CHAPTER 1: INTRODUCTION

1.1 General Introduction

Salmonellosis continues to be one of the global public health problems, which is caused by bacteria *Salmonella enterica* (*S. enterica).* Until 2007, more than 2500 different serovars of *S. enterica* have been identified which can cause extra-intestinal infections (Grimont, P.A.D. & Weill, F. X., 2007). According to the surveillance report from National Public Health Laboratory of Malaysia (2005), *S.* Enteritidis is the most common non-typhoidal *Salmonella*, followed by *S.* Weltevreden, *S.* Corvallis and *S.* Typhimurium.

Presence of different serovars in a country can be of worldwide importance due to travel and trade of breeding animals and food products around the world. Therefore, understanding of the presence and molecular epidemiology of different serovars in different regions may support in recognition and tracing of new emerging pathogens.

Antimicrobial resistance in *Salmonella* spp is a sober health problem in both human and food animal. Treatment is not necessary for most *Salmonella* infections that result in temporary gastroenteritis. However, invasive infections do need antibiotic treatment (Vugia *et al.*, 2004). Ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole are normally used but increasing antimicrobial resistance has decreased the efficacy of these drugs (Angulo *et al.,* 2000). Currently, extended-spectrum cephalosporins and fluoroquinolones are prescribed as treatment of extraintestinal infections in children and adults, respectively. However, emerging multidrug resistant (MDR) *Salmonella* strains that are co-resistant to these two types of antimicrobial agents may due to presence of

the β-lactamases and fluoroquinolones resistance genes. This is a sobering public health issue because this will limits treatment option for invasive salmonellosis. Therefore, antimicrobial susceptibility monitoring is important for surveillance of antimicrobial resistance. The emergence of MDR strains of *Salmonella* is because of excessive use of antimicrobials in food animals or other purposes. The widespread use of antimicrobial agents could be associated with selecting antimicrobials resistance mechanisms in *Salmonella*, such as acquisition of resistance genes, changes in bacterial cell wall permeability, energy-dependent removal of antimicrobials via membrane-bound efflux pumps etc (Schwarz & Chaslus, 2001). So, the selective pressure from the use of antimicrobials became an impetus behind the emergence of resistance.

An efficient route of acquisition, vertical, and horizontal dissemination of resistance genes is through mobile elements, which include plasmids, transposons, and gene cassettes in integrons. A clear relationship between MDR *Salmonella* strains and the presence of integrons has been discussed (Guerra *et al*., 2000). Class 1 integrons is the most common integron type in clinical strains of Enterobacteriaceae. Class 1 integrons and transferable elements like conjugative plasmids play an important role in disseminating antimicrobial resistance genes. These elements could incorporate or excise one or more resistance gene cassettes. Antibiotic resistance gene clusters in class 1 integrons located on *Salmonella* Genomic Island 1 (SGI1) have been demonstrated and many SGI 1 variant had been detected in a wide variety of *S. enterica* serovars such as serovars Agona (Boyd *et al.*, 2002), Albany (Doublet *et al*., 2003), Kentucky (Levings *et al*., 2007; Doublet *et al*., 2008), Newport (Doublet *et al*., 2004; Cloeckaert *et al*., 2006), Meleagridis (Ebner *et al*, 2004), Paratyphi B (Meunier *et al*., 2002), Cerro, Derby, Dusselderf, Emek, Infantis, and Kiambu (Levings *et al*., 2005). Therefore, these

mobile elements with resistance gene(s) play an important role in their transfer to other bacteria.

There is limited information on *Salmonella* strains that are resistant to both β-lactams and fluoroquinolones in Malaysia. Thus, the aim of the study was to determine the presence of selected β-lactamases and fluoroquinolones resistance genes (bla_{TEM}, $bla_{\text{PSE-1}}$, bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CMY-2}}$, *qnr*A, *qnr*B, *qnr*S) among selected MDR *Salmonella* strains of human origin in Malaysia. Detection of class 1 integrons, *Salmonella* Genomic Island 1 (SGI1) was carried out by using polymerase chain reaction (PCR). Plasmid profiling was also determined since plasmids could be involved in carriage of resistance traits. Therefore, the data obtained may provide a gimpse of the relative prevalence of both β-lactamases and fluoroquinolones resistance genes in MDR *Salmonella* strains of human origin in Malaysia.

