PHENOTYPIC AND GENETIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* ISOLATES FROM DIFFERENT SOURCES IN TURKEY

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ABSTRACT

PHENOTYPIC AND GENETIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* ISOLATES FROM DIFFERENT SOURCES IN TURKEY

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Salmonella enterica subsp. *enteric* serovars are responsible for causing the highest number of bacterial foodborne infections in the world. Antimicrobial resistance (AR) and virulence of *Salmonella* isolates play a critical role in systemic infections and they impose great concern to human health in severe salmonellosis cases when multidrug resistance interferes with treatment. Also, antimicrobial resistance genes might be shared with closely related human pathogens. Therefore, antimicrobial susceptibility monitoring of isolates from farm/field to fork is very crucial. The objective of this study was to determine the phenotypic and genetic variations of the AR among *Salmonella* isolates from different sources (i.e., animal, human, and foods). Disk diffusion and MIC methods were used for phenotypic characterization of AR in *Salmonella* isolates. For genotyping characterization, beta-lactam, chloramphenicol, aminoglycoside, sulfonamide and tetracycline resistance coding genes were studied. At the end, 21 regions of known

antimicrobial resistant coding genes (*bla*TEM-1, *bla*PSE-1, *bla*CMY-2, *ampC, cat1 ,cat2, flo, cmlA, aadA1, aadA2, strA, strB, aacC2, aphA1-Iab, dhfrI, dhfrXII, sulI, sulII, tetA, tetB, tetG*) were amplified to determine genetic variation of AR. The co-presence of some antimicrobial resistance genes had raised the question of mobile genetic elements presence, thus occurrence of plasmids and class 1 integrons on the isolates were analyzed. To investigate the virulence characteristics, *ctdB, gatC, gogB, hlyE, pefA, ssek3, sseI, sspH, sodC, sopE,* STM 2759, *tcfA* genes were screened on the isolates. The results were analyzed according to the source of isolate (food, animal, and human), the type of serovar. Our study fills the gap of limited relevant study about the antibiotic susceptibility profile of *Salmonella* isolates from farm/field to fork. Our study has the potential of being a progressive work conducted in the pathogenicity area.

Keywords: Antimicrobial Resistance, Mobile Genetic Elements, *Salmonella,* Virulence

ÖZ

TÜRKİYEDE FARKLI KAYNAKLARDA BULUNAN *SALMONELLA* İZOLATLARININ ANTİMİKROBİYAL DİRENÇLİLİKLERİNİN FENOTİPİK VE GENETİK KARAKTERİZASYONU

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Salmonella enterica subsp. enteric serovarları, dünyada en fazla bakteriyel gıda-kaynaklı enfeksiyonlarına neden olan mikroorganizmalardır. Çokluilaç-dirençli (ÇİD) *Salmonella*, bu dirençlilik tedavi ile çakıştığında insan sağlığı açısından büyük bir ilgi oluşturmaktadır. Ayrıca, bu direnç genleri yakın ilişkili diğer insan patojenleri arasında paylaşılabilmektedir. Bu nedenle, tarladan çatala kadar *Salmonella'*nın antimikrobiyal duyarlılığının kontrolü önemli bir konudur. Bu çalışmanın amacı doğrultusunda, farklı kaynaklardaki (hayvan, insan ve gıdalar) *Salmonella* izolatları arasında fenotipik ve genetik antimikrobiyal dirençlilik değişimleri belirlenmiştir. Fenotipik karakterizasyon için disk difüzyon ve minimum inhibisyon konsantrasyon metodları kullanılacaktır. Genetik karakterizasyon içinse, beta-laktam, kloramfenikol, aminoglikozit, sulfonamit ve tetrasiklin dirençlilik genlerini kodlayan genler çalışılmıştır. Sonuçta, genetik çalışma için antimikrobiyal dirençlilik genlerini kodlayan 21 bölge (*bla*$_{TEM-1}$, *bla*$_{PSE-1\ (AKA\ CARB-2)}$,

bla$_{CMY-2}$*, ampC, cat1, cat2, flo, cmlA, aadA1, aadA2, strA, strB, aacC2, aphA*$_{1-Iab}$*, dhfrI, dhfrXII, sulI, sulII, tetA, tetB, tetG*) çoğaltılmıştır. Bazı dirençlilik genlerinin birlikte bulunması, *Salmonella* izolatlarında mobil genetik elementlerin bulunma ihtimalini ortaya atmıştır. Bu nedenle, plazmid ve Sınıf 1 integronlar araştırılmıştır. Virulant özelliklerinin incelenmesi amacıyla da, *ctdB, gatC, gogB, hlyE, pefA, ssek3, sseI, sspH, sodC, sopE,* STM 2759*, tcfA* genleri bu izolatlarda aranmıştır. Sonuçlar, izolat kaynağına (gıda, hayvan ve insan) ve serovar tipine göre analiz edimiştir. Çalışma, tarladan çatala kadar izole edilen *Salmonella*'ların antimikrobiyal duyarlılık profilleri hakkında bilinmeyenleri açıklamaktadır. Çalışmamız patojenite alanında yapılmış ilerici bir araştırma olma potensiyeline sahiptir.

Anahtar Kelimeler: Antimikrobiyal Dirençlilik, Mobil Genetik Elementler, *Salmonella*, Virülans

To My Grandmothers and Grandfathers,

To My Parents and Brother,

and

To My Husband

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LIST OF ABBREVIATIONS

Ak: Amikacin

Amc: Amoxicillin-clavulanic acid

Amp: Ampicillin

AR: Antimicrobial resistance

C: Chloramphenicol

Cip: Ciprofloxacin

Cn: Gentamicin

Cro: Ceftriaxone

Eft: Ceftiofur

Etp: Ertapenem

Fox: Cefoxitin

Imp: Imipenem

K: Kanamycin

Kf: Cephalothin

MLST: Multi locus sequence typing

N: Nalidixic acid

PFGE: Pulsed field gel electrophoresis

S: Streptomycin

Sf: Sulfisoxazole

SGI: *Salmonella* Genomic Island

Sxt: Sulfamethoxazole-trimethoprim

T: Tetracycline

CHAPTER 1

INTRODUCTION

Foodborne diseases have been one of the major health issues worldwide. The global human effect of foodborne diseases has not been estimated clearly, but gastroenteritis is known to be the cause of morbidity and motility in general population. It is estimated that the incidence of diarrheal disease varied from 0.44 to 0.99 episodes per person per year; in other words, such an incidence would produce 2.8 billion cases of diarrheal illness each year worldwide (Scallan, Hoekstra et al. 2011). And, bacteria are responsible for the 39% of the cases. Moreover, bacteria are responsible for the 64% of both hospitalization cases and deaths. The leading pathogens causing deaths were nontyphoidal *Salmonella* spp., *T. gondii* and *L. monocytogenes* (Scallan, Hoekstra et al. 2011) *Salmonella* is a genus of rod-shaped, Gram-negative bacteria. It is a significant pathogen in foodborne diseases of animals and humans. The *Salmonella* genus has two species, *S. enterica* and *S. bongoria*, and these two species contain 2463 serotypes. (Brenner *et al.*, 2000) As Scallan *et al.* (2001) reported nontyphodial *Salmonella* spp. caused 28% of deaths and 35% of hospitalizations in foodborne diseases. In addition to species, subspecies and serovar types, *Salmonella* has been classified into host-restricted, host-adapted, and unrestricted serovars where the classification is based on host specificity.

Salmonellosis (the disease that *Salmonella* spp. causes) is very severe and mostly needs antimicrobial treatment. So, having resistance genes to antimicrobial drugs is a great concern for a treatment to be efficient. And it is been recorded that some *Salmonella* isolates that are obtained from human patients, foods and animals are resistant to multiple antimicrobial drugs such as ceftriaxone and cephalosporin (FDA, 2010). Also, according

to the recent studies, it is observed that there is an increase in antimicrobial resistance among *Salmonella* due to use or misuse of antimicrobial drugs in human and veterinary medicine and this cause a selective pressure for the proliferation of resistant bacteria (Foley and Lynne 2008).

1.1. *Salmonella* and salmonellosis

Salmonellosis is a critical medical problem that causes symptoms of gastroenteritis including diarrhea, nausea, abdominal pain, vomiting, mild fever and chills caused by *Salmonella enterica* subsp. e*nterica* nontyphodial serotypes. The number of salmonellosis infections reaches up to approximately 40,000 infections for each year in USA according to CDC records. *Salmonella*, are Enterobacteriaceae, gram-negative, zero-tolerant, rod shaped, facultatively anaerobic bacteria that are able to survive in low oxygen atmospheres. They are mesophile, and their growth rates are considerably low at temperature below than 15°C.

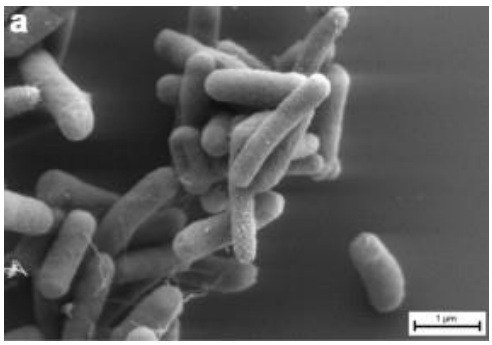


Figure 1 SEM micrographs of *Salmonella* Typhimurium (ST) in water control (Su, Howell et al. 2012)

Symptoms of salmonellosis are diarrhea, often fever and abdominal cramps after incubation period of 6 hours to 10 days. Differently, *Salmonella* Typhi, causes high fever, anorexia, malaise, headache and myalgia; sometimes diarrhea or constipation, is seen in 3-60 days. *Salmonella* Typhi is a host-restricted serotype, causing inflammation only in humans; thus its spread is limited compared to host-independent (i.e. *Salmonella enterica* subsp. *enterica* serovar Typhimurium) and host-adapted (i.e. *Salmonella enterica* subsp. *enterica* serovar Dublin) serovars.

Salmonella infections start with the ingestion of organisms that are found in contaminated food or water. *Salmonella* live in the intestinal tracts of humans and other animals, including birds. *Salmonella* are usually transmitted to humans by consuming foods contaminated with animal feces. Contaminated foods are usually of animal origin (beef, poultry, milk, or eggs), but any food, including vegetables, may also become contaminated. Food may also be contaminated by direct contact through the hands of an infected food handler who does not care personal hygiene.

Conditions that cause an increase in gastric acidity, reduce the *Salmonella* infectious dose, thus the gastric acidity plays a significant initial barrier for infection. In an interesting manner, *Salmonella*e demonstrate an adaptive acid-tolerance response on exposure to low pH, possibly encouraging the organism to be alive in acidic host environments such as the stomach. After entering the small bowel, *Salmonella*e must pass over the intestinal mucus layer before adhering to cells of the intestinal epithelium. *Salmonella*e have numerous fimbriae that lead to their capability to adhere to intestinal epithelial cells (Ohl and Miller 2001). All type of foods (meat, milk, ice-cream, etc.) plays a potential system as a host for *Salmonella.*

1.2. Isolation of *Salmonella* from food samples, i.e. analytical and molecular methods

The analytical methods are still the most known, highly-applied, traditional ways of controlling the food safety. Although the methods have some feedbacks such as being time-consuming and labor-intensive, they are well-standardized and the results obtained from them are accepted to be highly accurate.

The standard methods, which are used in food control and reference laboratories both in EU, USA and other countries to detect the pathogens in foods, are summarized in this section. The standard analytical methods can be reached from the Bacteriological Analytical Manual of US Food and Drug Administration (FDA/BAM), European Committee for Standardization (CEN), International Organization for Standardization (ISO) and Association of Analytical Communities (AOAC INTERNATIONAL). Protocols of CEN are basically adaptations of ISO methods. Protocols of both ISO and FDA/BAM are mainly based on cultural methods.

The analytical methods consist of several basic steps: sample collection, sample storage, sample preparation, detection and analysis, and finally result interpretation. Before sampling, it is crucial to consider the statistical considerations; for instance sample size, frequency and volume should be determined. Then, the sample is gathered by swabbing or by directly grabbing and stored at specific temperatures and for settled time intervals depending on the method and the microorganism. The samples should be processed to be homogenized by centrifugation or filtration. For some infections, purification and decontamination (i.e. from chemicals) may be required. Generally, non-selective enrichment, selective enrichment and selective agar plating is performed. The equipments, agars, broths are particularly chosen depending on the characteristics of the specific microorganism (growth conditions, able to use a sugar type etc.) and also the sample form (liquid/solid).

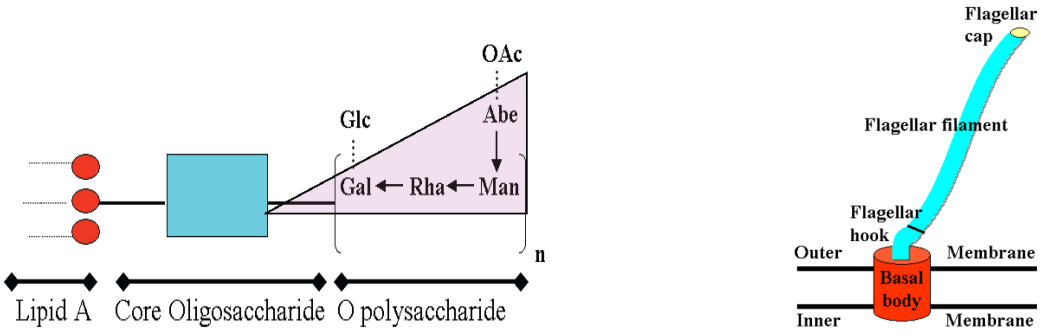
Rapid and accurate identification of pathogens is very important for foodborne outbreak detection together with epidemiological investigation. Recent multistate outbreak of *Salmonella* in cantaloupe in 2011 in the USA shows the sustained risk of pathogens and also the dispute of discovering the reason of widely-spread infections. Recent advances in molecular techniques have inspired the detection of pathogens in foods. For instance, PCR (polymerase chain reactions), synthesizing multiple copies of (amplifying) a specific piece of DNA, is the leading and mostly used technology (Naravaneni and Jamil 2005). These PCR-based methods consist of three parts: DNA extraction, DNA amplification, and detection. Sample enrichment, the start point of these assays, is the process where samples are incubated at enrichment broths to make all organisms to grow rapidly. After, sample preparation, in which step, the cells are lysed to extract DNA, the last process, PCR begins. In thermocycler, the DNA is amplified to produce sufficient copies of target sequence.

There are numerous methods to tract the bacterial source and determine the distribution of pathogens from the people that have foodborne illness. But there are some considerations to be a feasible subtyping method; for example, markers must be stable, reproducible, and exist in all outbreak isolates (van Belkum, Tassios et al. 2007). Besides the availability, the technique should have a high discriminatory power and also, illustrate similar outcomes with epidemiological results of an outbreak. On account of being operable to perform in different laboratories, the method should be rapid, adaptable to different conditions and pathogens. Likewise, it should have an affordable cost for the equipment, reagents, and consumables (van Belkum, Tassios et al. 2007).

Before the molecular subtyping methods, the previous methods, subspecies characterizations, have been done to identify the pathogens. The phenotyping methods such as serotyping and phage-typing have the ability to characterize bacteria but they have low discriminatory power compared to subtyping methods.

Serotyping is a definitive typing method used for epidemiological characterization of bacteria. Serotyping of *Salmonella* strains is carried out by identification of surface

antigens (LPS, O-antigens) and flagella antigens (proteins, H-antigens) (Figure 2). Most commonly, strains of *Salmonella* express two phases of H- antigens but aphasic, monophasic and triphasic variants are also known. The definition of the serotypes is based on the antigen combination present and is given in the "Kauffmann-White scheme" (Grimont and Weill 2007).



(a) (b)

Figure 2 Schematic view of (a) O-antigen and (b) H-antigen in *Salmonella* (Adapted from (Fields 2006)

On the other, for phenotyping methods, high amount of specialization is needed and their reagents may not be accessible for some laboratories. By the development of molecular techniques, it is now available to detect differences in the nucleic acid sequence of pathogens. Some of these subtyping methods are based on restriction analysis of bacterial DNA (i.e. ribotyping, pulsed field gel electrophoresis [PFGE]), and some uses polymerase chain reaction (PCR) amplification (i.e. amplified fragment length polymorphism [AFLP], multiple locus variable variable-number tandem repeat analysis [MLVA]) and the others identify DNA sequence polymorphism at specific loci in the

genome (i.e. multilocus sequence typing [MLST], single nucleotide polymorphism [SNP] analysis).

1.3. *Salmonella* and antibiotic usage

Salmonella infections usually settle in 5-7 days and mostly do not necessitate medical care other than oral fluids in healthy adults. Salmonellosis may cause severe diarrhea need rehydration with endovenous fluids. If infection spreads from the intestine, antibiotics, such as ampicillin, trimethoprim-sulfamethoxazole, or ciprofloxacin, are generally required. But some *Salmonella* bacteria have become resistant to antibiotics, mainly because of the use of antibiotics to encourage the growth of food animals.

It is a phenomenon that some strains of *Salmonella* show different antimicrobial resistant profiles and it receives a great attention in researches worldwide. The resistance profile may change depending on time, serovar, subtype, source of microorganism and also geographic region of isolate.

Antimicrobial resistance of zoonotic agents is screened through different agencies in developed countries. For example, in the US, the National Antimicrobial Resistance Monitoring System (NARMS) is a collaborative among the Food and Drug Administration, the Centers for Disease Control and Prevention (CDC) and the US Department of Agriculture (USDA) and it controls resistance of some main enteric bacteria to antibiotics (Tollefson, Angulo et al. 1998). The antibiotics tested by NARMS include amikacin, amoxicillin/ clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, tetracycline, nalidixic acid, streptomycin, sulfasoxazole and trimethoprim/sulfamethoxazole (FDA 2006). The NARMS Executive Report (2003) indicated that 22.5% of non-Typhi *Salmonella* isolates from humans were not susceptible to at least one antimicrobiotics, which shows a reduction from the 33.8% stated in 1996 (FDA, 2006). According to FDA, the most shared multidrug resistance phenotype was to ampicillin, chloramphenicol,

streptomycin, sulfonamides, and tetracyclines (ACSSuT), which was observed in 9.3% of isolates analyzed. When the veterinary samples were analyzed, it was seen that 44% of the *Salmonella* isolates, which were attained from animal slaughter and veterinary investigation sources, were found to be not susceptible to at least one antibotic (FDA 2006). The ACSSuT phenotype was again the most common multi-drug resistant profile (Figure 3).

