TTTT spacer and F2. Similarly BIP is composed of sequences B1c, TTTT spacer and B2. Sequences F2 is complementary to F2c and B2 is complementary to B2c.

FIP primer anneals to template DNA at the F2c region and begins complementary strand synthesis. F3 primer provided in a comparatively lower concentration hybridizes slowly to F3c in the template DNA and initiates strand displacement DNA synthesis. Due to the strand displacement activity of Bst polymerase, the FIP linked complementary strand is released and forms a loop at one end (structure 4). BIP primer will now get attached to this strand and DNA synthesis is initiated. Annealing of B3 primer initiates strand displacement DNA synthesis resulting in the production of a dumb-bell shaped strand (structure 6). Self primed DNA synthesis converts the dumb-bell shaped strand to a stem-loop structure which serves as the starting material for LAMP cycling in the next step.

FIP then hybridizes to the loop and initiates strand displacement DNA synthesis forming a gapped stem-loop structure having an additional inverted copy of the target sequence in the stem portion and a loop at the opposite end. Further strand displacement DNA synthesis produces a stem-loop structure which is twice as long as the target DNA (structure 9) and a complementary strand of the original stem-loop structure (structure10), which was produced at structure 7. These two products functions as templates for the subsequent reactions.
Subsequent amplification reactions result in the production of stem-loop structures with varying stem length. Multiple loops are also formed by annealing of alternately inverted repeats of sequences in the same strand. The use of four primers in the amplification process increases the specificity of the reaction.

Since the development, several improvements and modifications have been reported, increasing the ease of its use. Nagamine et al. (2001) reported the possibility of performing LAMP without the initial denaturation of the template DNA. The use of loop primer along with the other primers has accelerated the LAMP reaction (Nagamine et al., 2002). Moreover, it reduced the template requirement from more than $10^4$ to $10^3$. The reaction will produce magnesium pyrophosphate as a byproduct in excess amounts and hence, visualization of the positive result as a white precipitate is possible by naked eye (Mori et al., 2001). Increase in turbidity, due to continuous production of this precipitate, is proportional to the amount of DNA synthesized. Hence, a real-time monitoring of the reaction is possible by real-time measurement of the turbidity.

Detection of microbes has been an important application of LAMP. Aoi et al. (2006) reported the use of LAMP assay for detection of ammonia-oxidising bacteria. LAMP assay has been used as a rapid and sensitive diagnostic tool for several microbial pathogens like E. coli (Hara-Kudo et al., 2007; Hill et al., 2008), Vibrio spp. (Yamazaki et al., 2008a; Srisuk et al., 2010; Cai et al., 2010; Han and Ge, 2010), Yersinia spp. (Horisaka et al., 2004; Saleh et al., 2008), Mycobacterium tuberculosis (Pandey et al., 2008), Edwardsiella ictaluri (Yeh et al., 2005), Campylobacter spp. (Yamazaki et al., 2008b), Bacillus anthracis (Jain et al., 2011), Pseudomonas aeruginosa (Zhao et al., 2011), Listeria monocytogenes (Tang et al., 2011) Salmonella
LAMP has also been useful in virus surveillance by detecting Monkeypox Virus (Iizuka et al., 2009), foot-and-mouth disease virus (Dukes et al., 2006), Epstein–Barr virus (Iwata et al., 2006), human herpesvirus 8 (Kuhara and Yoshikawa, 2007) to name a few. *Salmonella* has been detected by LAMP assay from a variety of source: from pork (Techathuvanan et al., 2010), poultry (Ohtsuka et al., 2005; Okamura et al., 2008) and artificially contaminated water (Varghese et al., 2012). Several target genes like *fimY* (Zhang et al., 2011) and *invA* (Wang et al., 2008) have been used for detection of *Salmonella* by LAMP assay. Due to its cost effectiveness and high sensitivity it is widely used as an alternative to PCR for amplification and detection of specific genes. Lu et al. (2009) reported an Ethidium Monoazide-Loop Mediated Isothermal Amplification method, which can distinguish viable cells from dead cells. In order to increase the ease of observation, calcein and manganese ions are added to produce a characteristic color indicating a positive result (Tomita et al., 2008; Tang et al., 2011). Mori et al., (2006) reported a visual detection protocol using oligonucleotide probes labeled with fluorescent dyes.