1.2 Objectives

The objectives of this study were:

1. To determine the presence of selected resistance genes (*bla*$_{TEM,}$ *bla*$_{PSE-1,}$ *bla*$_{SHV,}$ *bla*$_{CTX-M,}$ *bla*$_{CMY-2,}$ *bla*$_{OXA-1,}$ *qnrA, qnrB* and *qnrS*)
2. To detect and characterize Class 1 Integron in MDR human *Salmonella* strains by using PCR and DNA sequencing
3. To determine the presence of *Salmonella* Genomic Island 1 (SGI1) by using PCR
4. To determine the plasmid profiles of the selected MDR *Salmonella* strains

1.3 Significance of the study

1. Study carried out by Benacer *et al.* (2010) showed that *Salmonella* Typhimurium was not resistant to third generation of cephalosporins and fluoroquinolones. However, resistances to these drugs were observed in this study.
2. ESBL genes and fluoroquinolones resistance genes are one reason that contributes to disseminate antimicrobial resistance.
3. Integrons and SGI1 are believed to confer multidrug resistance in Gram-negative bacteria.
4. Plasmids with resistance genes are usually able to be transferred and spread from bacteria to bacteria.

CHAPTER 2: LITERATURE REVIEW

2.1 *Salmonella* background

2.1.1 Nomenclature of *Salmonella*

The genus, *Salmonella*, named after D.E. Salmon, who first isolated *Salmonella choleraesuis* from a porcine intestine in 1884 (Smith, 1894). Originally, the organism was called "*Bacillus choleraesuis*", but changed to "*Salmonella choleraesuis*" by Lignieres in 1900. Based on the serologic classification that used an array of specific antisera, salmonellae are differentiated and identified by their combination of H and O antigens.

Classification of *Salmonella* has evolved overtime (Miller & Pegues, 2000) which was based on epidemiology, clinical manifestation, biochemical reaction, surface antigenic patterns and host range (Farmer, 2003). During 1970s, nucleotide sequence relatedness and other molecular analysis showed that typical *Salmonella* are 85-100% related (Crosa *et al.*, 1973; Brenner *et al.*, 1971, 1972). A single species, *Salmonella choleraesuis* was proposed because of the close genetic relatedness (Ewing, 1972). However, the species name was confusing because Choleraesuis is used both as a species name and as a serovar name. Further, the serovar Choleraesuis does not represent a majority of the serovars (Brenner *et al.*, 2000). In 2005, the Judicial Commission of the International Committee on Systemic Bacteriology finally approved that *Salmonella enterica* would replace *Salmonella choleraesuis* to become the type species of the genus *Salmonella*.

The antigenic classification used today was based on interactions between antibody and surface antigens of *Salmonella* organisms found out by Kauffman and White in

1934. All antigenic formulae of recognized *Salmonella* serovars are listed in the Kauffmann-White scheme (Popoff *et al.,* 2001). It is updated by the WHO Collaborating Centre for Reference and Research on *Salmonella* (WHOCC-Salm) at the Pasteur Institute, Paris, France. Newly recognized serovars are validated at WHOCC-Salm (Pasteur Institute) in collaboration with laboratories in Hambourg and Atlanta. Serovars are homologated when these three laboratories agree on their validation (Grimont, P.A.D. & Weill, F. X., 2007).