Antimicrobial resistance profiles of *Salmonella* are also varied depending on the location of isolation. In USA, between 1999 and 2003, there was increased sulfisoxazole resistance but decreased tetracycline resistance in non-human isolates (Kiessling, Jackson et al. 2007). Resistance to amphicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline is usual in *Salmonella* serovars, but also resistance to other antibiotics and other resistance patterns may be observed (Ridley and Threlfall 1998, Boyd, Peters et al. 2001). Randall and his colleagues (Randall, Cooles et al. 2004) studied antibiotic resistance, resistance genes and integrons in *Salmonella* for 397 strains containing 36 serovars in UK. The antibiotics that they have used were ampicillin, chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulfadiazine, trimethoprim and tetracycline.

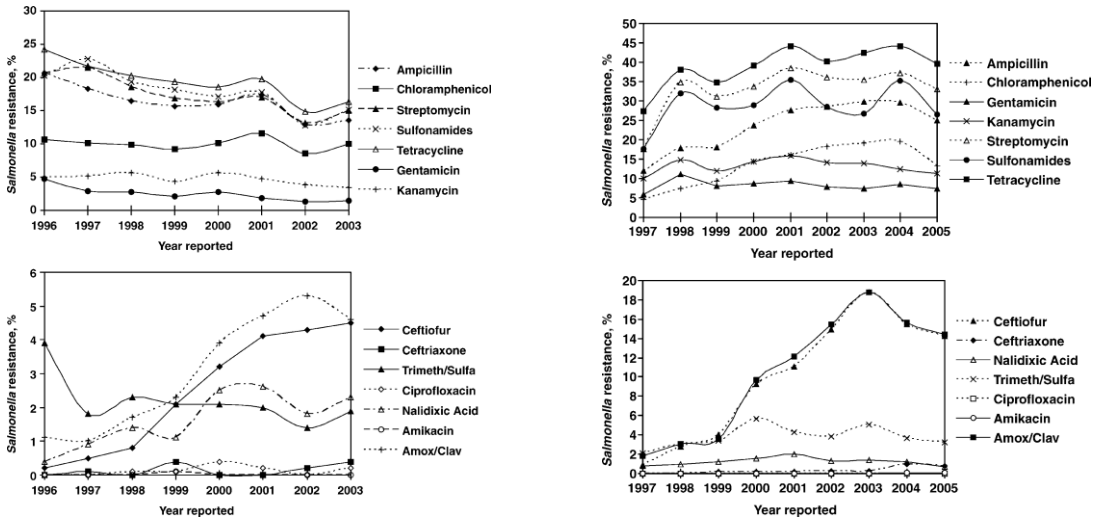


Figure 3 Changes in antimicrobial resistance profile with respect to time in *Salmonella* from human sources (a) and veterinary sources (b) during 1996 to 2005. Data are for 15 antibiotics tested for *Salmonella* resistance by the National Antimicrobial Resistance Monitoring System (FDA 2006, USDA 2007) (Trimeth/Sulfa: trimethoprim/sulfamethoxazole and Amox/Clav: amoxicillin/clavulanicacid)

From overall picture, it was seen that ampicillin, chloramphenicol and spectinomycin showed moderate antimicrobial activity, but streptomycin, sulfadiazine and tetracycline were the less effective antibiotics to *Salmonella* strains. A positive correlation exists between the presence of resistance genes and corresponding resistance phenotypes, proposing present resistance genes, are usually expressed (Table 1).

Table 1 Genes and mechanism of resistance (Adapted from (Randall, Cooles et al. 2004)

Resistance gene	Mechanism of resistance	Resistant to
aadA1	Streptomycin/spectinomycin adenytransferase	Spectinomycin, streptomycin
aadA2	Streptomycin/spectinomycin adenytransferase	Spectinomycin, streptomycin
aadB	Aminoglycoside transferase	Gentamicin
aphAI-IAB	Aminoglycoside phosphotransferase	Kanamycin
bla(Carb$_2$)	β-lactamase	Ampicillin
bla(Tem)	β-lactamase	Ampicillin
cat1	Chloramphenicol acetyl-transferase	Chloramphenicol
cat2	Chloramphenicol acetyl-transferase	Chloramphenicol
dhfr1	Dihydrofolate reductase	Trimethoprim
strA	Streptomycin phosphotransferase	Streptomycin
sul1	Dihydropteroate synthase	Sulfadiazine
sul2	Dihydropteroate synthase	Sulfadiazine
tetA(A)	Efflux	Tetracycline
tetA(B)	Efflux	Tetracycline
tetA(G)	Efflux	Tetracycline

1.4. Mechanisms of antimicrobial resistance in *Salmonella*

The antimicrobial resistance of *Salmonella* can be described by different mechanisms: (i) production of enzymes that inactivate antimicrobial agents, (ii) reduction of cell permeability to antibiotics, (iii) activation of antimicrobial efflux pumps, and (iv) modification of cellular target for drug (Sefton 2002). *Salmonella* produce β- lactamase enzymes, which can degrade the chemical structure of the antibiotics. The β-lactamases affect the antibiotic in different ways, some of them show affinities for the structures of

a restricted number of antibiotics, while others are called as extended- or broadspectrum β-lactamases, which can degrade a widespread collection of antibiotics (Bush 2003). The most concerning β-lactamases is the AmpC enzyme, which is generally encoded by bla_{cmy} and has been found to be related with the resistance antimicrobiotics such as ampicillin, ceftiofur, and ceftriaxone (Aarestrup, Hasman et al. 2004).

Some inactivating enzymes have the capability of modifying the structure of antimicrobial agents. To exemplify, most of the aminoglycoside resistance in *Salmonella* is related with aminoglycoside phosphotransferases, aminoglycoside acetyltransferases, and aminoglycoside adenyltransferases; which are known as modifying enzymes. They role in acetylating, phosphorylating and adenylating of known aminoglycosides (Poole 2005). *aph*A, which is known to play a function in aminoglycoside phosphotransferase, is associated wih kanamycin resistance, while *aac*C (aminoglycoside acetyltransferase encoded) can encourage gentamicin resistance, and lastly, *aad*A and *aad*B (aminoglycoside adenyltransferases encoded) are related with streptomycin and gentamicin resistance, respectively (Randall, Cooles et al. 2004, Welch, Fricke et al. 2007)

The other mechanism is the modification of the drug binding targets within the cell that ends up with antimicrobial resistance, again. For example, mutation in the genes encoding the topoisomerase enzymes needed for DNA replication, cause resistance to the quinolone and fluoroquinolone drugs. The mutations avoid the antibiotics from binding to their topoisomerase targets and thus they result in less and lack of antimicrobial activity (Heisig 1993). Efflux pumps, on the other hand, take away the antibiotic out of the cell, which are observed in resistance to tetracycline and chloramphenicol. Tetracycline resistance in most of the *Salmonella* isolates are due to efflux pumps and they are associated with *tet* genes. And chloramphenicol resistance in *Salmonella* is mostly related with efflux pumps due to *flo*R or *cm*l genes (Chopra and Roberts 2001, Butaye, Cloeckaert et al. 2003). On the other hand, rather than efflux-mediated resistance, drug target modification by chloramphenicol acetyltransferases due

to the *cat* genes, also cause chloramphenicol resistance in *Salmonella* (Murray and Shaw 1997). Enzymatic modification is also effective in sulfonamide and trimethoprim resistance, by the enzymes that function in changes in folic acid biosynthetic pathway; dihydropteroate synthetase (*sul1* and *sul2*) and dihydrofolate reductases (*dhfr*), respectively. (Huovinen, Sundstrom et al. 1995).

Mobile elements such as plasmids, phages, transposons, and mobilizable islands are also crucial for *Salmonella* evolution, including the occurrence of strains with new antimicrobial resistance and pathogenicity-gained phenotypes but more studies are required to understand that issue clearly (Switt, den Bakker et al. 2012)

1.5. Genetic mechanisms of antimicrobial resistance found in *Salmonella*

1.5.1. Aminoglycosides

The antimicrobial application of aminoglycosides have first seen in the middle of twentieth century as a treatment of severe infections related to Gram-negative bacteria (Maurin and Raoult 2001). Nowadays, aminoglycoside usage is decreased since their residuals can be found in animal tissues and they are toxic to nature. But, aminoglycosides such as streptomycin, gentamicin or neomycin have been applied as a treatment for intestinal diseases like swine dysentery and scours in weanling pigs (Maurin and Raoult 2001). In poultry, gentamicin has been given to cover *Salmonella* and *E. coli* infections. Also, aminoglycosides have been used together with macrolides and beta-lactams to treat mastitis in dairy cattle and enterococcal infections in human medicine (de Oliveira, Brandelli et al. 2006, Arias and Murray 2012).

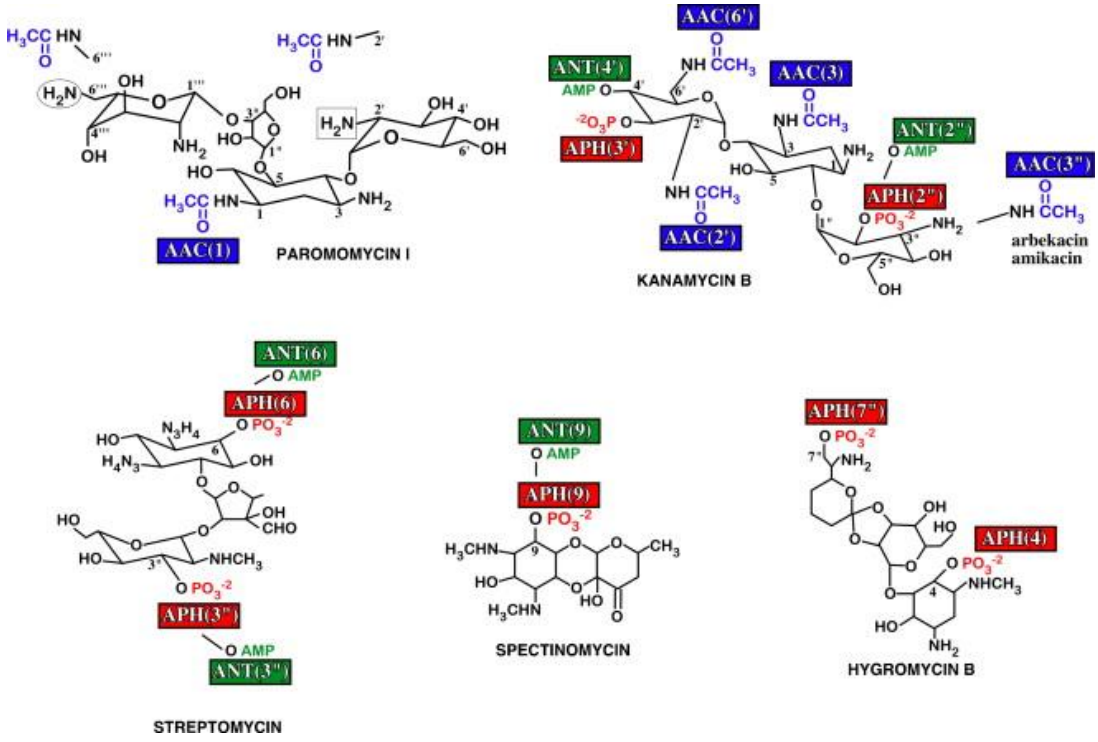


Figure 4 Representative aminoglycosides and modification sites by AAC (acetyltransferase), ANT (nucleotidyltranferases), and APH (phosphotransferases) enzymes. An example of each kind of modification is shown on one of the substrates (Adapted from (Ramirez and Tolmasky 2010)

The antimicrobial activity of aminoglycosides is due to their ability to bind to the 30S ribosomal subunit thus preventing protein translation. *Salmonella* species have gained resistance to aminoglycosides by enzymatic modification of the compound. The enzymes that play a role in resistance are acetyltransferases, phosphotransferases, and nucleotidyltransferases (Ramirez and Tolmasky 2010) (Figure 4).

Table 2 Common aminoglycoside antimicrobial genes found in *Salmonella* isolates from foods and animals

Antimicrobial group	Resistance related enzymes	Genes	References
Aminoglycoside	Acetyltranferases	*aacC(3'), aacC(3'')-IIa, aacC(6'), aacC2*	(Foley and Lynne 2008, Ramirez and Tolmasky 2010, Glenn, Lindsey et al. 2011, Folster, Pecic et al. 2012, Frye and Jackson 2013)
	Phosphotransferases	*aphAI,aphAI-IAB, aph(3')-Ii-iv,aph(3')-IIa, strA, strB*	
	Nucleotidyltransferases	*aadA,aadA1, aadA2, aadA12,aadB,ant (3'')-Ia*	

The aminoglycoside acetyltransferases, phosphotransferases, and nucleotidyltransferases are generally referred as *aac, aph,* and *ant* respectively (Frye and Jackson 2013). *aac* genes are usually related with resistance to gentamicin, kanamycin and tobramycin. Aminoglycoside phosphotransferases (*aph*), on the other hand, are associated with kanamycin and neomycin. But some *aph* genes are named differently such as *strA* and *strB* genes which confer resistance to streptomycin. Nucleotidyltransferase genes (*ant*) are found to have a role in resistance to antimicrobials such as gentamicin, tobramycin, or streptomycin and some of them are listed as *aad.* In total, the number of antimicrobial resistance genes is more than 50, but the common genes that are found *Salmonella* are given in Table 2.

1.5.2. B-lactams

Beta-lactam antimicrobials are the first antibiotics to be found, applied and described (due to discovery of penicillin in 1921 by Alexander Fleming). Thus, their resistance mechanism was the first to be understood. This group of antimicrobials are named due to their β-lactam rings which form irreversible bonds with enzymes that function in cell wall synthesis (Figure 5). And resistance to β-lactam group of antibiotics are developed by the enzymes; β-lactamases. They cleave the β-lactam ring and thus keep from binding and inactivating the cell wall enzymes (Kong, Schneper et al. 2010).

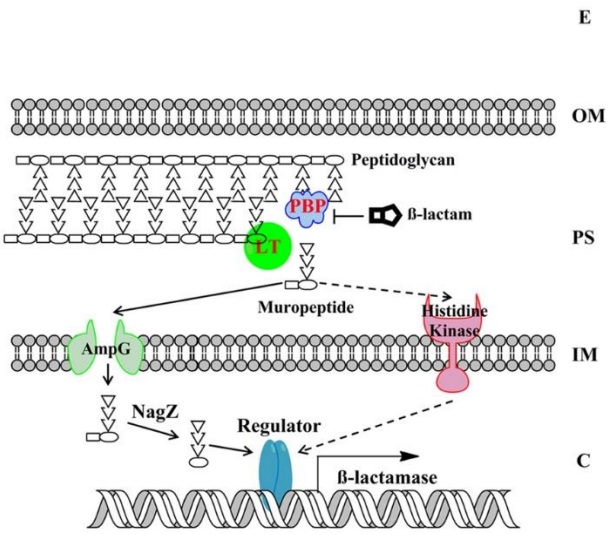


Figure 5 Beta-lactamase induction model in Gram-negative bacteria (Adapted from (Kong, Schneper et al. 2010) E, extracellular environment; OM, outer membrane; PS, periplasmic space; IM, inner membrane; C, cytoplasm.

New β-lactams are synthesized by modifying the chemical groups around the β-lactams ring to make them resistant to β-lactamases. Cephalosporins can be exemplified as cephalothin (1st generation), cefoxitin (2nd generation), ceftriaxone (3rd generation), and

cefipime (4th generation). Examples to carbapenems, on the other hand, are imipenem, ertapenem (Prescott 2000). But again, due to mutations in β-lactamase gene with the selective pressure done by the new antibiotics, extended spectrum β-lactamases (ESBLs) like cephalosporinases (Arlet, Barrett et al. 2006), and carbapenemases (Miriagou, Cornaglia et al. 2010) have been emerged. Still, some of the ESBLs can be inactivated by clavulanic acid-like inhibitors which can bind irreversibly to the specific β-lactamases and thus allow the β-lactam to be active such as in the case of Augmentin (ampicillin/clavulanic acid; Prescot, 2000).

Most ESBL-carrying *Salmonella* strains have been detected in Latin America, the Western Pacific, and Europe (Winokur, Canton et al. 2001). The first case was observed in the U.S. by 1994, because *S.* Typhimurium var. Copenhagen strain from an infant adopted from Russia was found to have *bla*$_{\text{CTX-5}}$ (Sjölund, Yam et al. 2008). Different ESBL *Salmonella* strains have been also reported, for example, one was obtained from a horse (*bla*$_{\text{SHV-12}}$) and one more from a 3-month-old child (*bla*$_{\text{CTX-M-5}}$) (Rankin, Whichard et al. 2005). Carbapenem resistance in *Salmonella* is also infrequent in the U.S. but has been detected in *Salmonella* serotype Cubana due to a plasmid-mediated *bla*$_{\text{KPC-2}}$ gene (Miriagou, Tzouvelekis et al. 2003). While ESBL-harboring *Salmonella* strains in U.S. is very rare, AmpC resistance encoded by *bla*$_{\text{CMY}}$ has been evolving in humans and also in food animals. The *bla*$_{\text{CMY}}$ mediates a cephalomycinase, which shows extended resistance to large number of beta-lactams, such as 1st, 2nd, and 3rd-generation cephalosporins (Zhao, White et al. 2001).

Beta-lactamases are generally transferred horizontally in *Salmonella* whereas other bacteria like *E. coli* may have intrinsic β-lactamases such as *ampC* (Siu, Lu et al. 2003). Most common β-lactamases in *Salmonella* are recorded as *bla*$_{\text{TEM-1}}$ and *bla*$_{\text{PSE-1}}$ (a.k.a. *bla*$_{\text{BARB2}}$) and they are associated with ampicillin, and *bla*$_{\text{CMY-2}}$ which is related with resistance to ampicillin and also 1st (i.e. cephalothin), 2nd (i.e. cefoxitin), and 3rd (i.e. ceftriaxone) generation of cephalosporins (Table 3). Apart from the mentioned genes, others (*bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{SHV}$, and *bla*$_{OXA}$ etc.) have been

observed worldwide to encode extended spectrum β-lactamases (ESBLs) or carbapenemase activity (Falagas and Karageorgopoulos 2009). Up to date, more than 340 β-lactamases genes have been recorded.