### 2.11. *Salmonella* Pathogenicity Islands (SPIs)

Pathogenicity islands (PAI) are distinct regions on chromosomes of pathogenic bacteria harboring clusters of virulence genes and acquisition of PAIs is considered as ‘quantum leaps’ in the process of bacterial evolution (Groisman and Ochman, 1996).

In *Salmonella* too, majority of the virulence genes are clustered in the PAIs, referred to as ‘*Salmonella* pathogenicity islands’ (SPI) (Marcus et al.,
2000). *Salmonella* has evolved as a successful pathogen, after its deviation from *E.coli*, by the acquisition of pathogenicity islands containing virulence determinants. These SPIs are acquired from other species through horizontal gene transfer, a fact which is well proved by the presence of significant differences in the G+C content of these islands and the remaining genome along with the presence of insertion sequences which flank them (Groisman and Ochman, 1996).

Several SPIs have been reported since the identification of the first one (SPI-1) by Mills et al. (1995), a 40kb fragment in *Salmonella* Typhimurium chromosome, which was absent from the corresponding region of *E.coli* K-12 chromosome. This region, located at centisome 63 of *Salmonella* chromosome, contains genetic information for a large number of proteins involved in the formation of a type III secretion system (TTSS), which is a specialized protein secreting system involved in the translocation of effector proteins into eukaryotic cells (Galan and Collmer, 1999; Cornelis and Van Gijsegem, 2000). Some of the effector proteins are involved in the modification of actin cytoskeleton thereby, helping in the uptake of bacteria by the host cells (Hayward and Koronakis, 2002). Another subset of the effector proteins are involved in enteropathogenesis and intestinal epithelial inflammation (Wallis and Galyov, 2000). SPI enjoys a wide distribution in almost all serotypes (Ochman and Groisman, 1996; Hensel et al., 1997a).

A study by Choi et al. (2007) showed that the expression of genes in SPI-1 requires the product of *luxS* gene. They showed that the cell-density-dependent induction of the *invF* gene is abolished if *luxS* is deleted. LuxS protein is involved in the synthesis of an auto inducer which activates the quorum sensing system (Meijler et al., 2004).
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SPI-2 is also a 40kb locus mapping downstream of a tRNA^Val locus at 31 min, encoding a second TTSS, enabling the bacteria to survive in epithelial cells and macrophages (Shea et al., 1996; Ochman et al., 1996; Hensel et al., 1997a). Hensel et al., (1999b) reported a mosaic nature in the structure of SPI-2, supporting multiple evolutionary events in the evolution of this island. Heterogeneity is observed in SPI-2 indicated by a marked difference in base composition and codon usage between the 25 kb portion, encoding a second TTSS and the 15 kb portion, with genes for tetrathionate reductase and other functions. SPI-2 is involved in anaerobic respiration by encoding tetrathionate reductase (Hensel et al., 1999a).

Even though both SPI-1 and SPI-2 encode various proteins for TTSS, SPI-2 genes show less similarity to homologues in SPI-1 than to those of other species (Hensel et al., 1997b). This is a clear indication of independent horizontal gene transfer rather than duplication of homologous gene clusters in SPI-1 (Hensel, 2000).

SPI-3 is an insertion of 17kb downstream of selC gene (Blanc-Potard and Groisman, 1997). This island maps at 82 centisome. SPI-3 harbors at least 10 ORFs, organized in six transcriptional units (Blanc-Potard et al., 1999) and it includes the mgtCB operon encoding a high affinity Mg^{2+} uptake system required for survival in the nutritional limiting intra-phagosomal environment (Snavely et al., 1991; Blanc-Potard and Groisman, 1997; Blanc-Potard et al., 1999). Variation in G+C content among the members along with the presence of functionally unrelated genes in the island suggest multistep horizontal gene transfer events (Blanc-Potard et al., 1999).