Molecular methods have shown that the genus *Salmonella* consists of only two species, which are *S. enterica* and *S. bongori*. The species called *S. subterranea* does not belong to the genus *Salmonella* (Grimont, P.A.D. & Weill, F. X., 2007). *S. enterica* is divided into the following six subspecies *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI). Each subspecies contains various serovars that defined by a characteristic antigenic formulae, include somatic (O) or cell wall antigens, surface (envelope) antigens and flagellar (H) antigens. A total of 2579 serovars in the genus *Salmonella* were identified (Table 2.1) (Grimont, P.A.D. & Weill, F. X., 2007).

Table 2.1: Number of serovars in each species and subspecies of *Salmonella*

Genus	Species	Subspecies	No. of serovars
Salmonella	*enterica*	*enterica* (I)	1531
		salamae (II)	505
		arizonae (IIIa)	99
		diarizonae (IIIb)	336
		houtenae (IV)	73
		indica (VI)	13
Salmonella	*bongori*	subspecies V	22

2.1.2 Morphology and Characteristics

Salmonella is a Gram-negative, facultative, catalase-positive, oxidase-negative, rod-shaped bacterium in the family of *Enterobactericeae*, grow optimum at 37°C and pH 7. Most *Salmonella* have long flagella for movement, and short hair-like surface pili for cellular attachment. As Gram-negative bacteria, it has an outer membrane and a cell wall that contains lipopolysaccharide (LPS), which is an important virulence factor. The O-specific polysaccharide tail that contains sugar variations is used to identify different *Salmonella* types. These somatic (O) antigens are heat stable and alcohol resistant. Surface antigens (i.e. Vi antigen) may be found in some *Salmonella* serovars. The Vi antigen occurs in only serovars of *S.*Typhi, *S.*Paratyphi C, and *S.* Dublin. However, strains of these serovars may or may not have the Vi antigen. (Todar, 2004).

2.1.3 Pathogenesis of *Salmonella* Infections in Humans

Infections of *Salmonella* in humans vary with the serovar, strain, infectious dose, nature of contaminated food, and host status. Strains of the same serovar are known to differ in the pathogenicity while certain serovars are pathogenic for humans.

In the pathogenesis of typhoid, the bacteria enter the digestive tract, penetrate the intestinal mucosa, and stopped in the mesenteric lymph nodes. Then, bacterial multiply and part of the population lyses. From the mesenteric lymph nodes, viable bacteria and endotoxin can be released to the bloodstream which caused septicemia. *Salmonella* excretion may continue long after the clinical cure. About 5% of patients clinically cured from typhoid remain as carriers for months or even years. Asymtomatic carriers are potentially dangerous when unnoticed. Normally, antimicrobials are ineffective on *Salmonella* carriage (even if salmonellae are susceptible to them) because the carriage site may not allow penetration of the antimicrobials (Todar, 2004).

Typhoid is strictly a human disease, and the incidence decreases when the development of a country increase. Foodborne *Salmonella* infections are caused by ubiquitous *Salmonella* serovars (for example, *S.* Typhimurium) within 12 to 24 hours with diarrhea, vomiting and fever that will last for two to five days. Usually, antibiotic treatment is unnecessary.

Normally, an individual infected by *S.* Enteriditis has symptoms of fever, abdominal cramps, and diarrhea from 12 to 72 hours after consuming a tainted food while the illnesses may last for up to seven days. Most patients may recover without any antibiotic treatment. Nevertheless, the diarrhea can become severe and hospitalization may be required. The infants, elderly, and those are immune-compromised may be suffered for a more severe illness. Therefore, the infection may spread from the

intestines to the blood stream and other parts of a body which can cause death unless the patient is treated with antibiotics (Todar, 2004).

The molecular pathogenesis of *Salmonella* has been much studied (Darwin & Miller, 1999; Fluit & Schmitz, 1999; Marcus *et al.*, 2000; Pfeifer *et al.*, 1999). The initial step in gaining access to the host cytosol involves modification of the action cytoskeleton (Hayward & Koronakiss, 2002). Bacteria products are also able to activate pathways allowing the pathogens to escape from the defense system. Unique *Salmonella* virulence traits are thought to be acquired by horizontal gene transfer and integration into the bacterial chromosome. For instance, a discrete chromosomal virulence gene insertion is termed *Salmonella* Pathogenicity Islands (SPI) (Marcus *et al.*, 2000).