Table 3 Common β-lactam antimicrobial genes found in *Salmonella* isolates collected from foods and animals

Antimicrobial group	Genes	References
Beta-lactams	*bla*$_{\text{CMY-2}}$, *bla*$_{\text{PSE-1}}$, *bla*$_{\text{TEM-1}}$	(Foley and Lynne 2008, Glenn, Lindsey et al. 2011, Frye and Jackson 2013)

1.5.3. Phenicols

Nowadays, by the new clinical developments, chloramphenicol is almost found to be inappropriate for human medicine. So it has been banned in the U.S. and some other countries for practice in humans and food animals because they have a possible toxic effects on humans. Also, its usage is restricted due to resistance in most of the developed countries, which may be a result from the low- cost of this antibiotic and not-controlled, extensive use. It had been used to treat systemic salmonellosis, eye infections and some other infections caused by anaerobic bacterial (Prescott 2000).

It has been reported that most of the resistance to phenicols are due to efflux pumps that are associated with the presence of *floR* and *cmlA* genes (Table 4). Inactivating enzymes such as chloramphenicol acetyltransferase (*cat1*) can also play a role in phenicols resistance.

Table 4 Common phenicol antimicrobial genes found in *Salmonella* isolates collected from foods and animals

Antimicrobial group	Genes	References
Chloramphenicols	*floR, cmlA, cat1, cat2*	(Foley and Lynne 2008, Glenn, Lindsey et al. 2011, Frye and Jackson 2013)

1.5.4. Quinolones

Quinolones and fluoroquinolones are produced synthetically and they had been firstly used over two decades ago. Since they have broad spectrum and low toxicity, fluoroquinolones such as genrofloxacin, difloxacin, marbofloxacin, enrofloxacin, orbifloxacin, and sarafloxacin (Hopkins, Davies et al. 2005) have been utilized in food animals such as cattle, chicken and turkeys. Fluoroquinolones are also used in human medicine as a treatment antibiotic against *Salmonella, E. coli,* and other bacterial infections. For instance, ciprofloxacin is mostly used nowadays to treat these types of infections. Because of high usage of these quinolones in human medicine and detection of ciprofloxacin-resistant *Campylobacter jejuni*, enrofloxacin usage had been withdrawn in EU since these two antimicrobials share the same resistance mechanism (Nelson, Chiller et al. 2007). Also in U.S., it is banned to use fluoroquinolones in poultry and limited usage is allowed in cattle.

Quinolones and fluoroquinolones bind to DNA processing enzymes such as helicase, and thus prevent DNA replication and maintenance. And resistance to these antimicrobials has been found to be associated with mutations in the genes that mediate the enzymes such as *gyrA*, *gyrB*, *parC*, and *parE* (Table 5). Rather than mutation, *qnr* efflux system, and an aminoglycoside acetyltransferase, *aac(6')-Ib*, can also modify and deactivate ciprofloxacin, which is also a quinolone (Cavaco and Aarestrup 2009, Cavaco, Hasman

et al. 2009); Cavaco and Aarestrup,2009) but these mechanisms are rare in *Salmonella* isolates.

Table 5 Common quinolone/fluoroquinolone antimicrobial genes found in *Salmonella* isolates collected from foods and animals

Antimicrobial group	Genes	References
Quinolones	Mutations in quinolone resistance determining regions (QRDR) of *gyrA, gyrB, parC, parE*	(Hopkins, Davies et al. 2005)

1.5.5. Sulfonamides and trimethoprims

The folate pathway inhibitors are the compounds which compete for the substrates of the primary folic acid pathway in bacteria. These can be divided into two: the sulfonamides that inhibit DHPS (dihydropteroate synthase) and trimethoprims that inhibit DHFR (dihydrodolate reductase). Sulfonamides are bacteriostatic alone but when they are used together with trimethoprims, the effect is bacteriostatic (Walsh, Maillard et al. 2003).

Sulfonamides are very old antimicrobials which are started to be used in 1930s (Sköld 2001). Sulfonamides and trimethoprims have been used as growth promoters in swine and as treatment drug for diseases such as colibacillosis in swine and coccidiosis in poultry (Prescott 2000). They are commonly used in combination to treat *Salmonella* infections that are resistant to other antimicrobials (Acheson and Hohmann 2001). And their combination is used as a second line treatment of salmonellosis in U.S. since resistance to both of them is rare.

Sulfonamide resistance is generally acquired by the genes *sul1, sul2* and *sul3* that encode an insensitive DHPS enzyme and trimethoprim resistance is harbored by the genes *dhfr* or *dfr* which encode DHPR enzymes (Table 6).

Table 6 Common folate pathway inhibitors antimicrobial genes found in *Salmonella* isolates collected from foods and animals

Antimicrobial group	Genes	References
Sulfonamides and trimethoprims	*sul1, sul2, sul3, dfr1, dfrA10, dhfrI, dhfrXII*	(Glenn, Lindsey et al. 2011, Zou, Lin et al. 2012, Frye and Jackson 2013)

1.5.6. Tetracyclines

Tetracyclines are introduced to global usage by invention of chlortetracycline in the late 1940s. Borreliosis, erlichiosis, rickettsiosis, tularemia and also infections such as pneumonia, brucellosis, and listeriosis have been treated with tetracyclines in food animals (Roberts 1996, Roberts 2005). Tetracyclines such as chlortetracycline and oxytetracycline are also used as growth promotion and feed efficiency promoter in cattle, swine, and poultry.

Its mechanism is based on targeting the 30S subunit of bacterial ribosome and thus preventing protein synthesis. Different resistance mechanisms have been determined; (i) efflux, (ii) modification of the rRNA target, and (iii) inactivation of the compound. But in *Salmonella,* mostly active efflux pump systems are found (Table 7) and they are generally related with the genes *tetA, tetB, tetC, tetD, tetG,* and *tetG.* It is a fact that

tetracycline resistance is high due to overuse of it in animals and in humans. Interestingly, they can also be found in the lists of growth promoters in animals (Jones-Lepp and Stevens 2007).

Table 7 Common tetracycline antimicrobial genes found in *Salmonella* isolates collected from foods and animals

Antimicrobial group	Genes	References
Tetracyclines	*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(G),and regulator *tet*R	(Roberts 2005, Foley and Lynne 2008, Glenn, Lindsey et al. 2011, Frye and Jackson 2013)

1.6. Mobile genetic elements of *Salmonella*

Mobile genetic elements (MGE) are parts of DNA that encode enzymes and other proteins that provide the movement of DNA within genomes (intra-cellular mobility) or between bacterial cells (inter-cellular mobility). Transformation, conjugation and transduction are the three ways of intercellular DNA movement in prokaryotes. Understanding the roles and origins of mobile genetic elements is very crucial nowadays due to its important roles in antibiotic resistance, infectious diseases, bacterial symbiosis, and biotransformation of xenobiotics (which is a foreign chemical material found within an organism) (Levin and Bergstrom 2000, Frost, Leplae et al. 2005).

Bacterial sequencing projects obviously designates that bacteria can adapt and genomes develop by positioning current DNA in a new arrangement and by acquisition of new

sequences. Therefore, MGEs have played an important role in the evolution of bacteria (Molbak, Tett et al. 2003).

1.6.1. Antimicrobial resistance associated mobile genetic elements in *Salmonella*

Plasmids are unnecessary extra-chromosomal fragments of DNA and they can duplicate with diverse autonomies from the replicative proteins of the host cell. Plasmids are existing in most of the bacterial species (Amabilecuevas and Chicurel 1992), but differ in size (1 to 1000 kb). Plasmids are also able to denote a big amount of the entire bacterial genome. In nature, plasmids are ablso responsible for genetic variety in bacteria and they help bacteria to to adapt to their environment possibly by horizontal gene transfer (Bergstrom, Lipsitch et al. 2000, Gogarten, Doolittle et al. 2002). Plasmids usually do not comprise genes vital for cellular functions, but some can mediate replicative roles and a variable collection of accessory genes role in routes, which are distinct from the chromosomal genome. The accessory gene traits can be collected in the cell and they are known to not alter the gene content of the bacterial chromosomal DNA. These traits can be virulence and/or resistance abilities, which affect the behavior of bacteria.

Plasmids contain the genes responsible for replication, controlling the copy number and inheritance at every cell division, which is also recognized as portioning. Plasmids thay have the identical replication mechanism cannot be present in the same cell. This phenomenon is called as incompatibility (Inc) and this trait is used for the classification of plasmids. They are identified as incompatible when they have repressors effective for preventing the replication of other plasmids. Generally, closely related plasmids are incompatible, and so they are involved in a dissimilar incompatibility groups. There are 26 incompatibility groups determined for enterobacteriaceae. Four main incompatibility groups have been determined so far based on the genetic similarity and pilus structure. The IncF groups contains InC, IncD, IncF, IncJ, IncS,; the IncP group is composed of

IncM, IncP, IncU, IncW; the Ti plasmid group consist of IncH, IncN, IncT, IncX, and lastly the IncI group has IncB, IncI and IncK (Garcillán-Barcia, Francia et al. 2009).

Functional properties of plasmids can also be used to characterize them effectively. For instance, the plasmids that carry *tra* gene that provides conjugation, transfer of DNA and thus expression of sex pili are named as F-plasmids, due to its fertility function. The replication organization of the plasmids outlines the pili and the incompatibility groups of them. The plasmids that contain resistance genes against antibiotics or poisons are known as R-plasmids. Col plasmids, on the other hand, have the code for bacteriocins, which are the proteins to kill other bacteria. Degradative plasmids have the capability of digestion of foreign molecules such as toluene and salicylic acid. And lastly, the virulence plasmids impose bacteria pathogenic properties.

Plasmid complexity maximizes with the size of the plasmid and these megaplasmids can have numerous co-integrated compatible replicons. Bacterial isolates mostly harbor minor, cryptic plasmids, which have a limited number of genes of anonymous role and replication genes. These small plasmids can be exchanged to an additional cell, where a conjugative plasmid, which are larger in size, or integrated conjugative elements (ICEs) occur by a process known as mobilization.

Salmonella enterica plasmids change in size from 2 to 240 kb. The virulence plasmids (50–100 kb) are best known and described ones, which are present in serovars Abortusovis, Choleraesuis, Dublin, Enteritidis, Gallinarum, Pullorum and Typhimurium. But the serovars such as Hadar, Infantis, Paratyphi, and Typhi and many of the exotic serovars generally do not harbor plasmids. But this case is correct for most *S. enterica* subspecies *enterica* serovars, while it is not true for the serovars that are often related with humans, and farm animals infections as described before (Rychlik, Gregorova et al. 2006).

High molecular weight plasmids are mostly associated with antibiotic resistance. Since most of the antibiotic resistance-associated plasmids are conjugative, they can share their genetic information and thus cause them to spread in larger proportions. Low molecular

weight plasmids, on the other hand, are known to have restriction modification systems, which at the end make them more resistant to phage infections. Ability to have these low molecular weight plasmids can be used to differentiate in epidemiological studies but there is no detaied information about their role.

The location of resistance genes is often fixed, they are on extrachromosomal genetic elements or in segments introduced within the chromosome. Genetic transformation is often needed for the acquisition of a new gene. Nevertheless, conjugative transfer is able to assemble the resistance genes on plasmids on different locations. The second can happen more regularly and efficiently, and thus numerous resistance genes can be assimilated at the same time (Garcillán-Barcia, Francia et al. 2009). Plasmids are thus notable for storage of genetic information and for circulation of genetic information as well as antimicrobial resistance.

Some antimicrobial resistance related plasmids are high molecular weighed like up to 200 kb. And these plasmids were observed in a collection of historical pre-antibiotic era isolates that were collected between 1917 and 1950. These pre-antibiotic era plasmids usually belong to IncF, IncI, and IncX incompatibility groups. And for the recent *Salmonella* isolates, the plasmids from IncF, IncI, and IncX incompatibility groups are the frequently-seen ones, and the incompatibility groups IncN, IncP and IncQ follows the previous ones.

Antimicrobial resistance is generally associated with conjugative plasmids, which are high molecular weight plasmids and confer resistance to multiple antimicrobials (R-plasmids). The resistance genes in plasmids are placed within transposons that function in relocate from plasmids to chromosome, and interchangeably. Generally, motile plasmids, which need co-resident conjugative plasmids, do not have the genes that encode the properties enabling the cell to couple prior to DNA transfer but they encode the proteins necessary for transfer of their own DNA. The motile resistance plasmids are usually small (less than 10 kb) but conjugative plasmids are larger in size with 30 kb or

more. On the other hand, resistance plasmids, which are 100 kb or more are not frequent in Gram negative bacteria (Bennett 2008).

Plasmids having beta-lactam resistance genes are one of the most well-defined and studied ones. During 1990-1997, reports alarmed the rapid development of beta-lactam resistance in several countries (Threlfall, Ward et al. 1997). In France, it was observed that there is a sudden increase from 0 to 42.5% between 1987 and 1994 in the prevalence of *Salmonella* isolates that are resistant to beta-lactams. And different beta-lactamases were found to be associated with plasmid with various incompatibility groups such as Q, P, F and HI (Llanes, Kirchgesner et al. 1999).

Table 8 Generally found chromosomal and plasmid-associated genes in *Salmonella* serovar Typhimurium

Antimicrobial resistance group	Chromosome associated genes	Plasmid associated genes
Ampicillin	*blapsE-i*	*blaTEM*
Chloramphenicol	*floR*	*cat*
Sulfonamides	*sul1*	*strA/B*
Streptomycin	*aadA2*	*sul2*
Tetracycline	*tetG*	*tetA, tetB, tetR*

Genomic islands are regions within the bacterial genome and they are originated from gene transfer. Their classification is based on their characteristics such as; G-C content which is different from the rest of the genome, alternative codon preferences, and mobility genes (Kelly, Vespermann et al. 2009). Pathogenicity islands, on the other hand, are the subset of genomic islands, which are related with virulence. Genomic islands play

a role in symbiosis, fitness, metabolism, antimicrobial resistance and pathogenicity (Dobrindt, Hochhut et al. 2004).

Salmonella genomic island (SGI) is an integrative mobilizable element related with multiple drug resistance and it has been found in many serovars; Agona, Albany, Paratyphi B, Newport, Kentucky, Virchow, Derby, Infantis (Boyd, Peters et al. 2001, Mulvey, Boyd et al. 2006, Doublet, Granier et al. 2009). SGI1 is able to conjugate and integrate specifically to site into other *Salmonella* strains and thus they may cause the increase of MDR *Salmonella* strains, which is a serious clinical issue. *Salmonella* Typhimurium DT104 has SGI1 that contains the genes responsible for ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline resistance (Mulvey, Boyd et al. 2006). Differently serovar Albany has trimethoprim resistance cassette rather than streptomycin resistance cassette in SGI1 (Hensel 2004). The changes and acquisitions of genes inside SGI1 imply that there is a continual evolution.

Naturally occurring gene expression elements called integrons, and they also are the vehicles for the acquisition of resistance genes carried by mobile genetic elements. These elements are also found to be involved in the genetic reassembly of resistance in multi drug resistant (MRS) pathogens. Three classes of integrons have been defined so far. Class 1 integrons are the most known and studied class, which is prevalent among clinical isolates, and they are composed of two stable region: 5'CS and 3'CS regions, and interposed variable region where gene cassettes for antimicrobial resistance are settled, at the same orientation and at the *attI* site. The 5'CS region of class 1 integrons has the *intI1* gene that encodes the type 1 integrase protein and it is related with site-specific insertion and removal of gene cassettes. Thus, there are many integron formations as a result of number, type and order of inserted genes. A gene cassette contains a recombination site (59 base element) and an open reading frame (Stokes, Holmes et al. 2001). And the 3'CS region has the *sul1* and *qacEΔ1* genes, which are associated with sulfonamides and quaternary ammonium compound resistances, respectively. Gene cassette array complementary to Class 1 integron were found in the transposon Tn7 with

intI2 instead of *intI1* and this group is named as Class 2 integrons. And recently, Class 3 integron has been identified with the putative integrase *intI3.*

1.6.2. Mobile genetic elements and chromosome\-associated virulence characteristics of *Salmonella*

Numerous *S. enterica* isolates are categorized by the existence of host-adapted virulence plasmids encoding genes giving them ability to colonize and have resistance to complement killing, such as the *spvA, spvB* and *spvC* (*Salmonella* plasmid virulence); the *rck* (resistance to complement killing) genes (Guiney, Fang et al. 1994); fimbriae-associated operons *fim, agf, lpf, sef* and *pef;* the *sopE* gene (type III secretion system, entry of bacterium into host cell) and lastly the *astA* gene (EAST1 toxin, enteroaggregative thermostable enterotoxin).

Recently, eight *Salmonella* serovars (Enteritidis, Typhimurium, Dublin, Paratyphi C, Choleraesuis, Gallinarum/Pullorum, Sendai and Abortusovis) (Table 9) are found to have virulence plasmids and these plasmids are serovar-specific. Despite many common properties among them, each virulence plasmids seem to be specific to its host, exemplified by the plasmid size unique to its serovar (Table 9). Although there are many common properties among them (Montenegro, Morelli et al. 1991), the virulence genes have some specific features about their host adaptability as their plasmid size also varies depending on their hosts. For instance the largest virulence plasmid originated from the serovar *S.* Sendai is 286 kb, while the smallest one is 50 kb, observed in *S.* Choleraesuis. This issue propose that the virulence plasmids cannot easily transfer between different serovars and more than one virulence plasmid can be present in one host. Every virulence plasmids have different degrees of degradation in the *tra* operon except in pSTV (Typhimurium). The deletion of *tra* operon causes different plasmid sizes and also demonstrates the reason of being non-conjugative. While some plasmids like pSCV (Choleraesuis) and pSDUV (Dublin) cannot be transferred by conjugation, pSGAV (Gallinarum) can move by the help of F or F-like plasmids (Ou, Lin et al. 1994) and

differently pSTV (Typhimurium) can transmit by itself although its conjugation frequency is very low (García-Quintanilla, Ramos-Morales et al. 2008). Thus it can be concluded that there are two lineages among *Salmonella* virulence plasmids. One contains pSCV, pSEV and pSTV. pSCV and pSEV are understood to be derived from pSTV by a deletion of some genes at two different locations. The other lineage contains pSDV and pSPV, which differ from other in a 12-kb DNA region that consist of *faeH* and *faeI* genes rather than the *pef* operon (Chu and Chiu 2006). Other than the mentioned *Salmonella* serovars, the serovars Newport, Derby, Give, Johannesburg and Kottbus were found to carry virulence plasmids (Rotger and Casadesús 2010).