SPI-4, a 27kb virulence region located at 93 min on the chromosome map, flanked by the ssb and soxSR loci, is needed for intra-macrophage
survival (Wong et al., 1998). It encodes 18 proteins; three of which shows homology with toxin secreting proteins.

In 1998 another SPI designated as SPI-5 was identified (Wood et al.). This locus maps at approximately 20 centisome of Salmonella Typhimurium chromosome. This island is flanked by tRNA$_{\text{Ser}}$ and copS/copR. It harbors at least six genes involved in enteric but not systemic salmonellosis (Wood et al., 1998).

Analysis of the complete genome of Salmonella Typhi CT18 reveals the presence of five more minor SPIs (Parkhill et al., 2001; Asten and Dijk, 2005). Islands with few virulence genes were also identified, based on the variability in G+C content. They were designated as “pathogenicity islets” (Groisman and Ochman, 1997)

There are several regulatory genes which are present in and outside these SPIs which controls the expression of various virulence genes. Gene hilA, present in SPI1 is one among them (Bajaj et al., 1995; Bajaj et al., 1996). It is a transcriptional regulator of several invasion genes, whose transcription in turn is activated by sirA (Johnston et al., 1996).

Choi et al. (2007) reported that luxS gene product is necessary for the expression of the virulence genes present in the pathogenicity islands.

### 2.12. Horizontal gene transfer

Bacterial genome is highly dynamic in nature. Horizontal gene transfer (HGT) has been attributed as the major cause of abrupt emergence of variants of bacterial strains. Transformation, conjugation and transduction are the three mechanisms by which microbes share their genomes (Jain et al., 2002). HGT takes place between species, genus or even kingdoms of organisms: bacteria to
archaea (Nelson et al., 1999), bacteria to eukaryotes (Doolittle, 1998), animals to bacteria (Wolf et al., 1999) and so on.

2.13. Virulence plasmids

Several Salmonella serotypes possess plasmids which provide virulence determinants involved in the process of pathogenesis (Gulig, 1990; Chiu et al., 1999; Chu et al., 1999; Chu et al., 2001). The size of the plasmid varies with the serotype and may range from 50 to 285kb (Ou et al., 1990). All of them share a common 7.8kb spv (Salmonella plasmid virulence) region which confers the virulence determinants. Other loci may play a role in the other stages of infection process (Gulig et al., 1993). Several loci of unknown function is also reported from these plasmids (Koski et al., 1992). Salmonella virulence plasmids were considered non-conjugative, until a self-transmissible plasmid was reported from Salmonella Typhimurium (Ahmer et al., 1999) and another one giving multiple antibiotic resistance (Guerra et al., 2002).

The virulence property is sometimes strengthened by the formation of resistance and virulence combined plasmids, where the virulence plasmids harbour the antibiotic resistance genes, spreading the antibiotic resistance genes among the bacterial community (Fluit, 2005). Several studies emphasizes the role of plasmids in the resistance phenotypes (Tosini et al., 1998; Guerra et al., 2001).

2.14. Phages

Salmonellae harbour several temperate bacteriophages, which help in lateral gene transfer by transduction. The incorporation of lysogenic phages into the bacterial gene can result in the lyogenic conversion of non-pathogenic bacteria to pathogenic forms by the addition of virulence genes.
Lysogenic conversion can result in the conversion of one phage type into another (Mmolawa et al., 2002). Mirold et al., (2001) reported the possibility of transfer of virulence determinants present in one phage to other unrelated phages.