Besides, *Salmonella* strains may secrete a thermolabile enterotoxin that bears relatedness to the cholera toxin in both antigenically and structurally. This enterotoxin causes water secretion in rat ideal loop. It is recognized by antibodies against both cholera toxin and the thermolabile enterotoxin (LT) of enterotoxinogenic *E. coli.* However, it does not bind in vitro to ganglioside GM1 (the receptor for *E. coli* LT and cholera ctx). Besides, a cytotoxin that inhibits protein synthesis and is immunologically distinct from Shiga toxin has been demonstrated. Both toxins are presumed to play a role in diarrhea symptoms of salmonellosis (Todar, 2004).

2.1.4 Emergence of Multidrug-resistant (MDR) of *Salmonella* serovars

The emergence of multidrug-resistant bacteria becomes a global concern. The abuse of antimicrobials for any purpose, such as disease treatments and growth promotion in domestic livestock may lead to distribution of antimicrobial-resistant bacteria (Anjum, M. F. *et al*., 2011).

Resistance to antimicrobial agents in bacteria is mediated by several mechanisms, which included (i) changes in bacterial cell wall permeability, (ii) energy-dependent removal of antimicrobials via membrane-bound efflux pumps, (iii) modification of the site of drug action, and (iv) destruction or inactivation of antimicrobials (Schwarz & Chaslus, 2001). Acquisition of antimicrobial resistance phenotypes most often develops via conjugative transfer of plasmids (Gebreyes & Altier, 2002; Guerra *et al*., 2002). Plasmids may carry class 1 integrons that is important for the proliferation of bacterial multidrug resistance (MDR) (Arduino *et al.*, 2002; Poirel *et al.*, 1999; Verdet *et al.*, 2000).

Molecular genetic techniques have been used to characterize antimicrobial resistant salmonellae, especially *S.* Typhimurium DT 104 (Boyd *et al.*, 2002; Randall & Woodward, 2001). For instance, variant *Salmonella* Genomic Island 1 (SGI1) consist integrons that encode different resistance genes have been found in the chromosomal DNA of *S.* Typhimurium DT 104, *S.* Agona (Levings *et al.*, 2005), *S.* Paratyphi B (Meunier *et al*., 2002), and *S.* Albany (Doublet *et al*., 2003). The presence of these MDR regions is hypothesized to favor expression of a huge number of resistance genes and to enhance their transfer to other bacteria. Further, class 1 integrons had integrated into the chromosome in those serovars carrying SGI 1, which made them able to persist even in the absence of antimicrobial selection (Boyd *et al.*, 2002; Cloeckaert *et al.*,

2000). This had led *S.* Typhimurium DT 104 became a stable and widely disseminated clone of multidrug resistant *S.* Typhimurium (Chen *et al.,* 2004).

2.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a revolutionary method invented by Kary Mullis in the 1980s. PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from DNA building-blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides as primers, which are required for initiation of DNA synthesis. Majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temperature in a process called DNA melting. At a lower temperature, each strand is then used as the template in DNA synthesis by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. Overall, PCR is based on repeated cycles of denaturation of the template DNA, annealing of the oligonucleotide primers and synthesis of new DNA with the presence of a heat stable polymerase. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons) (Brown, 2006).