Table 9 Virulence associated *Salmonella* plasmids (Refined from ncbi.nlm.nih.gov)

Plasmid	Specific serovar	Plasmid size	Virulence genes	Disease	Host
pSCV	Choleraesuis	49.56 kb	*spvR, spvA, spvB, spvC, spvD, VsdF*	Septicemic disease	Pigs
pSAV	Abortusovis	50-67 kb	*spv operon*	Abortion	Sheep
pSPCV	Paratyphi C	55.41 kb	*spvA, spvB, spvC, spvD*	Paratyphoid fever	Humans
pSEV	Enteritidis	59.37 kb	*spvR, spvA, spvB, spvC, spvD,* virulence plasmid *DNA2C*	Murine typhoid	Rodents
pSDUV	Dublin	80 kb	*spvR, spvA, spvB, spvC, spvD*	Septicemic disease	Cattle
pSPV	Gallinarum/ Pullorum	87.37 kb	*spvR, spvA, spvB, spvC, spvD, vagC, vag D*	Fowl typhoid/ Pullorum disease	Poultry
pSTV	Typhimurium	94.7		Murine typhoid	Rodents
pSSV	Sendai	285 kb			

The increase of virulence plasmids has the capability of extension of host range of plasmids and thus leads to occurrence and spread of more virulent and resistant non-typhoid *Salmonella* (Fluit 2005).

Infections caused by *Salmonella enterica* change by serovar and the nature of the infected host. The major components for *Salmonella* to cause infection are carried on discrete regions of the chromosome called *Salmonella* pathogenicity islands (SPIs) and 14 SPIs have been identified so far (Table 10). SPI1 is required for bacterial penetration of the epithelial cells of the intestine. SPI2, 3 and 4 are necessary for growth and survival of bacteria with the host. Virulence factors of SPI5 are found to mediate the inflammation and chloride secretion and thus characterization of enteric phase of disease. Type III secretion system is both encoded by SPI1 and SPI2 and the secretion causes translocation of bacterially encoded proteins into the host cell cytosol.

Virulence plasmids, on the other hand, are required for growth of the bacteria within host macrophages and they function in prolonged survival.

Bacteriophages take attention since they are source of DNA transfer causing an evolution (Figueroa- Bossi, Uzzau et al. 2001). In *Salmonella,* several bacteriophages have been identified, which play roles in fitness and virulence, and the phages are grouped in five; P27-like, P2-like, lambdoid, P22-like, and T7-like (Kropinski, Sulakvelidze et al. 2007). And there are three outliers; KS7, Felix O1 and ε15. Most of the *Salmonella* phages belong to the P22 family and can enable horizontal transfer of bacterial virulence genes by transduction (Mirold, Rabsch et al. 1999).

Gifsy 1 and Gifsy 2 are two lambdoid prophages found in *Salmonella* serovar Typhimurium (Slominski, Wortsman et al. 2007). Gifsy 2 takes attention since it is definitely associated with virulence (Figueroa- Bossi and Bossi 1999) and it has the genes *sodC, gtgB/sseI* and *gtgE* (Coombes, Wickham et al. 2005).

Table 10 The roles of *Salmonella* pathogenicity islands (SPIs)

Region	Related genes	Functional properties	Serovar(s)
SPI1	*sopA, sopB, sopD, sopE, sitABCD*	Type III secretion system, Fe^{2+} and Mn^{2+} uptake system	Broad
SPI2	*Ttr, ssr, ssc, ssa, sse*	Type III secretion system, tetrathionate respiration	Broad
SPI3	*mgtCB, misL, marT*	Magnesium transport system, colonization of GI tract	Typhi, Typhimurium
SPI4	*soxSR, ims98*	Type I secretion system, superoxide response regulatory genes, colonization of cattle GI system	Different structure among serovars
SPI5	*sopB/sigD, pipB*	SPI1 and SPI2 encoded type III secretion system, enteropathogenic responses	Broad
SPI6	*pagN*	Invasion, Saf and Tcf fimbriae	Typhi, Typhimurium
SPI7	*viaB, sopE*	Capsular exopolysaccharide biosynthesis, type III secretion system, invasion, enteropathogenesis, type IV pili, SopE prophage	Typhi, Dublin, Paratyphi C
SPI8		Bacteriocin, integrase	Typhi
SPI9		Type I secretion system, toxin-like protein, biofilm formation, intestinal colonization	Typhi, Typhimurium
SPI10	*sef*	Cryptic bacteriophage, Sef fimbriae, virulence in chicks	Typhi, Enteritidis
SPI11		Macrophage survival, serum resistance	Choleraesuis
SPI12		Type III secretion system effector	Choleraesuis
SPI13		Virulence in chicks	Typhimurium
SPI14		Virulence in chicks	Typhimurium

SodC, on the other hand, is significant for bacterial survival within macrophages since it is a periplasmic Cu/Zn superoxidase dismutase and it functions in the production of hydrogen peroxide from superoxide radicals (Farrant, Sansone et al. 1997, Tidhar, Rushing et al. 2015).

SseI is an effector protein and related with SPI2 encoded type III secretion system and found generally on Gifsy 2 phages. It inhibits normal host cell migration, which finally prevents the ability of the host to eliminate the systemic bacteria; in this case *Salmonella* serovar Typhimurium, Enteritidis or Paratyphi C, for instance (Thomson, Clayton et al. 2008, McLaughlin, Govoni et al. 2009, Huehn, La Ragione et al. 2010).

Ssek3 is a new identified protein, which is again a phage-encoded effector that takes a role in SPI2 type III secretion system (Brown, Coombes et al. 2011).

Gifsy 1 is also related with virulence, but the effect of virulence genes is not observable in the presence of Gifsy 2, because their genes are functionally identical. Gifsy 1 encodes GipA and GogB; where GipA is specifically induced in the small intestine of the host animals and the lack of this protein is related with reduction in the growth and survival of *Salmonella* in Peyer's patches (aggregated lymphoid modules) and GogB is able to localize to the cytoplasm and has a nearly same sequence with virulence associated proteins in other bacteria (Coombes, Wickham et al. 2005). GogB protein is also known as leucine-rich protein, and is secreted by both type III secretion systems encoded in SPI1 and SPI2. On the other hand, Translocation of GogB into host cells is a SPI2-mediated process since its regulation is controlled by SPI2-related transcriptional activator, SsrB.

Fels1, Fels2, Gifsy 3, and SopEΦ are the other phages found in *Salmonella* serovars (Chan, Baker et al. 2003). Gifsy 3 is found in the serovar Typhimurium ATCC 14028 and carries the gene *pagJ*; associated with PhoP/PhoQ (a regulatory system correlated with virulence). Fels1 is seen in the serovar Typhimurium LT2 and encodes NanH and SodCII whereas; Fels2 is commonly observed in the serovars Typhimurium, Typhi, Sendai, and Enteritidis.

SspH, is another effector protein and can be found among different serovars of *Salmonella* (i.e. Enteritidis, Typhimurium, Typhi, and others). It functions as a ubiquitin protein ligase, and it is associated with Gifsy-3 phage and SPI2. SspH provokes host cellular immune response and prolongs intracellular bacterial survival (Rohde, Breitkreutz et al. 2007, Le Negrate, Faustin et al. 2008).

SopEΦ is generally found in the serovar Typhimurium and Typhi and encodes SopE, which is related with bacterial invasiveness (Kropinski, Sulakvelidze et al. 2007). SopE protein is also commonly associated with SPI1 and function as a guanine nucleotide exchange factor in SP1 type III secretion system to transfer effector proteins into host cells and to control host cell signal transduction. Excitingly, *sopE* gene has sequences resembling tail and tail-fiber genes of P2-like phages and is found in one *S.* serovar Typhimurium strain and lacking from another (Hardt, Urlaub et al. 1998). SopE protein is commonly found in *S.* serovar Typhimurium STM 910, but not in strain STM 709 (Cordeiro, Yim et al. 2013).

Table 11 The bacteriophages found on *Salmonella* serovars

Region	Related genes	Serovar(s)
Gifsy 1	*gigA, gogB*	Typhimurium
Gifsy 2	*sodC, gtgB/sseI* and *gtgE*	Typhimurium, Typhi
Gifsy 3	*pagJ, sspH*	Typhimurium
Fels1	*nanH, sodC*II	Typhimurium
Fels2	*int, fII*	Typhimurium, Typhi, Sendai, Enteritidis

Nonetheless, not all virulence genes are transferred through mobile genetic elements, some of them such as *cdtB, tcfA, hlyE*, *gatC* and STM2759 are chromosome associated.

CtdB protein is a cytolethal distending toxin (Williams, Gokulan et al. 2015) and mostly found in *S.* serovar Typhi (Hodak and Galan 2013) and thus known as typhoidal toxin. But nowadays, it is found in nontyphoidal serovars such as Enteritidis, Typhimurium, Montevideo, Poona and Chester (Lienau, Strain et al. 2011, Timme, Pettengill et al. 2013).

TcfA, on the other hand, is a fimbrial protein, which takes part in cell wall organization and again it is mostly associated with *S.* serovar Typhi. But a recent study has shown that a megaplasmid of *S.* serovar Infantis from Israel has pathogenic characteristics such as harboring *tcfA* gene (Aviv, Tsyba et al. 2014). Also, *tcfA* is found in other non-typhoidal serovars like Enteritidis, and Kentucky (Beutlich, Jahn et al. 2011, Allard, Luo et al. 2013).

HlyE is a pore-forming hemolysin and it accumulates in the periplasm of S. serovar Typhi (Oscarsson, Westermark et al. 2002). This periplasmic *S.* serovar Typhi hemolysin (*hlyE*) is found to be necessary for efficient invasion of host cells and colonization in deep organs for *S.* serovar Typhimurium in mice model (Fuentes, Villagra et al. 2008). Recently, hemolysin protein is also detected in *S.* serovar Kentucky isolated from broiler chickens (Dhanani, Block et al. 2015).

STM2759, is again a periplasmis protein, functioning as a dipeptide/oligopeptide/nickel ABC-type transporter and is associated with enteritic and invasive *S.* serovar Typhimurium LT2 isolates so far (McClelland, Sanderson et al. 2001, Suez, Porwollik et al. 2013).

GatC, a galactitol transmembrane transporter protein, is very common among *Salmonella* serovars such as Typhi, Typhimurium, Kentucky, Enteritidis, Infantis, and Paratyphi C (Liu, Feng et al. 2009, Fricke, Mammel et al. 2011, Timme, Pettengill et al. 2013, Aviv, Tsyba et al. 2014). It encodes a component of the phosphoenolpyruvate (PEP)-dependent phospho-transferase system for galactitol uptake (Fabich, Leatham et al. 2011).

1.7. Aim of the study

In literature, there are high numbers of examples that show the distribution of pathogens (i.e. *Salmonella*) changing geographically. In addition, the antimicrobial resistance profile of *Salmonella* alters in different serovars, geographic regions and in various hosts. Identification of distribution of antimicrobial susceptibility profile is crucial for human health, and economical issues for different countries.

There are few studies related with the antimicrobial susceptibility profile of *Salmonella* from different sources in Turkey. In the study conducted by Erdem et al., (Erdem, Ercis et al. 2005), 620 *Salmonella* clinical human cases were analyzed from ten cities (Ankara, Antalya, Bursa, Eskişehir, Edirne, İstanbul, İzmir, Kayseri, Konya and Trabzon) with 8 antimicrobial agents (ampicillin, amoxicilin-clavulanate acid, cloramphenicol, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin and cefotaxime) using MIC method. The ratio of susceptible pathogens to all antimicrobials were found as 35.1%, 14.9%, 88.9%, 75.0% for *S.* Paratyphi B, *S.* Typhimurium, *S.* Typhi, and *S.* Enteritidis, respectively. And in the study of Avsaroglu (Avsaroglu 2007), 59 epidemiologically unrelated *Salmonella* strains isolated from foods in Turkey and 29 in Germany were analyzed for serotyping, phage typing, antimicrobial typing and molecular biological characterization. Among 72 resistant strains, the most prevalent resistance genotypes were observed as bla_{tem-1} (56 %, ampicillin resistance); *floR* (100 %, chloramphenicol and florfenicol resistance); *aphA1* (100 %, kanamycin and neomycin resistance); *tet*A (53 %, tetracycline resistance); *aadA1* (82 %, spectinomycin and streptomycin resistance); *sulI* (78 %, sulfonamide resistance).

There is an increase in the number of antimicrobial resistant strains (especially multi-resistant *Salmonella* strains) and it presents a threat for human health. In Turkey, there is a high potential of redundant and unconscious usage of antibiotics, especially in humans and animals that causes the pathogens (i.e., *Salmonella*) to get antimicrobial resistance genes to their genetic material. In clinical veterinary cases antibiotic usage is performed without doing any test in most of the regions of Turkey. And this influences the pathogen

chain that comes from farm to the fork. In this study, the analyses were performed in three types of sources; foods, humans and animals by phenotypic and genetic methods.

The phenotypic and genotypic distribution of antimicrobial resistance profile of *Salmonella* in Turkey was determined by using the antimicrobials and the resistance genes. Findings of strains that were resistant to antimicrobials and the type of antimicrobial had given informational results for the health promotion activities. The results were analyzed according to the source of isolate (food, animal, and human), the type of serovar. Our study fills the gap of limited relevant study about the antimicrobial susceptibility profile of *Salmonella* isolates from farm/field to fork.

CHAPTER 2

MATERIALS AND METHODS

2.1. Bacterial strains

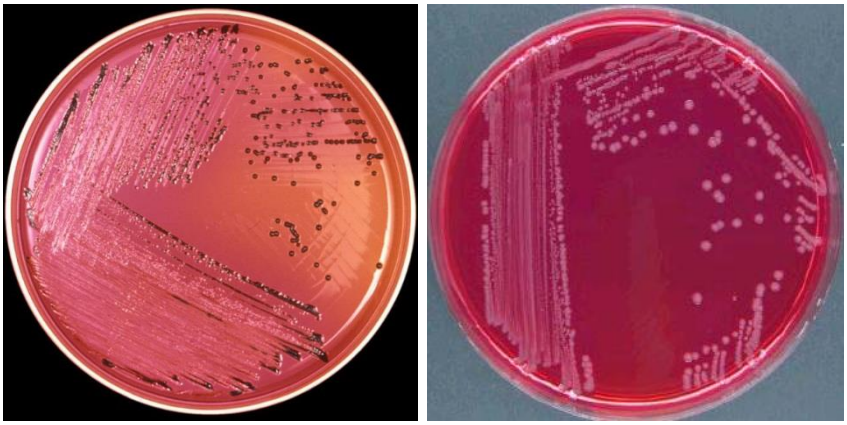
Strains were gathered from Turkey (especially from Southeast Anatolian Region and Median Anatolian Region). The isolates were from veterinary, human and food (i.e. different kind of meat, cheese, nut, spices) sources.

2.1.1. Food isolates

All isolates were obtained from Sanliurfa, Southeast Anatolian Region of Turkey. From April 2012 to January 2013, food samples were collected from eight different food types: (i) ground lamb, (ii) ground beef, (iii) chicken meat, (iv) unripened cheese, (v) Urfa (ripened) cheese, (vi) pistachio, (vii) pepper and (viii) isot (paprika). Samples were collected from two different locations and three different quality types, which was determined according to their prices. In each season (summer, autumn, winter and spring) 48 samples (8 type X 2 location X 3 quality type) were collected. All food samples were transported to Middle East Technical University (METU) Food Engineering Department (Ankara, Turkey) overnight in cold chain for isolation and further studies (Appendix 1). At a total 192 samples were studied for *Salmonella* isolation according to ISO 6579 procedure in METU, Ankara (Durul, Acar et al. 2015).

According to the ISO 6579:2002, the isolation step was performed in three stages: non-selective pre-enrichment, selective enrichment, and selective agar plating. For non-selective enrichment, 25 g of sample was weighted with a sterilized spoon and then put into a stomacher bag with 225 ml buffered peptone water (ISO) (CM1049, Oxoid,

Thermo Fisher Scientific Inc.). The sample was put into stomacher () for 30 sec, and then incubated for 16-20 h at 37°C. In selective enrichment, 0.1 ml of the mixture in stomacher bag was transferred into 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth (CM0866, Oxoid, Thermo Fisher Scientific Inc.) in parallels and incubated at 41.5 ± 1°C for 24 ± 3 h. RVS broth (Rappaport, Konforti et al. 1956) has a specific formulation for *Salmonella* species, such as (i) it has the capability to persist at relatively high osmotic pressure, (ii) to survive at relatively low pH values, (iii) to be comparatively resistant to malachite green, and (iv) to include relative less challenging nutritional requirements. After RVS step, 10 µl of broth was spread into xylose-lysine-desoxycholate (XLD) agar (CM0469, Oxoid, Thermo Fisher Scientific Inc.) and brilliant green agar (BGA) (CM0263, Oxoid, Thermo Fisher Scientific Inc.) separately in parallels. Usage of XLD agar relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulfide for the primary differentiation of *shigellae* and *salmonellae* from non-pathogenic bacteria. BGA, on the other hand, is selective agar for isolation of *salmonellae*, other than *Salmonella* serovar Typhi. After labeling the agar petri dishes, they were incubated 37 ± 1°C for 24 ±3 h. A positive typical *Salmonella* colony had a slightly transparent zone of reddish color and a black center on XLD and a grey-reddish to red/pink color and a convex structure on BGA. The presumptive *Salmonella* colonies were transferred into brain heart infusion (BHI) agar (CM1136, Oxoid, Thermo Fisher Scientific Inc.) for long-storage until confirmation by PCR.