*S. Typhimurium* is well known for the presence of different phages like Gifsy-1, Gifsy-2 and Gifsy-3, Fels-2 and a P2-like phage, SopE that encodes the *sopE* gene (Figueroa-Bossi et al., 1997; Hardt et al., 1998b; Figueroa-Bossi and Bossi, 1999; Miao and Miller, 1999; Mirold et al., 1999). Prophage-like elements Gifsy-1 and Gifsy-2 are present at 57 and 24 units of *Salmonella* genome and their sequence is identical over a portion (Figueroa-Bossi et al., 1997). They contribute to the virulence of their bacterial hosts (Stanley et al., 2000; Ho and Slauch, 2001). Association of virulence gene with phage genes or to non functional phage attachment sites suggests the role of phages in the spread of virulence genes (Blanc-Potard and Groisman, 1997; Hensel et al., 1997a; Hardt et al., 1998b; Gunn et al., 1998; Wood et al., 1998).

### 2.15 Antibiotic resistance

Acquisition of resistance phenotype in microbes is by some common mechanisms (Fig. 2.2). Increasing antibiotic resistance gains a global attention (Su et al., 2004; Alcaine et al., 2007).

**Fig 2.2. Chemical and genetic mechanisms in antibiotic resistance phenotype**

Salmonellae resistant to antimicrobials have become a serious health care issue
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(Butaye et al., 2006; Parry and Threlfall, 2008). Since the early 1990s the increase in antibiotic resistance in non-typhoidal Salmonellae is cause for concern (Su et al., 2004; Alcaine et al., 2007).

2.15.1. Multiple antibiotic resistance in *Salmonella*

Multidrug resistant *S.* Typhimurium increased more than double between 1981 and 1990 in Great Britain (Threlfall et al., 1993). Threlfall et al. (1996) showed that *S.* Typhimurium resistant to ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole increased from 1% to 25%, 1.5% to 25%, and 0% to 25% respectively, from 1986 to 1993 in UK. Emergence of *S.* Typhimurium DT104 with its multiple antibiotic resistance has become the source of deep concern (Threlfall et al., 1994) as they harbour multiple antibiotic resistance determinants giving resistance to at least ampicillin, chloramphenicol-florfenicol, streptomycin-spectinomycin, sulfonamides, and tetracycline (ACSSuT phenotype) (Sandvang et al., 1997; Glynn et al., 1998). This strain was first isolated from cattle in UK but has now been isolated from a wide range of sources (Besser et al., 1997; Low et al., 1997).

Gebreyes and Altier (2002) reported a new phage type of *Salmonella* Typhimurium with AKSSuT type penta resistance giving resistance against ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. The antibiotic resistance genes were found on a plasmid. A study on antibiotic susceptibility profiling of one hundred and eighty seven isolates representing eighty seven *Salmonella* serotypes isolated from imported seafood collected from various field laboratories of U.S. Food and Drug Administration, showed that 8% of the isolates were resistant to at least one antibiotic and 2.7% were resistant to three or more antibiotics (Zhaoa et al., 2003). Isolates which are resistant to two or more antibiotics originates from high-risk sources of
contamination. Reports from a study conducted in Spain showed that out of the 133 Salmonellae isolated from chicken slaughterhouse, 65.4% were resistant to multiple antibiotics (Carraminana et al., 2004).

Quinolones have been successfully used for the treatment of salmonellosis, caused especially by multiple drug resistant strains (Barnass et al., 1990). It has been very effectively used in the treatment of enteric fever (Tran et al., 1995; Vinh et al., 1996). But the increasing emergence of quinolone resistant Salmonellae poses a major public health concern (Piddock et al., 1993; Molbak et al., 1999; Threlfall and Ward, 2001; Ling et al., 2003; Ahmed et al., 2009). Quinolone resistance is conferred by point mutations in the gyrA gene which encodes the GyrA subunit (Belland et al., 1994; Heisig et al., 1995; Griggs et al., 1996). These mutations are clustered in a region of the gene product between amino acids 67 and 106, termed quinolone resistance-determining region (QRDR) (Yoshida et al., 1990). In nalidixic resistant bacteria, amino acid serine at position 83 is changed to phenylalanine, tyrosine or alanine or aspartic acid at 87th position is changed to glycine Asn or tyrosine. Yoshida et al. (1991) reported another QRDR region from gyrB gene of E.coli which results in reduced quinolone susceptibility compared to gyrA mutations.