2.3 Plasmids in *Salmonella enterica*

Plasmids are autonomous self-replicating extra-chromosomal molecules of deoxyribonucleic acid (DNA) which have been identified in many bacterial genera and exist as covalently closed circular (CCC) molecules. Plasmids of *Salmonella enterica* is vary in size from 2kb to 200kb. Plasmids are classified into incompatibility groups according to their mode of replication and maintenance in a bacterium. High molecular weight plasmids are responsible for the antibiotic resistance. The presence of the plasmid is associated frequently with easily selectable antibiotic resistance than with any other phenotype. Majority of high molecular weight plasmids is conjugative and confers resistance to multiple antibiotics (R-plasmids). The antibiotic resistance genes are often located within transposons which can transpose from plasmids to chromosome, and vice versa. Therefore, plasmids are important not only for storage of genetic information but also for dissemination of genetic information including the antibiotic resistances (Rychlik *et al*., 2006).

2.4 Extended-spectrum β- lactamase (ESBL)

Until recently, three kinds of ESBL deifinitions have been proposed. First, the classical definition includes variants that derived from TEM-1, TEM-2, or SHV; K1 (KOXY) of *Klebsiella oxytoca.* Second, the broadened definition includes: (1) b-lactamases (CTX-M-ESBLs, GES-ESBLs, and VEB-ESBLs), with spectra similar to those of TEM and SHV variants (designated as TEM- and SHV-ESBLs, respectively) but derived from other sources; (2) TEM and SHV variants with borderline ESBL activity; i. e., TEM-12; and (3) various b-lactamases conferring wider resistance than their parent types but not meeting the definition for group 2be; i. e., OXA-types (OXA-ESBLs) and mutant AmpC-types (AmpC-ESBLs), with increased activity against

oxyimino-cephalosporins and with resistance to clavulanic acid. Third, the all-inclusive definition includes: (1) $ESBL_A$ (named for class A ESBLs); (2) $ESBL_M$ (miscellaneous ESBLs), which has been subdivided into $ESBL_{M-C}$ (class C; plasmid-mediated AmpC) and $ESBL_{M-D}$ (class D); and (3) $ESBL_{CARBA}$ (ESBLs with hydrolytic activity against carbapenems), which has been subdivided into $ESBL_{CARBA-A}$ (class A carbapenemases), $ESBL_{CARBA-B}$ (class B carbapenemases), and $ESBL_{CARBA-D}$ (class D carbapenemases) (Lee *et al*., 2012).

According to the β-lactamase functional classification scheme by Bush, Jacoby and Medeiro, ESBLs are located in two subgroups of group 2, namely subgroups 2be (Ambler's class A enzymes) and 2d (Ambler's class D ESBLs) (Bush *et al.*, 1995). With classical definition, most ESBLs are derivates of TEM and SHV β-lactamase families, which are inhibited by clavulanic acid, sulbactam and tazobactam, a feature that is used as a criterion for classification of β-lactamases and for diagnostic ESBL detection purposes (Bradford *et al*., 2001). However, Livermore (2008) has broadened the classical definition of ESBL, which includes: (1) β-lactamases with spectra similar to those of TEM and SHV variants, but derived from other sources (CTX-M-ESBLs: the CTX-M-types, rapidly spreading worldwide (Bonnet *et al*., 2004); (2) TEM and SHV variants with borderline ESBL activity; for example, TEM-12 that have only slightly increased activity against oxyimino-cephalosporins; and (3) various β-lactamases conferring wider resistance than their parent types but not meeting the definition for group 2be; for example, OXA-types (designated as OXA-ESBLs) and mutant AmpC-types (designated as extended-spectrum AmpC [AmpC-ESBLs]) with increased activity against oxyimino-cephalosporins and with resistance to clavulanic

acid (Livermore, 2008). Until recently, more than 600 ESBL variants are known (http://www.lahey.org/Studies).