(a) (b)

Figure 6 Representative *Salmonella* positive agar plates (a) XLD agar (b) Brilliant Green agar

2.1.2. Animal isolates

For each season, from April 2012 to January 2013, fecal samples were collected from clinical animal cases in Animal Hospital of Veterinary Faculty, Harran University. Moreover, fecal samples were collected from poultry, bovine and, sheep farms and also from slaughterhouses. Overall, 83 animal-related isolates were collected from chicken, cow, sheep and goat fecal samples according to ISO 6579 procedure in Harran University, Sanliurfa and collected suspicious *Salmonella* isolates were sent to METU in *Salmonella Shigella* (SS) agar in cold chain for confirmation and advance studies.

2.1.3. Clinical human isolates

Fecal and/or blood samples were taken from patients with salmonellosis or suspicious salmonellosis diagnosis were in Medicine Faculty of Harran University for four seasons during April 2012 to January 2013. Fecal samples were inoculated into blood agar, eosin

methylene blue (EMB) agar and SS agar sequentially. Lactose negatives colonies in SS agar were then taken for biochemical tests. Suspicious colonies were inoculated into Simmons' citrate agar, urea agar, triple sugar iron (TSI) agar and also motility agar to characterize the isolates according to their citrate, urea, iron and motility properties (Davis and Morishita 2005). Blood samples, on the other hand, were directly taken in BD BACTEC 9050 Blood Culture System (BD Diagnostics, New Jersey, U.S.) in sterile conditions. Depending on reproduction abilities of colonies, they were incubated in EMB, blood and chocolate agar. Lactose negative colonies were further analyzed according to the methods mentioned above. A total of 50 presumptive *Salmonella* isolates were sent to METU in cold chain for further confirmation and characterization.

2.2. Confirmation of presumptive *Salmonella* isolates by *inv*A gene in PCR

Firstly, *invA* primer concentrations was adjusted according to the protocol that was used. And DNA was prepared by selecting a single colony per isolate of *Salmonella* from BHI agar and scraped into a PCR tube which contained 95µl sterile distilled water. The mixture was exposed to microwaving for 30 sec in oven to lyse the cells.

PCR master mix was prepared with distilled sterile water, buffer, $MgCl_2$, dNTPs, forward primer, reverse primer and Taq enzyme with the concentrations mentioned in Taq enzyme set in 1.5 ml Eppendorf tube. 49 µl of the master mix was pipetted into 0.2 ml PCR tube and 1 µl of presumptive *Salmonella* DNA was added for each sample. This step was repeated for positive and negative control.

The PCR tubes were placed into thermocycler (Eppendorf Mastercycler DNA Engine, Scientific Support, CA, US and T100 Thermal Cycler, Bio-Rad, CA, US) and the following protocol was applied;

94ºC for 8 minutes	[1X]

94ºC for 30 seconds	
60ºC for 30 seconds	[35X]
72ºC for 30 seconds	

72ºC for 5 minutes	
4°C until stopping the reaction	[1X]

2.3.Storing the confirmed *Salmonella* isolates

The confirmed isolates were streaked into BHI agar and incubated at 37°C overnight. One colony was selected and incubated into 5 ml BHI broth (CM1032, Oxoid, Thermo Fisher Scientific Inc.) and incubated again at 37°C overnight. After labelling the vials, 850 µl isolate suspension was added to a 2-ml screw-cap vial and 150 pre-sterilized glycerol was added to the vial and mixed gently. Confirmed *Salmonella* isolate was stored in %15 glycerol solution at -80°C freezer (Thermo Fisher Scientific, US).

2.4. Serotyping

Serotyping of *Salmonella* was done according to Kauffman-White Procedure (Grimont 2007). The studies was performed by collaboration with Public Health Institution of Health, Turkish Ministry of Health (Türkiye Halk Sağlığı Kurumu).

For O-typing, a loop full of growth from the inoculated nutrient agar was mixed with a saline drop on the slide ensuring a smooth, opaque suspension. The step was repeated for negative control test. Then a drop of poly O antisera with or without Vi antiserum was added and antisera and antigen are mixed with a loop or stick for one minute. A loop full of culture from the nutrient agar was mixed with a drop of an O-serum on a slide and the

slide was mixed gently for a maximum of 2 minutes. A negative reaction was a homogenous suspension whereas, a positive reaction was lumping (agglutination). First, the strains were tested in the O-sera-pools and afterwards individual O-sera test were done. The positive and negative reactions were both noted.

Table 12 Serotypes of *Salmonella enterica* subsp. *enterica* with their antigenic formulae found in this study

Serotype	O-Antigen	H-antigen Phase 1	H-antigen Phase 2	Other
Corvallis	8, 20	z4, z23	[z6]	-
Infantis	6, 7, 14	r	1,5	R1...],[z37],[z45],[z49]
Montevideo	6, 7, 14	g,m,[p],s	[1,2,7]	-
Othmarschen	6, 7, 14	g,m,[t]	-	-
Virchow	6, 7, 14	r	1,2	-
Mikawasima	6, 7, 14	y	e,n,z15	[z47], [z50]
Mbandaka	6, 7, 14	z10	e,n,z15	[z37], [z45]
Hadar	6, 8	z10	e,n,x	-
Kentucky	8, 20	i	z6	-
Sandiego	1, 4, [5], 12	e, h	e,n,z15	-
Enteritidis	1, 9, 12	g, m	-	-
Newport	6, 8, 20	e, h	1, 2	[z67], [z78]
Typhi	9, 12[Vi]	d	-	[z66]
Typhimurium	1, 4, [5], 12	i	1, 2	-
Paratyphi B	1, 4, [5], 12	b	1, 2	[z5], [z33]
Reading	1, 4, [5], 12	e, h	1, 5	R1…]
Caracas	[1],6,14,[25]	g, m, s	-	-
Charity	[1],6,14,[25]	d	e,n,x	-
Anatum	3,10,15,15,34	e, h	1, 6	[z64]
Poona	1,13,22	z	1, 6	[z44], [z59]
Salford	16	l, v	e,n,x	-
Telaviv	28	y	e,n,z15	-

For H-typing, firstly subculturing was done to swarm agar from nutrient agar and incubation was performed for one night at 37^0 C. On the second day, from the edge of

motility zone on swarm agar, a loop full of growth was removed and mixed to the first drop of saline. A negative test was also performed similar to O-antigen testing. Poly H antisera was added and mixed with a loop. From the edge of motility zone on swarm agar, a loop full of growth and a drop of an H-serum was mixed on the slide and it was mixed for 2 minutes. Again, positive and negative results were noted (agglutination gives positive result, and homogenous suspension was a negative result). After the 1st phase of H-antigen detection, 10 µl of antisera against the detected H-antigen was added to petri dish together with 5 ml of swarm agar. When the agar was solidified, one spot at the centre of the agar was inoculated and incubation is performed at 37^0 C. 2nd phase H-antigens were then tested by the same methods used in 1st phase.

At the end, O- and H- reactions were combined and the serotype was identified according to the Kauffmann-White scheme (ISO6579 2002) (Table 12).

2.5. Antimicrobial susceptibility test (AST) for *Salmonella* by disc diffusion method

The culture is transferred to 4 ml Mueller-Hinton broth by sterile loop and the broths are incubated at 37^0 C for 18 hours. After incubation, dilution is done in 1:100 portions and then transfer of diluted cultures is performed into Mueller-Hinton agar. Paper discs (6mm) that contain antimicrobials are put into the surface of agar and the petri dishes are incubated at 37^0 C for 16- 18 hours. For disk diffusion method, 19 different antimicrobial elements are used. The quality control strain is *E. coli* ATCC 25922 for AST testing. The limits are determined by the Clinical Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) (Table 13).

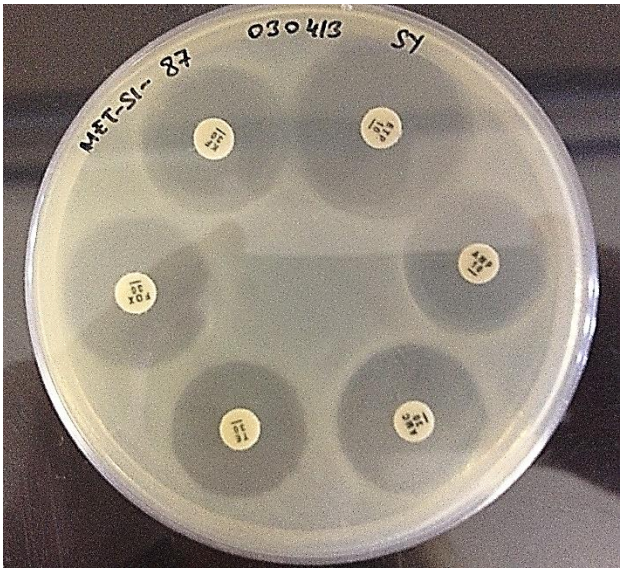


Figure 7 An example from disk diffusion antimicrobial susceptibility result

Table 13 Zone diameter standards for antimicrobial susceptibility test (AST) for *Salmonella* by disc diffusion method

Antimicrobial group	Antimicrobial agent	Disk content	Zone diameter (mm)		
		(µg)	S	I	R
Aminoglycosides	Amikacin [1]	30	≥17	15-16	≤14
	Gentamicin [1]	10	≥15	13-14	≤12
	Kanamycin [1]	30	≥18	14-17	≤13
	Streptomycin [1]	10	≥15	12-14	≤11
Beta lactams	Ampicillin [1]	10	≥17	14-16	≤13
	Ceftiofur[2]	30	≥21	18-20	≤17
	Cefoxitin [1]	30	≥18	15-17	≤14
	Ceftriaxone [1]	30	≥23	20-22	≤19
	Cephalothin [1]	30	≥18	15-17	≤14
	Amoxicillin-clavulanic acid [1]	20/10	≥18	14-17	≤13
	Ertapenem [1]	10	≥23	20-22	≤19
	Imipenem [1]	10	≥23	20-22	≤19
Phenicols	Chloramphenicol[1]	30	≥18	13-17	≤12
Quinolones and	Nalidixic acid [1]	30	≥19	14-18	≤13
Fluoroquinolones	Ciprofloxacin [1]	5	≥21	16-20	≤15
Tetracyclines	Tetracycline [1]	30	≥15	12-14	≤11
Sulfanomides and	Trimethoprim-sulfamethoxazole[1]	1.25/23.75	≥16	11-15	≤10
trimethoprims	Sulfisoxazole [1]	300	≥17	13-16	≤12

[1] CLSI, 2011. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement, Vol:31, ISBN 1-56238-742-1

[2] CLSI, 2002. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second Edition , Vol: 22, ISBN 1-56238-461-9

2.6. Determination of antimicrobial resistance profile of *Salmonella* isolates by minimum inhibitory concentrations (MIC) method

Salmonella isolates firstly were transferred into Muller-Hinton agar and then were incubated at 37^0 C for 18 hours. They were taken into sterile salty water containing 0.85% NaCl by the help of sterile plastic inoculating loops. The concentrations of inoculums were set to 10^5 cfu using the spectrophotometer (Shimadzu UV-1700 Pharma Spec). 15 µl of prepared suspension were transferred to tubes containing 11 ml Muller-Hinton broth and vortex were performed. 100 µl of mix was put into well of micro-titer plaques that have increasing concentrations of 18 different antibiotics (Table 14). Plaques were incubated at 37^0 C for 18 hours and after incubation the minimum inhibitory concentration (MIC) was determined from the first well in which no growth is observed. The MIC of that antibiotic was compared by the CLSI and EUCAST break point values and at the end; it was coded as susceptible, intermediate or resistant.

2.7. Determination of antimicrobial resistance profile of *Salmonella* isolates by genotypic method

The isolates that are studied in genotypic methods are determined according to the results of phenotypic methods, PFGE and MLST profiles. Firstly, the phenotypically resistant *Salmonella* isolates were studied.

Purified *Salmonella* DNA were practiced to study antimicrobial resistance profile genetically. PCR master mix concentrations were given in Table 15. The genes and primers that were used in this study are as in Table 16.

Table 14 The minimum inhibitory concentrations of antimicrobial agents. (CLSI, EUCAST)

Antimicrobial agent	**MIC breaking point (μg/mL)**		
	Susceptible	Intermediate	Resistant
Amikacin	≤ 16	32	≥ 6
Gentamicin	≤ 4	8	≥ 16
Kanamycin	≤ 16	32	≥ 64
Streptomycin	≤ 32	N/A	≥ 64
Ampicillin	≤ 8	16	≥ 32
Amoxicillin-clavulanic acid	≤ 8 / 4	16 / 8	≥ 32 / 16
Ceftiofur	≤ 2	4	≥ 8
Ceftriaxone	≤ 8	16 - 32	≥ 64
Cephalothin	≤ 8	1	≥ 32
Cefoxitin	≤ 8	16	≥ 32
Sulfamethoxazole-sulfisoxazole	≤ 256	N/A	≥ 512
Trimethoprim-sulfamethoxazole	≤ 2 / 38	N/A	≥ 4 / 76
Chloramphenicol	≤ 8	16	≥ 32
Ciprofloxacin	≤ 1	2	≥ 4
Nalidixic acid	≤ 16	N/A	≥ 32
Tetracycline	≤ 4	8	≥ 16
Imipenem	≤ 13	14-15	≥ 16
Ertapenem	≤ 15	16-18	≥ 19

Table 15 PCR Master Mix

PCR solutions [concentration]	**Volume (µl)**
dH_2O	71.5
10X PCR buffer	10.0
$MgCl_2$ [25mM]	6.0
dNTPs [10mM]	2.0
Primer*-F [12.5 µM]	4.0
Primer*-R [12.5 µM]	4.0
Taq DNA polymerase	0.5
TOTAL	98

*: the sequences of primers are given in Table 16.

Table 16 The genes, primers and primer concentrations of *Salmonella* that are related with antimicrobial resistance

Gene	Primer Sequence	The location that it shows resistance	Primer Binding Temp. (°C)	Reference
bla$_{TEM-1}$	F: CAG CGG TAA GAT CCT TGA GA R: ACT CGC CGT CGT GTA GAT A	Class A beta-lactamase	53.9	(Chen, Zhao et al. 2004)
bla$_{PS13E-1}$	F: TGCTTCGCAACTATGACTAC R: AGCCTGTGTTTGAGCTAGAT	Class A beta-lactamase	52.4	(Chen, Zhao et al. 2004)
*bla*CMY-2	F: TGGCCGTTGCCGTTATCTAC R: CCCGTTTTATGCACCCATGA	Ceftiofur, Ceftriaxone	60.8	(Chen, Zhao et al. 2004)
ampC	F: AACACACTGATTGCGTCTGAC R: CTGGGCCTCATCGTCAGTTA	Beta-lactamases	60	(Pérez-Pérez and Hanson 2002)
cat1	F: CTTGTCGCCTTGCGTATAAT R: ATCCCAATGGCATCGTAAAG	Chloramphenicol	Touch down 55-45	(Chen, Zhao et al. 2004)
cat2	F: AACGGCATGATGAACCTGAA R: ATCCCAATGGCATCGTAAAG	Chloramphenicol	60	(Chen, Zhao et al. 2004)
flo	F: CTGAGGGTGTCGTCATCTAC R: GCTCCGACAATGCTGACTAT	Chloramphenicol	54.4	(Chen, Zhao et al. 2004)
cmlA	F: CGCCACGGTGTTGTTGTTAT R: GCGACCTGCGTAAATGTCAC	Chloramphenicol	58.5	(Chen, Zhao et al. 2004)

Table 16 Continued

Gene	Primer Sequence	The location that it shows resistance	Primer Binding Temp. (°C)	Reference
aadA1	F: TATCAGAGGTAGTTGGCGTCAT R: GTTCCATAGCGTTAAGGTTTCATT	Streptomycin	53.6	(Randall, Cooles et al. 2004)
aadA2	F: TGTTGGTTACTGTGGCCGTA R: GATCTCGCCTTTCACAAAGC	Streptomycin	57.3	(Randall, Cooles et al. 2004)
strA	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	Streptomycin	51.8	(Gebreyes and Altier 2002)
strB	F: ATCGTCAAGGGATTGAAACC R: GGATCGTAGAACATATTGGC	Streptomycin	57	(Gebreyes and Altier 2002)
aacC2	F: GGCAATAACGGAGGCAATTCGA R: CTCGATGGCGACCGAGCTTCA	Gentamicin, Kanamycin	57.9	(Chen, Zhao et al. 2004)
aphA$_{1\text{-}Iab}$	F: AAACGTCTTGCTCGAGGC R: CAAACCGTTATTCATTCGTGA	Kanamycin	54	(Frana, Carlson et al. 2001)
dhfrI	F: CGGTCGTAACACGTTCAAGT R: CTGGGGATTTCAGGAAAGTA	Trimethoprim	51.7	(Chen, Zhao et al. 2004)
dhfrXII	F: AAATTCCGGGTGAGCAGAAG R: CCCGTTGACGGAATGGTTAG	Trimethoprim	57.9	(Chen, Zhao et al. 2004)
sulI	F: TCACCGAGGACTCCTTCTTC R: CAGTCCGCCTCAGCAATATC	Sulfoxazole	55.6	(Chen, Zhao et al. 2004)

Table 16 Continued

Gene	Primer Sequence	The location that it shows resistance	Primer Binding Temp. (°C)	Reference
sulII	F: CCTGTTTCGTCCGACACAGA R: GAAGCGCAGCCGCAATTCAT	Sulfoxazole	56	(Chen, Zhao et al. 2004)
tetA	F: GCGCCTTTCCTTTGGGTTCT R: CCACCCGTTCCACGTTGTTA	Tetracycline	57.7	(Chen, Zhao et al. 2004)
tetB	F: CCCAGTGCTGTTGTTGTCAT R: CCACCACCAGCCAATAAAAT	Tetracycline	58.4	(Chen, Zhao et al. 2004)
tetG	F: AGCAGGTCGCTGGACACTAT R: CGCGGTGTTCCACTGAAAAC	Tetracycline	60	(Chen, Zhao et al. 2004)

Amplification conditions:

94ºC, 8 minutes [1X]

94ºC, 30 seconds

Annealing Temperature-seconds* [35X]

72ºC, 30 seconds

72ºC, 5 minutes

4 ºC ∞ [1X]

*: It changes for every gene (shown in Table 16)

A mix of 5 µl were taken from the PCR result and then it were run with markers, for which DNA molecular weight is known, in 1.5 % agarose gel at 110V for half an hour.