Topoisomerase IV is reported as a secondary target for quinolone resistance in many Gram negative bacteria. The subunits, which are homologous to the GyrA and GyrB subunits respectively, are sites for quinolone action (Khodursky et al., 1995). Mutations in the genes of ParC and ParE at the corresponding positions of GyrA and GyrB are responsible for high-level quinolone resistance (Heisig, 1996; Vila et al., 1996; Breines et al., 1997).
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The prevalence of *Salmonella* strains resistant to at least one antibiotic is increasing day by day (MacDonald *et al.*, 1987). An increasing trend of multiple antibiotic resistance has been reported from several serotypes (Threlfall *et al.*, 1994; Wondwossen *et al.*, 2000; White *et al.*, 2001). It is quite alarming that even the third generation cephalosporins are not spared by *Salmonellae* (Rossi *et al.*, 1995; Fey *et al.*, 2000; White *et al.*, 2001). Ciprofloxacin is also becoming useless in their treatment (Threlfall and Ward, 2001; Adhikari and Baliga, 2002).

The indiscriminate use of antimicrobials in the production of food has been attributed to the emergence of antimicrobial resistance (Aarestrup, 1995). The use of single antibiotic can also give resistance to other antibiotics whose genes are present in the same mobile genetic element (Aarestrup *et al.*, 2001).

2.16. *Salmonella* Genomic Island 1

In the 1990s, emergence of a new multidrug resistant *Salmonella* Typhimurium strain, *Salmonella* Typhimurium definitive phage type 104 (DT104), was reported from the United Kingdom (Threlfall *et al.*, 1994). Since then, this phage type has been reported from several countries like Canada (Poppe *et al.*, 1996), the United States (Besser *et al.*, 1997), Israel (Metzer *et al.*, 1998), Denmark (Baggesen and Aarestrup, 1998) etc. The emergence of epidemic strain is a matter of great concern in the clinical field (Glynn *et al.*, 1998) as they harbor multiple antibiotic resistance determinants (Ridley and Threlfall., 1998; Briggs and Fratamico, 1999). All these resistance genes are packed in a 43kb genomic island designated as *Salmonella* Genomic Island 1 (SGII) (Boyd *et al.*, 2001) (Fig 2.2).
Fig 2.3. Map of *Salmonella* Genomic Island 1

![Diagram of Salmonella Genomic Island 1](image)

Figure courtesy (Ebner *et al.*, 2004)

In *Salmonella* Typhimurium SGI1 is located between *thdf* and *int2* genes. The *int2* gene is a part of the retron sequence which is not present along with the genomic islands in other serotypes. In them SGI1 is located between *thdf* and *yidY* genes (Boyd *et al.*, 2001; Mulvey *et al.*, 2006). SGI1 is flanked by direct repeats at the boundaries supporting the site specific recombination events which might have resulted in the transfer of it to the *Salmonella* genome. This island harbours two class 1 integrons; one has an aminoglycoside resistance gene (*aadA1*) and the other had the β-lactamase gene (*pse-1*) (Briggs and Fratamico, 1999). Genes conferring resistance to florfenicol-chloramphenicol (*floR*) and tetracycline (*tetR* and *tetA* [class G]) are present in between the two integrons (Briggs and Fratamico, 1999; Boyd *et al.*, 2000; Boyd *et al.*, 2001). Majority of the SGI1 has the above mentioned ACSSuT type of resistance pattern even though variants are also present (Threlfall *et al.*, 1998; Ng *et al.*, 1999; Daly and Fanning, 2000; Frana *et al.*, 2001).

It has been shown that the antibiotic resistance genes of *S.* Typhimurium DT104 can be efficiently transduced by ES18, a P22-like phage and by phage PDT17, which is released by DT104 isolates (Schmieger and Schicklmaier, 1999).
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2.17. Integron

Integrons are site specific recombination systems which help in the acquisition and dissemination of genes (Stokes and Hall, 1989), common in gram negative bacterial pathogens in general and Enterobacteriaceae in particular (Sallen et al., 1995; Jones et al., 1997; Martinez-Freijo et al., 1998).