Recently, Lee and co-workers (2012) proposed a definition includes three classes of β-lactamases for ease of communication between various groups of healthcare professionals. The first class is an $ESBL_A$ (named for class A ESBLs; relevant to TEM-, SHV-, CTX-M-, GES-, and VEB-ESBLs in the broadened ESBL definition), which has been subdivided into high prevalent $ESBL_A$ and low prevalent $ESBL_A$. The guidelines for detection of classical ESBLs would still apply to the class $ESBL_A$. The second class is an $ESBL_M$ (named for miscellaneous ESBLs), which has been subdivided into $ESBL_{M-C}$ (class C; plasmid-mediated AmpC; relevant to AmpC-ESBLs in the broadened ESBL definition) and $ESBL_{M-D}$ (class D; relevant to OXA-ESBLs in the broadened ESBL definition). Other genotypic methods (for the detection of $ESBL_{M-D}$), in addition to phenotypic approaches able to detect $ESBL_{M-C}$, would be needed for the detection of $ESBL_M$ enzymes. The third class is an $ESBL_{CARBA}$ (named for ESBLs with hydrolytic activity against carbapenems; β-lactamases excluded in the broadened ESBL definition), which has been subdivided into $ESBL_{CARBA-A}$ (class A carbapenemases), $ESBL_{CARBA-B}$ (class B carbapenemases), and $ESBL_{CARBA-D}$ (class D carbapenemases).

The true incidence of ESBL-producing pathogens and the precise detection of ESBLs are difficult to estimate because no consensus on three ESBL definitions mentioned above is available. Gram-negative pathogens producing ESBLs are found to truly be multidrug-resistant pathogens causing severe clinical problems. Because of the worldwide emergency and dissemination of these enzymes, it is necessary for health care professionals to exactly and rapidly detect emerging ESBLs in Gram-negative pathogens, and thus to precisely define ESBL (Lee *et al*., 2012).

2.5 Quinolones / Fluoroquinolones

Quinolones or fluoroquinolones is a family of synthetic broad-spectrum antibacterial drugs derived from quinoline compounds (http://medical-dictionary.thefreedictionary.com/quinolone). Early quinolones, i.e. nalidixic acid, had poor systemic distribution, limited activity and were used primarily for urinary tract infections. The next generation of quinolones, the fluoroquinolones (i.e. ciprofloxacin) were more readily absorbed and increased activity against Gram-negative bacteria. Newer fluoroquinolones (e.g. levofloxacin, trovafloxacin) are broad-spectrum agents with enhanced activity against many Gram-positive and Gram-negative organisms (http://www.cdc.gov/HAI/settings/lab/Quinolones-Clinical-Laboratory.html).

Fluoroquinolones are one of the choices for treating invasive infections caused by *Salmonella* that resistant to multiple earlier antimicrobials (Chiu *et al.*, 2002, 2004). Nevertheless, cases of fluoroquinolones resistance have been frequently reported in many countries (Su *et al.*, 2004). Fluoroquinolone resistant *Salmonella* isolates had shown to use several mechanisms of resistance, including change of membrane permeability, activated efflux pumps (Poole, 2000), mutations in the DNA gyrase, DNA topoisomerase IV and spread of *qnr* resistance genes.

Fluoroquinolone resistance is characterized by a step-wise process. A single mutation in any of mutation sites may cause resistance to nalidixic acid but only a slightly reduced susceptibility to fluoroquinolones (MIC, < 4 μg/ml) (Cloeckaert & Chaslus, 2001). Once double or more mutations are present concurrently, full resistance to fluoroquinolones is achieved (Chu *et al.*, 2005).

DNA gyrase and DNA topoisomerase IV are the targets of quinolone action. Both are complex because of two pairs of subunits. The subunits of DNA gyrase are Gyr A, a