The bands were photographed after waiting them in ethidium bromide solution. The presence of band had shown whether there was the resistance gene or not. The information about the isolates were downloaded into publicly available database, Pathogen Tracker, located at Cornell University.

2.8. Agreement analysis for phenotypic and genotypic profiles

The agreement of two studies; phenotypic and genotypic profiles; was determined by Kappa statistics in Minitab 17 Statistical Software (Minitab, Inc., State College, PA). Cohen's Kappa which is a statistical measure of inter-rater agreement or inter-annotator agreement (Carletta 1996) for qualitative (categorical) items was calculated according to the formula given below.

$$\kappa = \frac{\Pr(a) - \Pr(e)}{1 - \Pr(e)},$$

Equation 1 Kappa statistics formula

where Pr(a) is the relative experimental agreement among raters, and Pr(e) is the theoretical probability of chance agreement, using the experimental data to compute the probabilities of each observer arbitrarily indicating each category. Scores of kappa value lower than 0.20 indicated poor, between 0.21 and 0.40 indicated fair, between 0.41 and 0.60 indicated moderate, between 0.61 and 0.80 indicated good and lastly between 0.81 and 1.00 indicated a very good agreement.

2.9. Plasmid isolation and antimicrobial resistance gene detection in plasmids

Plasmids were analyzed to understand the source of antimicrobial resistance in *Salmonella* isolates. Plasmid DNA extraction were performed by Qiagen mini spin miniprep kit (Qiagen Finland). The extracted plasmid was run with markers at 0.7 % agarose gels at 90 V. CHEF-DR III system was also used for larger plasmid DNA fragments with S1 nuclease enzyme (Life Technologies, Themo Fisher Scientific, US). After waiting in gel ethidium solutions, bands were visualized by Quantity One software. Presence of band showed the existence of it in the selected isolate and the appropriate band size. *E. coli* 39R861 (7, 36, 63, 147 kb) were used as a reference for determination of the size of the bands (Nogrady et al., 2012). Detection of antimicrobial resistance genes in plasmids were determined by the method described in 2.4. Hereby, the source of resistance was specified as plasmid or chromosomal.

2.10. Detection of Class I Integrons

Salmonella cultures were used to extract DNA from where the cultures were subcultured into fresh LB broth and grown aerobically to late-exponential-phase using the Qiagen DNeasy Purification kit (Qiagen) according to the manufacturer's instructions.

Class I integron studies were performed with a bacterial colony that is handled in 1.0 ml phosphate-buffered solution, centrifuged and then kept in 100 mM Tris, 1 mM EDTA buffer (pH 8.0) for 10 minutes. After preparation of solution, it was stored at -20°C. 2 µl solution was used for each reaction, and the primers that were used are given in Table 17.

Table 17 The primers used to determine the presence of Class 1 integrons

Gene	Primer sequence	GenBank DataBank Code	Coordinates	Reference
int1	F:GGC ATC CAA GCA GCA AGC	U12338	1416→1433	(Hall and Collis 1998)
	R:AAG CAG ACT TGA CCT GAT	U12338	4831→4814	
sul1	F:CTT CGA TGA GAG CCG GCG GC	X12869	924→943	Sundström et al. .1988
	R:GCA AGG CGG AAA CCC GCG CC	X12869	1360→1341	
qacEΔ 1	F:ATC GCA ATA GTT GGC GAA GT	X15370	211→230	Stokes and Hall, 1989
	R:CAA GCT TTT GCC CAT GAA GC	X15370	436→417	
ant (3")	F:GTG GAT GGC GGC CTG AAG CC	M10241	514→533	Hollingshead and Vapnek, 1985
	R:ATT GCC CAG TCG GCA GCG	M10241	1040→1023	
pse-1	F:CGC TTC CCG TTA ACA AGT AC	M69058	323→342	Huovinen and Jacoby, 1991
	R:CTG GTT CAT TTC AGA TAG CG	M69058	742→723	

2.11. Detection of virulence genes by real-time PCR

Salmonella cultures were again used to extract DNA from them where they were initially on fresh LB broth and grown aerobically to late-exponential-phase using the Qiagen DNeasy Purification kit (Qiagen) according to the manufacturer's instructions. 2 µl purified DNA were used as a template for a PCR amplification using the primers (Suez, Porwollik et al. 2013) listed in Table 18.

Table 18 Virulence genes and their primers used in this study

Primer	**Sequence (5' to 3')**	
	Forward	**Reverse**
ssek3	TATCAATCTCAAATCATGG	CGCGTTTATATCATACGTTTGC
sspH1	GGTCACAGGACACGTTCTACG	GCGCTTCTTCGTAATTTTCC
sopE	CATAGCGCCTTTTCTTCAGG	ATGCCTGCTGATGTTGATTG
pefA	TAAGCCACTGCGAAAGATGC	GCGTGAACTCCAAAAACCCG
sodC	ATGACACCACAGGCAAAACG	AGATGAACGATGCCCTGTCC
sseI	CGCCATCATCAGTAACCGCC	CTGCTGACCACATCCTCCC
STM2759	ACCATTTTCACCTGGGCTCC	CGTTCAGGTTTTGTCGCTGG
gatC	ATTGGTATCGGCTTCGTGGG	ATCCCCAGCCAGTATGAACC
gogB	ACGAGGCGACATCAAACCTT	GACCGTTCCCTCAATCGTGT
tcfA	TCGCTATGTTTGCATGTGGT	TTCAGGAACAGCCTCGAAGT
hlyE	GCGTGATTGAAGGGAAATTG	CGAAAAGCGTCTTCTTACCG
cdtB	CACTCGGCTATTGATGTTGG	ATTTGCGTGGGTTCTGTAGG
tcfA	AGGAGGTACCAGCAGGGAAT	TTCAGGAACAGCCTCGAAGT
hlyE	GCAGCAATTGGGGAGATAAA	CGAGAAGCGTCTTCTTACCG
cdtB	ATTTGCGTGGGTTCTGTAGG	GGATGCTGCAGCTATTGTCA

Real-time PCR was performed in 96 well plates with FASTStart SYBR Green Master (ROX) (Roche Life Science, IN, US) with ABI 7500 Real-time PCR System (Applied Biosystems). Data acquisition and analysis of the real-time PCR assays were done using the 7500 System SDS Software Version 1.2 (Applied Biosystems).

2.12. Statistical analyses

Relations between isolate sources groups (i.e. human, food, animal), subgroups (i.e., food groups, animal species, gender) and resistance types (i.e., susceptible, intermediate, and resistant) were evaluated by Fisher's exact test. Analyses were carried out using R-project (*www.r-project.org/*).

Odds ratio (OR) was used to determine the association of resistance genes that were significantly different with 95% confidence intervals (CI) (Altman 1990). Bonferroni corrections were used as a conservative modification for multiple comparisons getting the level of statistical significance at $p < 0.05/n$, where n is the number of comparisons done for each outcome (Dohoo, Martin et al. 2009). An OR of >1 showed a positive association between the outcome and predictor variable, while an OR of <1 showed a negative association between the outcome and predictor variable.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. *Salmonella* serovar distribution in farm to fork chain

From April 2012 to January 2013, 792 food samples were collected from different food types: sheep ground meat, cattle ground meat, chicken meat, offal, un-ripened cheese, Urfa cheese, green vegetables, tomato, pistachio, pepper and isot in the southeastern and middle part of Turkey. 83 animal-related isolates were collected from chicken, cow, sheep and goat fecal samples.

During sampling period, 50 *Salmonella* isolates were collected from human clinical cases in Harran University (HU) Medical School, which is also located in the south part of Turkey. A total of 175 *Salmonella* isolates from three different sources were used in this study.

The distributions of *Salmonella* serovars for 3 different sources were varied (Table 22), and the variations of serovars for food and animal isolates were higher (SID_{food}= 0.833, SID_{animal}=0.814) than human isolates (SID human=). The most frequently observed serovar was different in each sample groups; *S.* Infantis (30.0 %), *S.* Montevideo (35.9 %) and *S.* Paratyphi B (64.0 %) were the most common serovars in food, animal and human *Salmonella* isolates, respectively. Although most of the animal isolates were obtained from bovine group (62.3 %), the dispersed diversity of *Salmonella* serovars (n=13) in ovine fecal samples were noteworthy.

In clinical human isolates, the variation of serovars was narrower considering the food and animal isolates, only 6 serovars were detected. The parameters, such as location of the cases and gender, did not affect the serovar distribution in clinical human isolates ($p > 0.05$), among which *S.* Paratyphi B was the most common serovar at suburban areas of city Sanliurfa. This might be due to asymptomatic hosts or low hygienic conditions of the environment. Also, since the number of paratyphoid fever cases had been increased and was higher than that of rather than typhoid fever in Asia, and developing countries, it was not surprising to observe *S.* Paratyphi B at a high prevalence rate in Turkey, due the development status of Sanliufa (Hawker, Begg et al. 2012). In the city center, besides few *S.* Paratyphi B and *S.* Typhi isolates, nontyphodial serovars such as *S.* Enteritidis, *S.* Kentucky, *S.* Othmarschen, and *S.* Typhimurium were collected from human salmonellosis cases, most likely due to the contaminated food.

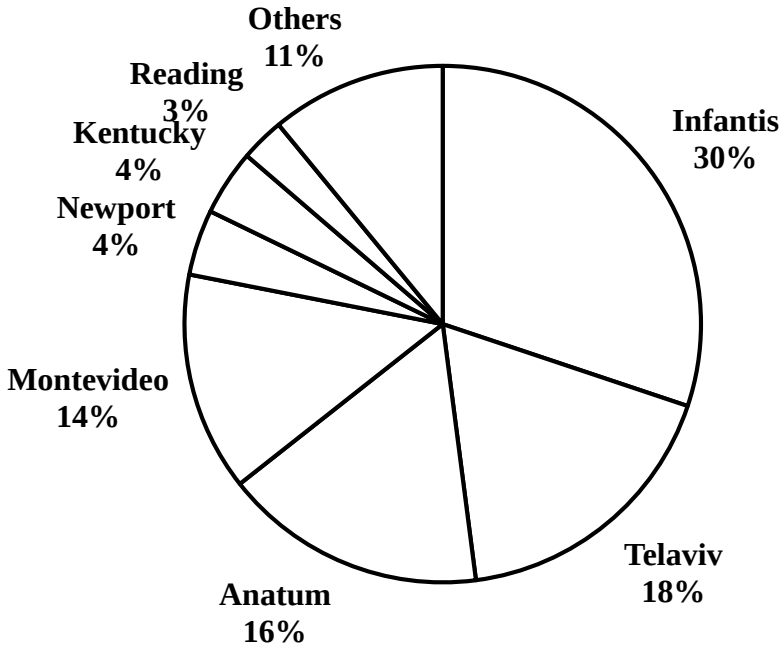
3.1.1. Serotype distribution with respect to isolate source: food, animal, clinical human

All of the *Salmonella* isolates (175/175) had been serotyped (Table 19). At a total, 15 different serovars had been observed. Mostly-seen food-related serovar was *Salmonella* serovar Infantis (30.0 %), which was obtained all from chicken samples (breast, wing, offal). Telaviv (17.8 %) and Anatum (16.4 %) were the other leading serotypes.

For the animal origin isolates, similarly 13 different serotypes had been obtained from 53 samples (Table 20). Montevideo was the leading serovar with a percentage of 35.9%. Telaviv (18.9 %) and Kentucky (13.2 %) had followed it afterwards.

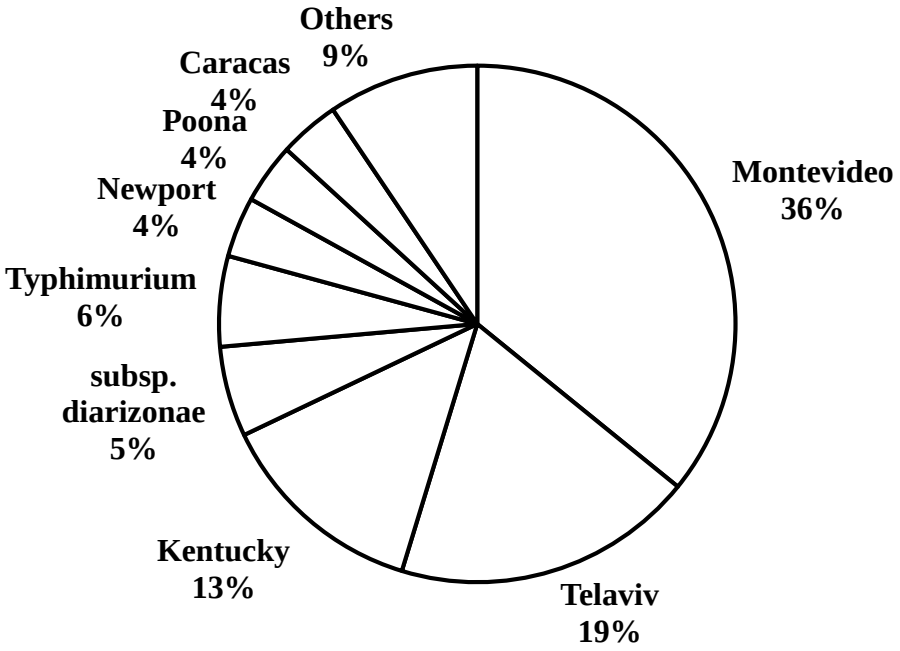
Lastly for the clinical human samples, 6 different serotypes had been observed (Table 21). Most of the isolates (68.0 %) were the serovars; Paratyphi B and then Typhimurium (14.0 %) and Kentucky (10.0 %).

Table 19 Serovar distribution of *Salmonella* isolates that were obtained from different food samples (sheep ground meat, cattle ground meat, chicken meat, offal, un-ripened cheese, Urfa cheese, green vegetables, tomato, pistachio and isot) in Turkey



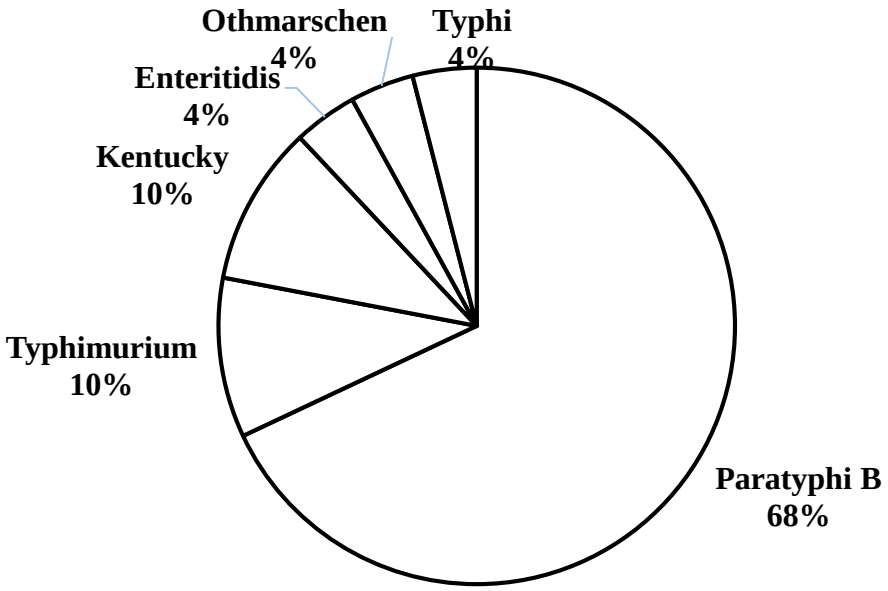
Serotype	Number of isolate	Percentage (%)
Infantis	21	**30.0**
Telaviv	13	**18.0**
Anatum	12	**16.6**
Montevideo	10	**13.8**
Newport	3	**4.2**
Kentucky	3	**4.2**
Reading	2	**2.8**
Enteritidis	1	**1.4**
Othmarschen	1	**1.4**
Hadar	1	**1.4**
Mbandaka	1	**1.4**
Salford	1	**1.4**
Charity	1	**1.4**
Mikawasima	1	**1.4**
Chester	1	**1.4**
TOTAL	72	**100**

Table 20 Serovar distribution of *Salmonella* isolates that were obtained from different animal samples (cattle, sheep, chicken) in Turkey



Serovar	Number of isolate	Percentage (%)
Montevideo	19	**35,8**
Telaviv	10	**18,9**
Kentucky	7	**13,2**
subsp. *diarizonae*	3	**5,7**
Typhimurium	3	**5,7**
Newport	2	**3,8**
Poona	2	**3,8**
Caracas	2	**3,8**
Reading	1	**1,9**
Anatum	1	**1,9**
Enteritidis	1	**1,9**
Hadar	1	**1,9**
Saintpaul	1	**1,9**
TOTAL	53	**100**

Table 21 Serovar distribution of *Salmonella* isolates that were obtained from clinical human samples in Turkey



Serovar	Number of isolate	Percentage (%)
Paratyphi B	34	**68**
Typhimurium	5	**10**
Kentucky	5	**10**
Enteritidis	2	**4**
Othmarschen	2	**4**
Typhi	2	**4**
TOTAL	50	**100**

Salmonella serovar Telaviv and Montevideo were predominant in food and animal samples. Importantly, *S.* serovar Kentucky serovar was observed in all type of sources; food (3/73), animal (7/53) and clinical human (5/50) samples. Interestingly, *Salmonella* serovar Othmarschen had been isolated from the two type of sources; food and clinical

human samples with 1.4% (1/73), 4.0 % (2/50) respectively. Also, *Salmonella* serovar Enteritidis had been seen in clinical human samples (4.0 %) and animal samples (1.9 %).

3.1.2. Serotype distribution with respect to different source subgroups

Serotype distribution was investigated according to the isolate source subgroups.