Integrons usually harbour antibiotic resistance genes and hence play a vital role in the emergence of new multidrug resistant bacteria (Hall and Stokes, 1993). Several classes of integrons have been identified so far based on the integrase gene they harbor (Hall and Collis, 1995). Five distinct integron classes have been found associated with cassettes that contain antibiotic resistance genes.

Class 1 integrons are the most frequently found ones. They have a 5’ conserved segment (5´CS), a 3’ conserved segment (3´CS) and an internal variable region. The 5’ conserved region has an integrase gene (intI) belonging to the tyrosine recombinase family (Nunes-Duby et al., 1998), a site for recombination (attI) and a promoter (Stokes and Hall, 1989). The 3’ region is defined by a truncated version of quaternary ammonium compound resistance gene, quacEΔ1, a sulphonamide resistance gene, sul1, and an open reading frame orfC of unknown function. The 3’ conserved region can have a varied structure in isolates from different locations (Hall et al., 1994) or may be absent altogether (Recchia and Hall, 1995). Two unusual class 1 integrons In6 and In7, having two copies of sul1 have been reported (Stokes et al., 1993).

The target for the integrase protein are mobile gene cassettes which normally carries a single open reading frame (Recchia and Hall, 1995) and a recombination sequence termed as attC or 59-base element (Stokes et al., 1997). The 59-base element comprise a family of diverse sequences differing in their sequence and length but has a consensus sequence at their boundaries.
which correspond to the inverse core site (RYYYYAAC) and the core site (GTTRRRY; R - purine, Y - pyrimidine).

The cassettes are devoid of any promoter and are expressed from a strong promoter located in the 5’ conserved region of the integron. Due to the presence of the promoter, integrons can act as a natural expression vector for the antibiotic resistance genes which are inserted in the correct orientation (Stokes and Hall, 1989). The same integrase can excise the gene cassette from the integron which will be integrated in another integron by site-specific recombination (Collis and Hall, 1992b). They are excised as covalently closed circular molecules (Collis and Hall, 1992a). Fluit and Schmitz (1999) reported about 60 antibiotic resistance gene cassettes. Stockpiling of antibiotic resistance gene cassettes in the integron can lead to the emergence of multidrug resistant bacteria which may pose a potential risk to public health (Rowe-Magnus et al., 2002). Integrons harbouring up to eight antibiotic resistance gene cassettes has been reported previously (Naas et al., 1999).

Class 2 integrons are similar in organization with that of class 1 but it is found associated with Tn7 transposon. They carry three conserved resistance genes, dfrA1, sat1 and aadA1, which confer resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively (Hansson et al., 2002). Biskri and Mazel (2003) reported a plasmid borne integron 2 with unusual gene cassettes. It has an erythromycin esterase gene inserted in between sat1 and aadA1 at the expense of the dfrA1 gene. A similar work by Ahmed et al., (2005) showed the presence of another unusual class 2 integron from Salmonella Enteritidis. This integron has another sat gene which replaced the dfrA1 gene of the classical class 2 integron.
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Class 3 integrons are not found so frequently as the other two. The configuration of the three distinctive features of the integron, integrase gene, recombination site and the promoter, is similar to that found in the corresponding 5’ region of class 1 integron (Collis et al., 2002). Integron 3 was first reported from a carbapenem-resistant strain of *Serratia marcescens* (Arakawa et al., 1995). Correia et al., (2003) reported the presence of a class 3 integron from a small plasmid in *Klebsiella pneumoniae*.

Mazel et al., (1998) reported a new integrase gene, *intI4*, from *Vibrio cholerae* which codes for a previously unknown integrase similar to that of the other well characterized integrons. Later, presence of a part of class 5 integron was reported from *Vibrio mimicus* (Clark et al., 2000).