97 kDa protein encoded by the *gyrA* gene and Gyr B, a 90 kDa protein encoded by the *gyrB* gene. DNA gyrase plays roles in introducing negative supercoils into DNA, removing both positive and negative supercoils and catenation and decatenation of the closed circular molecules. On the other hand, subunits of topoisomerase IV are ParC (75 kDa) and ParE (70 kDa). DNA topoisomerase IV can also remove positive and negative supercoils and is considered better in decatenation than gyrase. Point mutations in these genes are generally restricted to certain codons within the 'quinolone resistance determining region' (QRDR) (Yoshida *et al.*, 1990). In *Salmonella,* some common point mutations found to be associated with resistance to quinolones occur in the *gyrA* gene results in substitutions at the Ser-83 position, often to Tyr, Phe or Ala and Asp-87 substitutions to Asn, Gly, or Tyr. The most common amino acid substitution found in ParC is Thr-57 to Ser, Thr-66 to Ile or Ser-80 to Arg, which being observed as occasional second substitutions (Eaves *et al.,* 2004). These two enzymes work together in the replication, transcription, recombination, and repair of DNA, which break both strands of the double-stranded DNA. Further, in an ATP-dependent reaction, pass a second DNA double helix through the break, which is then resealed. Quinolones block the reaction and trap gyrase or topoisomerase IV to become a drug-enzyme-DNA complex; later, double stranded DNA breaks (Jacoby, 2005).

However, over-expression of AcrAB-TolC efflux systems is also required to achieve high level fluoroquinolone resistance (MIC, ≥ 32μg/ml) (Baucheron *et al.*, 2002). Besides, regulatory systems of *marRAB* and *soxRS*, have been associated with multiple antimicrobial resistance among members of *Enterobacteriaceae*, which include *Salmonella* (Cloeckaert & Chaslus, 2001). The *marA* and soxS genes were coded for homologous proteins that up-regulate the AcrAB-TolC efflux system. The

marR and *soxR* genes were coded for repressor proteins that down-regulate the expression of *marA* and *soxS* genes, respectively (Cloeckaert & Chaslus, 2001). Besides, *marA* and *soxS* also up-regulate to yield an antisense RNA, *micF* which is responsible to reduce the formation of a major porin, OmpF, resulting in decreased outer membrane permeability and resistance to multiple antibiotics, including fluoroquinolones (Cloeckaert & Chaslus, 2001).

In 1998, quinolone resistance was first described as horizontally transmissible resistance (Martinez *et al.*, 1998) despite chromosomal mutations in genes encoding target enzymes or affecting drug accumulation (Jacoby, 2005). Qnr A, locus responsible for the plasmid-mediated quinolone resistance was identified on the conjugative plasmid of a clinical *Klebsiella pneumoniae* isolate. It encodes a peptapeptide repeat protein protecting type II topoisomerase and found to remain within class 1 integrons that harboring other antimicrobial resistance cassettes (Wang *et al.*, 2003; Tran & Jacoby, 2002). The *qnr A* gene confers nalidixic acid and fluoroquinolones (i.e. ciprofloxacin) resistance, and its presence has been identified to promote selection of chromosomal mutations that confer higher levels of resistance (Martinez *et al.*, 1998).

Besides, other plasmid-mediated members of the pentapeptide repeat family, Qnr B and Qnr S, have been identified in *Enterobacteriaceae* species (Nordmann & Poirel, 2005). *qnrB* was found in clinical isolates of *K. pneumoniae*, *Citrobacter koseri*, *Enterobacter cloacae*, and *E. coli* from India and the United States (Jacoby *et al.*, 2006). *qnrS* was discovered in a strain of *Shigella flexneri* 2b from an outbreak of food poisoning in Japan (Hata *et al.*, 2005).

2.6 Quinolones/Fluoroquinolones Resistance Genes Epidemiology

Qnr determinants were identified in a series of enterobacterial species in areas, including America, Europe and Asia. In the earlier study, a QnrA determinant was identified only from *K. pneuminiae* in Alabama (Martinez *et al.*, 1998). During a six month period in 1994, it was not identified between 350 Gram-negative isolates that included strains producing reference plasmid-mediated cephalosporinases and clavulanic-acid ESBLs and originating in 18 countries and 24 states in the US states (Jacoby *et al.*, 2003). After that, another study noted that 11% of QnrA-positive isolates among ciprofloxacin-resistant *K. pneumoniae* isolates from six US states are collected from 1999 to 2002 (Wang *et al.*, 2004).