3.1.2.1.Serovar distribution with respect to food subgroups

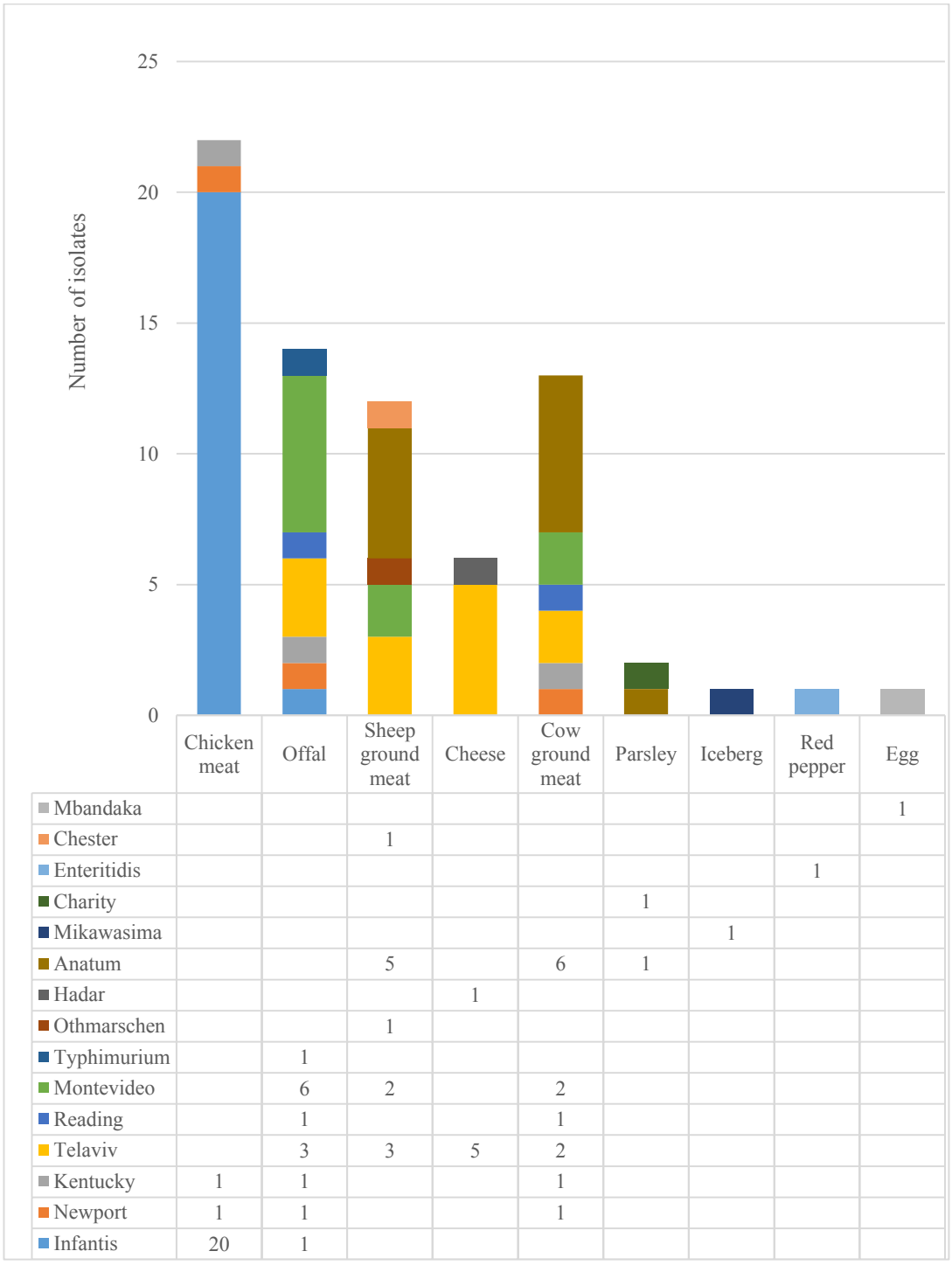
The serovar distribution was analyzed to observe the main food source for a specific serovar of Turkey. For instance, *Salmonella* serovar Infantis was mostly related with chicken samples (chicken breast, chicken skin, and chicken wing). 20/22 of serovars found in chicken samples were the serovar Infantis and most of the isolates found in food samples were associated with chicken (Figure 6). Offal, cow/sheep ground meat had followed the chicken samples in terms of incidence of *Salmonella,* but the diversity of serovars isolated from these sub-sources was denser compared to the serotypes found in chicken (21 Infantis, 1 Kentucky, 1 Newport). In offal samples, the following serovars; Montevideo (6), Telaviv (3), Newport (1), Reading (1), Infantis (1), Typhimurium (1), Kentucky (1) had been isolated in descending order. The serovar distribution was very similar for sheep ground meat and cow ground meat; Anatum and Telaviv were the most prevalent serovars among them. In cheese samples, again Telaviv was the predominant serovar (83.3 %). From 100 of egg samples, only 1 *Salmonella* isolate had been found; *Salmonella* serovar Mbandaka. In raw vegetables, 3 isolates have been determined; Charity and Anatum from parsley and Mikawasima from iceberg. And, from red pepper 1 *Salmonella* serovar Enteritidis was observed. The serovar diversity of isolates for pistachio samples was very different compared to other sub-sources; Salford and Corvallis.

3.1.2.2.Serotype distribution with respect to animal subgroups

There were three sub-sources in animal group: cattle, chicken and sheep (Figure 7). Most of the veterinary isolates were got from cattle sources. Most of the isolates found in that group were *Salmonella* serovar Montevideo (16/33). Telaviv (9/33) and Kentucky (6/33) had been seen after Montevideo in cattle group. Also, 1 *Salmonella* serovar Typhimurium was observed. On the other hand, in sheep source, the diversity was very high (13 different serovars/19 isolates). And in chicken sample, *Salmonella* serovar Montevideo was isolated.

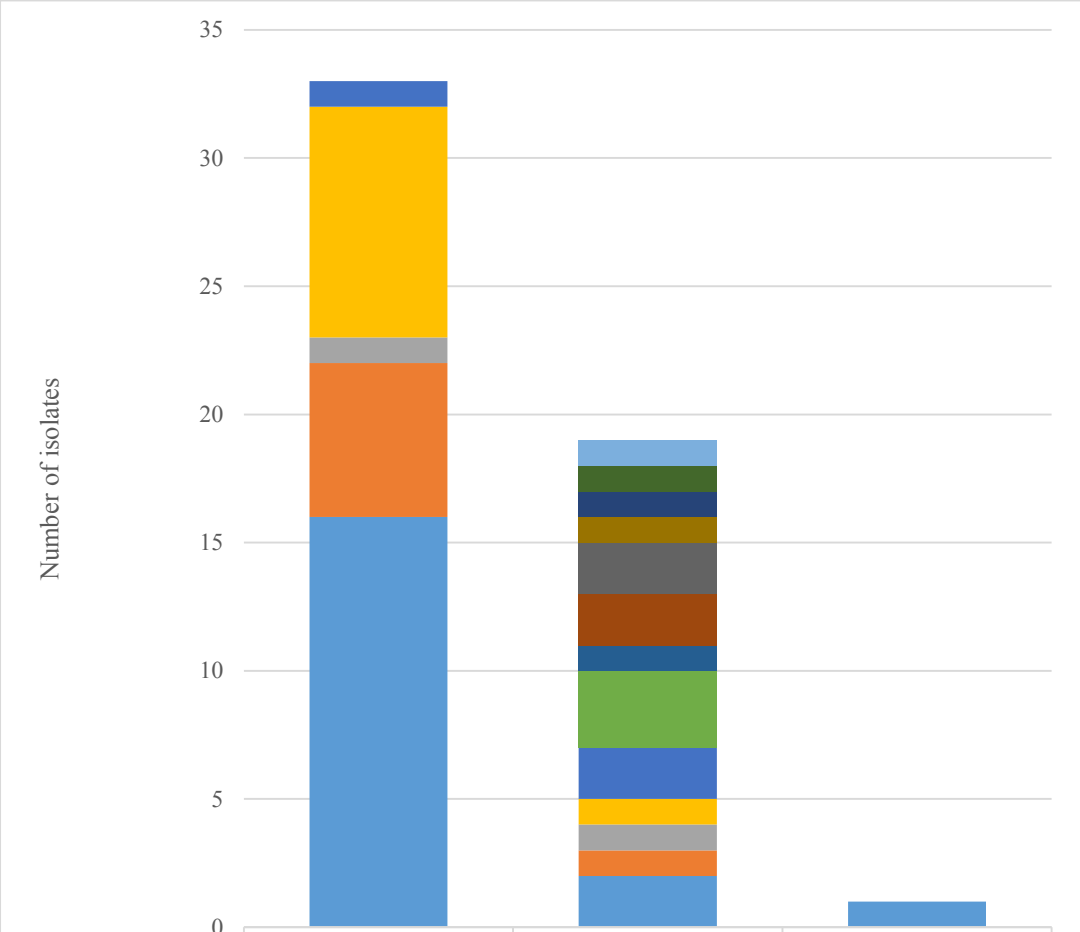
3.1.2.3. Serovar distribution with respect to clinical human subgroups

Clinical human samples were analyzed in two different trends: gender and age (Figure 8 and Figure 9). The distribution of serovars were similar in each group; in man and woman. *Salmonella* Paratyphi B was predominant in both of the gender groups. In age groups (0-10 years, 10-20 years, 20-30 years, 30-50 years, 50-80 years), it was seen that the patients are mostly elder people. And again, mostly Paratyphi B was isolated in all age groups except 0-10 years.



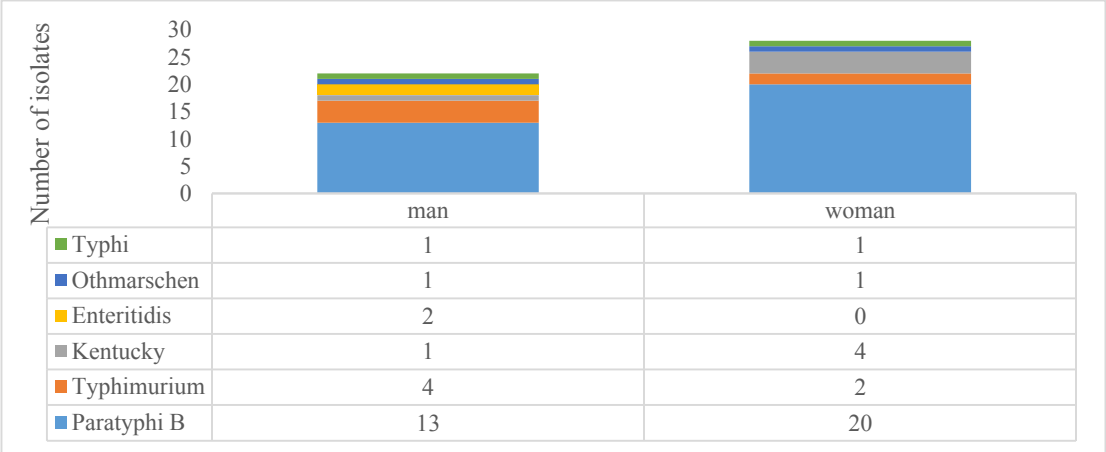
	Chicken meat	Offal	Sheep ground meat	Cheese	Cow ground meat	Parsley	Iceberg	Red pepper	Egg
Mbandaka									1
Chester			1						
Enteritidis								1	
Charity						1			
Mikawasima							1		
Anatum			5		6	1			
Hadar				1					
Othmarschen			1						
Typhimurium		1							
Montevideo		6	2		2				
Reading		1			1				
Telaviv		3	3	5	2				
Kentucky	1	1			1				
Newport	1	1			1				
Infantis	20	1							

Figure 8 The distribution of the food subgroups according to the serovars for food isolates



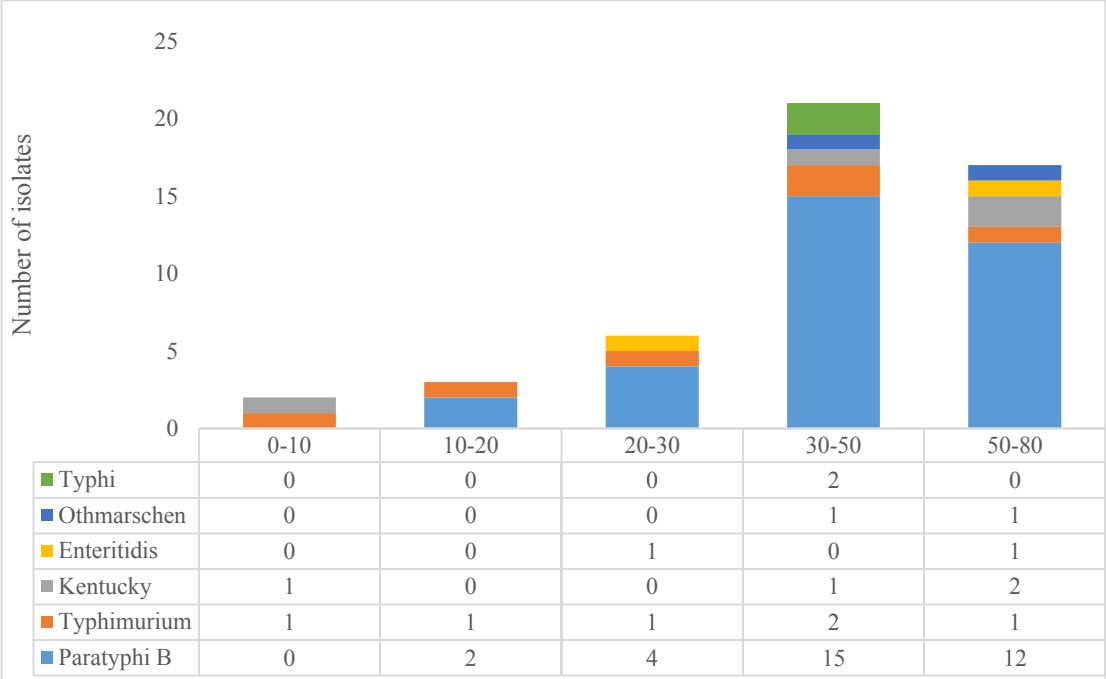
	Cattle	Sheep	Chicken
Chester		1	
Hadar		1	
Enteritidis		1	
Anatum		1	
Caracas		2	
Poona		2	
Reading		1	
subsp. diarizonae		3	
Typhimurium	1	2	
Telaviv	9	1	
Newport	1	1	
Kentucky	6	1	
Montevideo	16	2	1

Figure 9 The distribution of animal subgroups according to the serovars for animal isolates



	man	woman
Typhi	1	1
Othmarschen	1	1
Enteritidis	2	0
Kentucky	1	4
Typhimurium	4	2
Paratyphi B	13	20

Figure 10 The distribution of human gender according to the serovars for clinical human isolates



	0-10	10-20	20-30	30-50	50-80
Typhi	0	0	0	2	0
Othmarschen	0	0	0	1	1
Enteritidis	0	0	1	0	1
Kentucky	1	0	0	1	2
Typhimurium	1	1	1	2	1
Paratyphi B	0	2	4	15	12

Figure 11 The distribution of age clusters (0-10, 10-20, 20-30, 30-50 and 50-80) according to the serovars for clinical human isolates

Table 22 Distribution of serovar and antimicrobial resistance profile of 175 isolates

Subspecies	Serovar	Total number of isolates	Antimicrobial resistance profile	Number of isolates from			Detailed source
				Food	Animal	Clinical human	
enterica	Infantis	21	K-S-T-Sf-N	9	-	-	Chicken meat (wing, breast, liver, drumstick, offal)
			S-T-N	1	-	-	
			S-T-Sf-N	6	-	-	
			K-S-T-Amp-Sf-N	1	-	-	
			K-S-T-Amp-Kf-Sf-Sxt-C-N	1	-	-	
			S-Amp-Kf-N	1	-	-	
			S-Sf-N	1	-	-	
			T-N	1	-	-	
	Telaviv	23	Susceptible	-	1	-	Sheep feces
			Susceptible	3	-	-	Sheep ground meat
			Susceptible	-	9	-	Cattle feces
			Susceptible	2	-	-	Cow ground meat
			Susceptible	5	-	-	Cheese
			Susceptible	3	-	-	Offal
	Anatum	13	Ak-Sf	-	1	-	Sheep feces
			Sf	3	-	-	Sheep ground meat
			Susceptible	2	-	-	Sheep ground meat
			Susceptible	-	6	-	Cow ground meat
			Susceptible	1	-	-	Parsley

Table 22 Continued

Subspecies	Serovar	Total number of isolates	Antimicrobial resistance profile	Number of isolates from			Detailed source
				Food	Animal	Clinical human	
enterica	Montevideo	29	Sf	1	-	-	Sheep ground meat
			Susceptible	1	-	-	Sheep ground meat
			Susceptible	-	2	-	Sheep feces
			Susceptible	2	-	-	Cow ground meat
			Fox-Kf-Etp	-	1	-	Cattle feces
			Fox-Kf	-	1	-	Cattle feces
			T-Etp	-	1	-	Cattle feces
			Sf	-	2	-	Cattle feces
			Susceptible	-	11	-	Cattle feces
			Susceptible	6	-	-	Offal
			Sf	-	1	-	Chicken feces
	Reading	3	Sf	-	1	-	Sheep feces
			Susceptible	1	-	-	Cow ground meat
			Susceptible	1	-	-	Offal
	Newport	5	N	1	-	-	Chicken meat
			Susceptible	1	-	-	Cow ground meat
			Susceptible	1	-	-	Offal
			Sf	-	1	-	Cattle feces
			Susceptible	-	1	-	Sheep feces

Table 22 Continued

Subspecies	Serovar	Total number of isolates	Antimicrobial resistance profile	Number of isolates from			Detailed source
				Food	Animal	Clinical human	
enterica	Kentucky	15	Susceptible	1	-	-	Chicken meat
			Sf	1	-	-	Cow ground meat
			Susceptible	1	-	-	Offal
			Susceptible	-	6	-	Cattle feces
			Sf	-	1	-	Sheep feces
			Sf	-	-	4	Human
			Susceptible	-	-	1	Human
	Hadar	2	S-T-Amp-Kf-N	1	-	-	Cheese
			S-T-Amp-Amc-Fox-Kf-Etp-N	-	1	-	Sheep feces
	Othmarschen	3	Susceptible	1	-	-	Sheep ground meat
			Sf	-	-	1	Human
			Susceptible	-	-	1	Human
	Typhimurium	11	T-Amp	1	-	-	Offal
			Ak-S-T-Amp-Kf-N	-	1	-	Cattle (bull) feces
			S-T-Amp-Amc-Sf-C-N	-	1	-	Sheep feces
			T-Amp-Kf	-	1	-	Sheep feces
			K-S-Sf-Sxt-C	-	-	1	Human
			T-Amp	-	-	2	Human
			Sf	-	-	2	Human
			Susceptible	-	-	2	Human

Table 22 Continued

Subspecies	Serovar	Total number of isolates	Antimicrobial resistance profile	Number of isolates from			Detailed source
				Food	Animal	Clinical human	
enterica	Caracas	2	Sf	-	1	-	Sheep feces
			Susceptible	-	1	-	Sheep feces
	Poona	2	Susceptible	-	2	-	Sheep feces
	Charity	1	Sf	1	-	-	Parsley
	Chester	2	Amc-Fox-Kf-Etp	-	1	-	Sheep feces
			Susceptible	1	-	-	Sheep ground meat
	Mbandaka	1	Susceptible	1	-	-	Egg
	Mikawasima	1	Susceptible	1	-	-	Iceberg
	Enteritidis	4	Susceptible	1	-	-	Red pepper
			Susceptible	-	1	-	Sheep feces
			Susceptible	-	-	2	Human
	Typhi	2	Sf	-	-	2	Human
	Paratyphi B	32	Ak-K-S-Sf-Sxt-C	-	-	1	Human
			Fox-Kf-Sf	-	-	1	Human
			Fox-Sf	-	-	1	Human
			Sf	-	-	17	Human
			Sf-N	-	-	1	Human
			S-Sf	-	-	2	Human
			Susceptible	-	-	9	Human
diarizonae		3	Susceptible	-	3	-	Sheep feces
Total number of isolates		**175**		**72**	**53**	**50**	Food, animal and clinical human samples

The common serovars, which were *S.* serovar Montevideo (n= 29; 15.4 %) and *S.* Telaviv (n= 22; 13 %) in all isolates, had risen to notice, since neither *S.* Montevideo, nor *S.* Telaviv was commonly collected serovar worldwide. Association of serovar *S.* Telaviv with bovine was early reported both in Turkey and England (Richardson 1975, Erol 1999). In our study, *S.* Telaviv (ST 1068) was frequently found in a variety of foods (i.e., ground beef meat, ground lamb meat, unripened cheese, Urfa cheese) and food animals (i.e., bovine and ovine feces). Since it is not a dominant serovar in Europe and United States, the prevalence of *S.* Telaviv in Turkey shows the possible emergence of this serovar in this geographic area (Durul 2015).