Integrons equip bacteria to scavenge foreign genes, especially antibiotic genes. As the antibiotic resistance gene cassettes are subjected to episodic selection, unnecessary genes are removed through excision events catalysed by integrase (Collis and Hall, 1992b), which will result in the reduction of genetic burden.

Integrons by themselves are not mobile (Brown et al., 1996; Rowe-Magnus et al., 1999), but they may be part of mobile elements like transposons, plasmids etc (Heikkila et al., 1993; Correia et al., 2003) which further enhance the spread of antibiotic resistance genes. Large conjugative plasmids have been reported from *Salmonella* previously which harbours both class 1 and class 2 integrons (Rodriguez et al., 2006).

2.18. Typing

Typing is an important step in the surveillance program of any food-borne pathogen. Epidemiologically, it is increasingly important to be able to
type *Salmonella* isolates, because it helps in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated with a particular type.

### 2.18.1. Phage typing

Several bacteriophages are known to infect *Salmonella*. The infection is a selective process due to compatibility of phage and phage receptors present on the surface of the hosts. A strain is assigned a phage type based on the array of the typing phage which is able to infect the bacterium and form plaques (Hickman-Brenner *et al.*, 1991).

Phage typing has been used for epidemiological study of *Salmonella* (Hickman-Brenner *et al.*, 1983; Fernandez *et al.*, 2003). It has been successfully used for the subtyping of *Salmonella* Enteritidis (Hickman-Brenner *et al.*, 1991; Katouli *et al.*, 1993). Phage typing has limitations as it could be performed only by reference laboratories and the epidemiological implications derived from this method are limited as the discriminative index is low. Several methods have been standardized for analyzing genetic variability, which is useful for epidemiological studies. The application of molecular techniques has revolutionized epidemiological studies. Intraserovar typing is being performed for epidemiological studies.

Molecular typing plays a key role in understanding disease transmission and tracking and has been used in identification of clinical strains isolated from different sources (Gudmundsdottir *et al.*, 2003; Kubota *et al.*, 2005; Yong *et al.*, 2005).
2.18.2. Ribotyping

Ribotyping has successfully been applied for molecular typing of several *Salmonella* serotypes (Altwegg *et al.*, 1989; Martinetti and Altwegg, 1990; Pignato *et al.*, 1992; Esteban *et al.*, 1993; Usera *et al.*, 1994). In spite of the technical difficulties the results of ribotyping is comparable even with PFGE (Navarro *et al.*, 1996). This method involves the analysis of restriction fragment length polymorphisms in rRNA gene, a complex technique which is beyond the technical resource of many laboratories.

2.18.3. PCR-Ribotyping

Kostman *et al.* (1992) developed a new technique, called PCR-Ribotyping, based on the amplification of the internal transcribed spacer (ITS) sequences between the 16S and 23S genes in the rRNA transcriptional units. The spacer regions hold enough variation with respect to their sequence and length, which can be effectively utilized for characterization of bacteria at the genus (Jensen *et al.*, 1993), species (Dolzani *et al.*, 1995) and subspecies (Kostman *et al.*, 1992; Dolzani *et al.*, 1994) level. The ease of its use made it a choice of researchers engaged in surveillance and epidemiological studies. Even though it was used for differentiating *Salmonella* at the serotype level and intraserovar level (Lagatolla *et al.*, 1996; Christensen *et al.*, 2000), the D value is low compared with other typing methods (Lim *et al.*, 2005). Sequencing of the PCR-Ribotype amplicons could reveal the clonal relationship of *Salmonella* serotypes isolated from different source and time (Oliveira *et al.*, 2009).

2.18.4. Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995) proved to be a promising molecular fingerprinting technique, having
application in epidemiological studies of Salmonella. It has been used in the surveillance of Salmonella Typhimurium (Tamada et al., 2001; Hu et al., 2002; Lawson et al., 2004; Mikasova et al., 2005), Salmonella Abortusequi (Akiba et al., 2003), Salmonella Enteritidis (Scott et al., 2001), Salmonella Havana (Reche et al., 2003) etc. Nair et al. (2000), reported a high discriminative index for AFLP, which was comparable with PFGE.