QnrA determinants in ciprofloxacin-resistant *E. coli* isolate collected from 2000 to 2002 were about 7.7% in Shanghai, China (Wang *et al.*, 2003). Besides, a *qnrA* gene was detected in 11 out of 23 $bla_{\text{VEB-1}}$-positive enterobacterial isolates collected from Bangkok, Thailand in 1999. This made South East Asia on the lists of regions in which QnrA determinants have spread (Poirel *et al.*, 2005). Besides, QnrA determinants also detected in *E. coli* isolates in South Korea (Kim *et al.*, 2004).

In France, the *qnrA* gene was detected in *E. coli* and *E. cloacae* (Mammeri *et al.*, 2005; Poirel *et al.*, 2005). QnrA-positive *Enterobacter* spp. and *Citrobacter freundii* isolates were identified in patients in intensive care units from 2000 to 2003 (Jonas *et al.*, 2005). QnrA determinants also being detected in *C. freundii, E. coli, Enterobacter amnigenus, E. cloacae* and *K. pneumonia* in Netherlands (Paauw *et al.*, 2004). On the other hand, *qnrB* gene was identified from India and US and *qnrS* gene was detected from Japan (Jacoby *et al.*, 2004; Hata *et al*, 2005).

2.7 Integrons

Integrons are mobile genetic elements that have a site, *attI*, at which additional DNA, in the form of gene cassettes that can be integrated by site-specific recombination, which can encode integrase to mediate these site-specific recombination events. Today, five classes of mobile integrons were identified to play a role in spreading antibiotic-resistance genes. These integrons are classified based on the sequence of the encoded integrases, which show 40-58% identity. All classes of integrons are physically linked to mobile DNA elements, including insertion sequences (ISs), transposons and conjugative plasmids that serve as vehicles for the intraspecies and interspecies transmission of genetic material. These mobile integrons are not only found in Gram-negative species but also detected in Gram-positive bacteria (Nandi *et al*, 2004).

Class 1 integrons are most prevalent among clinical isolates and most of the known antibiotic resistance gene cassettes are belong to this class of integrons. Until recently, only cassettes that differ in nucleotide sequence by more than 5%, over 80 different gene cassettes from class 1 integrons was described. These elements were found to confer resistance to all known β-lactams, all aminoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin and antiseptics of the quaternary-ammonium-compound family (Rowe-Magnus & Mazel, 2002; Fluit & Schmitz, 2004; Mazel, 2006).

2.8 *Salmonella* Genomic Island 1 (SGI 1)

Many clusters of resistance genes have been identified that formed "*antimicrobial resistance islands*" in salmonellae. *Salmonella* Genomic Island1 (SGI 1) has become the best known multidrug antibiotic resistance locus that maps to the chromosome of *S.* Typhimurium DT 104. This locus confers resistance to a core group of antimicrobials, including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (usually abbreviated as ACSSuT phenotype). This region has been cloned from a genome of Canadian isolate and had been shown to be consisted of a 43 kb region between *thdF* and *int2* genes. The *int2* gene is part of the retron sequence that reported only in serovar Typhimurium strains (Boyd *et al*, 2000, 2001). In other serovars, SGI 1 is located between *thdF* and *yidY* gene (Boyd *et al.*, 2001, 2002; Doublet *et al.,* 2003; Meunier *et al.,* 2002). Many SGI 1 variants had been detected in a wide variety of *S. enterica* serovars such as serovars Agona (Boyd *et al.*, 2002), Albany (Doublet *et al*., 2003), Kentucky (Levings *et al*., 2007; Doublet *et al*., 2008), Newport (Doublet *et al*., 2004; Cloeckaert *et al*., 2006), Meleagridis (Ebner *et al*, 2004), Paratyphi B (Meunier *et al*., 2002), Cerro, Derby, Dusselderf, Emek, Infantis, and Kiambu (Levings *et al*., 2005).