2.18.5. Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) is a typing method based on allelic differences in the nucleotides of housekeeping genes (Maiden et al., 1998). It has been widely used as a typing method for several bacterial pathogens including Salmonella (Kotetishvili et al., 2002; Noller et al., 2003; Nemoy et al., 2005; Torpdahl et al., 2005). Several studies show that MLST shows a mixed type of discriminative power. Kotetishvili et al., (2002) showed that MLST has a better discriminatory ability than serotyping and PFGE typing with various Salmonella strains. Even though MLST shows good discriminative power among different Salmonella serotypes it is not considered suitable for distinguishing closely related strains in a particular serovar (Fakhr et al., 2005; Sukhnandan et al., 2005).

2.18.6. ERIC-PCR

Multiple copies of Enterobacterial repetitive intergenic consensus (ERIC) sequences are present in the genomes of Escherichia coli, Salmonella Typhimurium and other Enterobacteriaceae (Hulton et al., 1991). These highly conserved elements are 126bp long and include a central core inverted repeat. Since the description of ERIC sequences (Versalovic et al., 1991), polymorphism in ERIC patterns has been widely used for genotyping bacteria. Van Lith et al., (1994) reported that ERIC-PCR can be used for exploring
variations between serotypes, as genotypes obtained are serotype-specific. But reports of ERIC profiles showing more than one genotype within a serotype (Urlings et al., 1998) make it a valuable tool for assessing intra-serotype variations, which is a very useful in epidemiological studies. The usefulness of ERI-PCR for genotyping different Salmonella serotypes has been reported earlier (Millemann et al., 1996; Chmielewski et al., 2002; Lim et al., 2005). The discriminative index of ERIC-PCR is comparatively very high (Chmielewski et al., 2002).

2.18.7. Random Amplified Polymorphic DNA

RAPD technique (Williams et al., 1990) relies on a single arbitrary primer, which binds to random segments of genomic DNA to reveal polymorphism. It detects polymorphism over the entire genome. Even though RAPD has strongly been criticized for lack of reproducibility, it is widely used for Salmonella strain differentiation (Hilton et al., 1996; Hilton and Penn, 1998; Lim et al., 2005; Shabarinath et al., 2007; Albufera et al., 2009).

2.18.8. Pulse field gel electrophoresis

Pulse field gel electrophoresis is considered as the “gold standard” in molecular typing of Salmonella (Olsen et al., 1994; Murase et al., 1995; Weide-Botjes et al., 1998). This method is of great help in the investigation of clonal relatedness within and between serotypes. Ridley et al. (1998) reported a high discriminative value to PFGE than other genotypic methods for epidemiological studies of Salmonella Enteritidis.
2.19. *Salmonella* monitoring and surveillance

Many animals harbour *Salmonella* and act as sources for infections in humans. They enter the food chain through contamination of the carcass by animal feces at the time of processing (White *et al.*, 2001) or through those who handle food. Measures taken to prevent contamination by these routes are an effective way to prevent salmonellosis. Some serotypes are exclusively associated with particular hosts. Prevalence data of strains isolated from each source is an important component of any surveillance program. The changing antibiotic resistance profile is also one important aspect to be monitored routinely.

Contaminated food produced in one area or country can cause disease in another area or country demonstrating the importance of a proper surveillance program. Surveillance programs at various level including international, national, regional and local, levels are being established to track *Salmonella* outbreaks, their epidemiology, and antimicrobial resistance patterns (Marano *et al.*, 2000; Threlfall *et al.*, 2003). FoodNet and PulseNet are other important surveillance programs for food-borne pathogens and/or susceptibility.

Even though many countries have succeeded in minimizing the incidence of *Salmonella* in water and food materials to a greater extent by adopting better sanitary measures, it is still endemic in several countries. *Salmonella* spreads mainly through the oral-fecal route and hence it is a major health concern to the entire human population. An integrated robust approach in surveillance is the need of the time.