As for *S.* serovar Montevideo, the food association was more diverse in literature, *S.* serovar Montevideo was found in bovine feces, cheese, red and black peppers, and pistachio samples in elsewhere (Allard, Luo et al. 2012, Edrington, Loneragan et al. 2013). These food animal and food types are commonly consumed products in Turkey, as well as in Sanliurfa region.

Only three serovars, *S.* serovar Kentucky, *S.* serovar Enteritidis and *S.* serovar Typhimurium were obtained from all three sources (Table 22). Notably, a rare seen serovar worldwide, *S.* Othmarschen, had been isolated from the two sources; food and clinical human samples with 1.7 % (1/59), and 4.0 % (2/50), respectively.

Another noteworthy serovar was *S.* serovar Infantis, which had been associated with chicken samples (chicken breast, chicken skin, and chicken wing). Among 23 isolates collected from chicken samples, 21 represented the serovar *S.* Infantis and these isolates dominated the number of isolates from all food samples (p-value< 0.05); all the *S.* serovar Infantis isolates were from chicken sources such as wings, skin, and breast. Similarly, European Food Safety Authority (EFSA) (ECDC 2015) reports indicated that *S.* serovar Infantis has been very common among breeding flocks (second order) and also human (forth order). While this serovar was very persistent among food related sources, in our study it was not observed in animal and clinical human samples.

S. Anatum and *S.* Telaviv were the most dominant serovars among the isolates obtained from ground beef and ground lamb samples. *S.* Anatum was associated with meat (p-value<0.05) since all the food-related (n=11) were either from cow ground meat (n=6) or sheep ground meat (n=5). In addition, one isolate was gathered from ovine fecal sample, indicating the transmission route of the farm. According to a previous study, performed in Ankara, *S.* serovar Anatum was the most prominent serovar in cow's mesenteric lymph nodes (Küplülü 1995) and it may be the explanation of the relation of *S.* Anatum with bovine and bovine meat products in this study. In cheese samples, again *S.* Telaviv had been the predominant serovar. Interestingly, the most common serovars among food or animal isolates were less frequently collected from clinical human cases. The result of the clinical human isolates revealed that major serovar among clinical human cases was *S.* serovar Paratyphi B, since 64 % of the clinical human isolates represented *S.* that serovar.

3.2. Phenotypic antimicrobial resistance profiles according to disk diffusion test method

Phenotypic antimicrobial susceptibility profile tests were analyzed according to the source of isolate. In food-related isolates (Figure 12), *Salmonella* serovar Infantis had attracted attention since all of the Infantis isolates had shown a resistance at least to one antimicrobial agent. Every Infantis isolate was resistant to nalidixic acid and tetracycline; and nearly all of them were resistant to streptomycin and sulfisoxazole. None of the food-related isolates showed resistance to amikacin, gentamicin, ciprofloxacin, amoxicillin-clavulanic acid, cefoxitin, ceftriaxone, ceftiofur, imipenem, and ertapenem. *Salmonella* serotypes Reading, Othmarschen, Mbandaka, and Mikawasima were found to be susceptible to all 18 different antimicrobial agents.

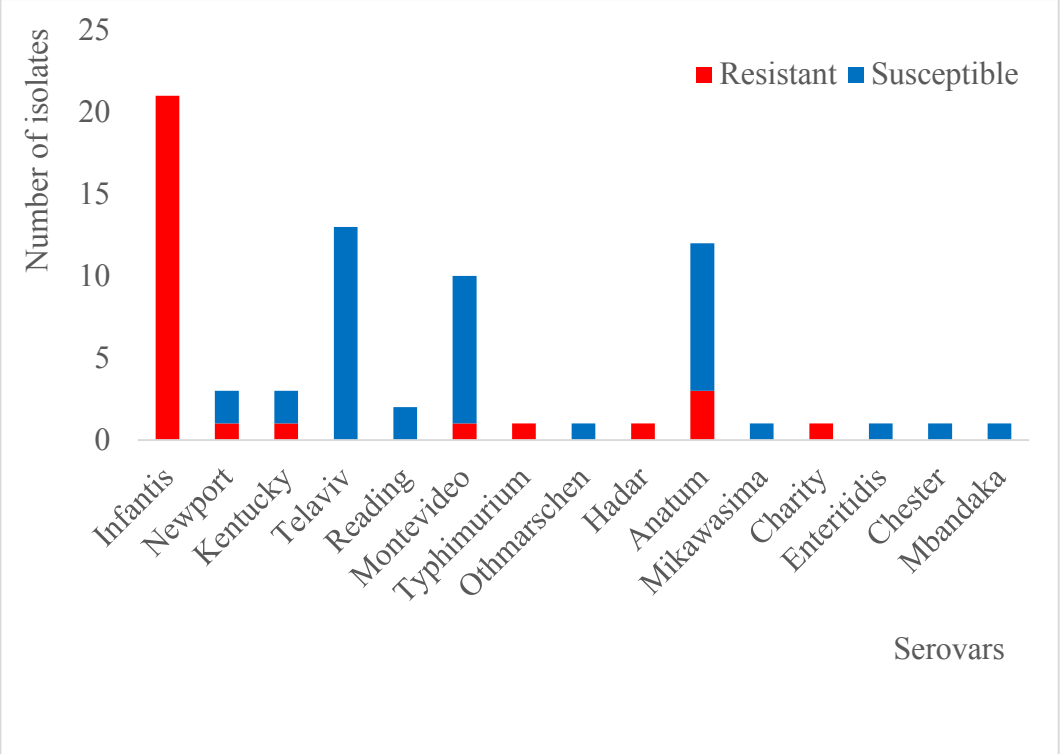


Figure 12 The number of resistant and nonresistant *Salmonella* serotypes isolated from food samples for the selected antimicrobial agents

The diversity of antimicrobial resistance profile of animal-related *Salmonella* isolates was different (Figure 13) than the food-related and human ones. The antimicrobial agents; gentamicin, ciprofloxacin, imipenem and sulfamethoxazole-trimethoprim were observed to be effective on the isolates. The beta lactams did not have the same impact on the animal-origin isolates compared to food-origin isolates. All of the Typhimurium isolates (3/3) had shown resistance to ampicillin and tetracycline. On the other hand, the serotypes; Enteritidis, Paratyphi B, Poona and *Salmonella* subsp. *diarizonae* were seen to be susceptible to 18 different antimicrobial agents.

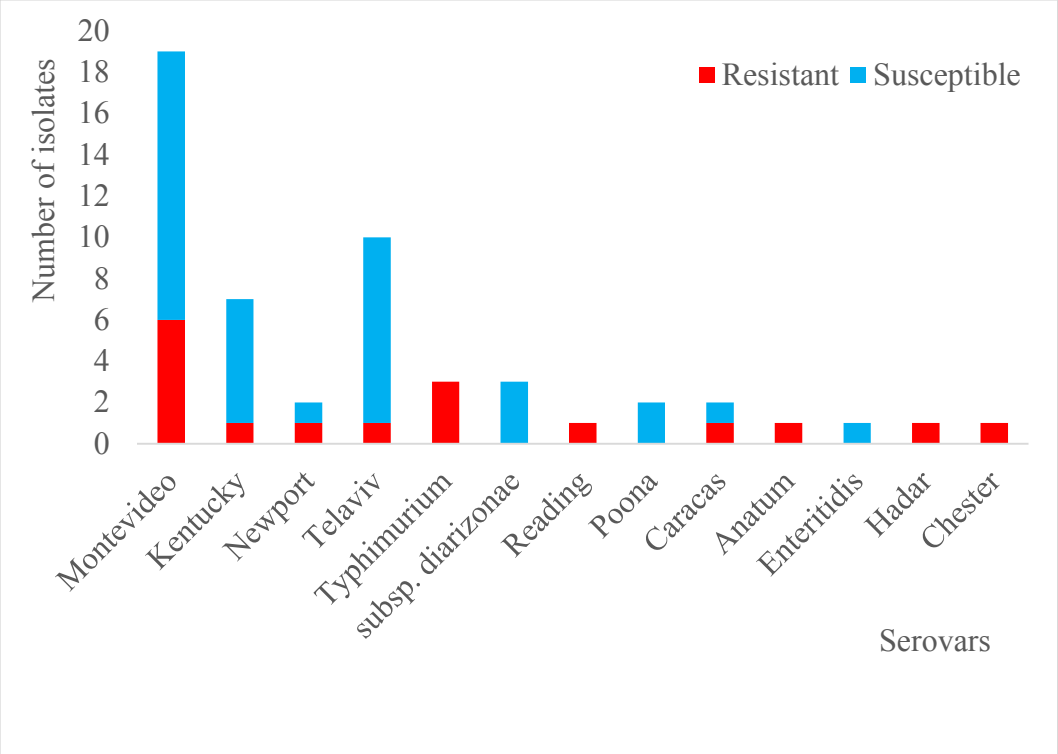


Figure 13 The number of resistant and nonresistant *Salmonella* serotypes isolated from animal samples for the selected antimicrobial agents

The distribution of antimicrobial resistance profiles was wider in animal isolates compared to food isolates. Nearly all isolate had different antimicrobial profiles. FoxKfEtp was observed in one Montevideo and one Telaviv serotype that were isolated from cattle feces. And FoxKf was seen in four serotypes; Montevideo, Telaviv, Hadar, and Saintpaul. The food-origin Hadar serotype had shared the same antimicrobial resistance with animal-origin one; SNAmpTKf (streptomycin, nalidixic acid, ampicillin, tetracycline and cephalothin). In addition to these groups of

antimicrobials, in animal-origin one, amoxicillin-clavualic acid, cefoxitin, and ertapenem resistance were also observed.

Recently, an increase in extended-spectrum cephalosporins (ceftiofur and ceftriaxone) resistance among *Salmonella* has grown into an important municipal health problem since severe salmonellosis in children is usually treated by ceftriaxone, which is, thus a significant antimicrobial agent (Rabsch, Tschape et al. 2001). Ceftiofur, on the other hand, is the single extended-spectrum cephalosporin drug accepted for veterinary practice in the U.S. (Bradford, Petersen et al. 1999). In addition to all, ceftriaxone-resistant organisms are also resistant to ceftiofur, which at the end, shows the importance of the studies analyzing the occurrence and spreading of resistance to these antimicrobial agents in *Salmonella* and other infection-related microorganisms. (Alcaine, Sukhnanand et al. 2005). In our study, we did not observe any ceftiofur resistance.

For the clinical human antimicrobial susceptibility results, all antibiotics; except gentamicin, ciprofloxacin, ceftriaxone, ceftiofur, ertapenem and imipenem; could not cause a susceptible profile for the isolates. The serovars, rather than *Salmonella* serovar Enteritidis, had resulted in a resistance to at least one antimicrobial agent (Figure 14).

3.3. Significance of resistant *Salmonella* isolates according to antimicrobials drug categories in human medicine

The Center for Veterinary Medicine (CVM) suggested a classification sheme for antibiotics founded on their significance in human medical therapy (9). The first class, Category I drugs, are vital for treatment of life-threatening diseases of humans, or are significant for treatment of foodborne diseases of humans, or are the drugs of an exceptional class that are used in humans (e.g., fluoroquinolones, glycopeptides). Secondly, Category II drugs, are mainly practiced for the treatment of human diseases,

which are possibly severe, on the other hand appropriate replacements of them are also present (e.g., ampicillin, erythromycin). Lastly, Category III drugs, have slightly or no important effect for the usage in human medicine, or are not the drugs of primary choice for human infections (e.g., ionophores).

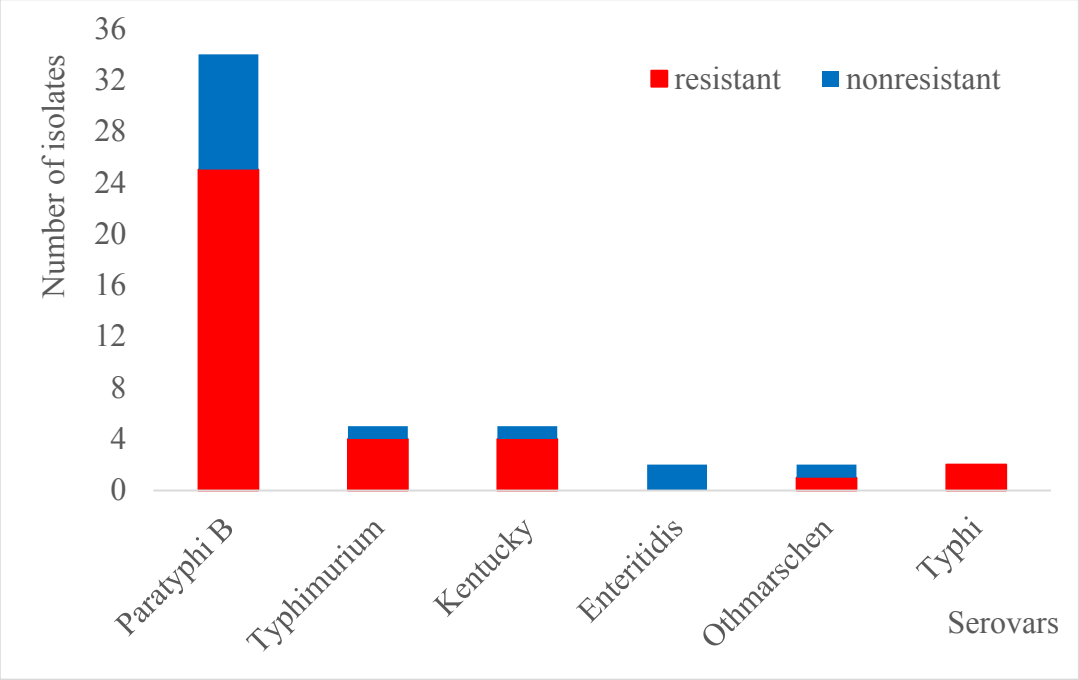


Figure 14 The number of resistant and nonresistant *Salmonella* serotypes isolated from clinical human samples for the selected antimicrobial agents

Furthermore, antimicrobial agents can also be ranked into high, medium, and low categories by looking at the probability of human contact by resistant human pathogens due to the use of these antimicrobial agents in food animals. Classification may thus consist of three main elements (i) the characteristics of antimicrobial agent

such as the resistance mechanism, degree of acquisition and expression, or cross-resistance; (ii) the predictable use of antimicrobial agent such as period of treatment, species of food animal, number, type of animals treated), and lastly (iii) the likelyhood of bacteria-human contact such bacteria of concern, environmental and food contamination, food processing effects.

Table 23 Prevalence of antimicrobial resistance in *Salmonella* isolates recovered from food sources

Antimicrobial category	Antimicrobials	Overall n= 36 (%)	Chicken meat n=21 (%)	Sheep ground meat n=5 (%)	Cow ground meat n=2 (%)	Offal n=5 (%)	Cheese n=1 (%)	Parsley n=1 (%)	Pistachio n=1 (%)
I	Amc	-	-	-	-	-	-	-	-
	Eft	-	-	-	-	-	-	-	-
	Cro	-	-	-	-	-	-	-	-
	Cip	-	-	-	-	-	-	-	-
	Imp								
	Etp	-	-	-	-	-	-	-	-
II	Ak	-	-	-	-	-	-	-	-
	Fox	-	-	-	-	-	-	-	-
	Amp	5 (14)	3 (14)	-	-	1	1	-	-
	Cn	-	-	-	-	-	-	-	-
	K	11 (31)	11 (52)	-	-	-	-	-	-
	N	23 (64)	21 (100)	-	-	1	1	-	-
	S	23 (64)	18 (86)	-	1	2	1	-	-
	Sxt	2 (6)	1 (5)	-	-	-	-	-	1
	Kf	3 (8)	2 (10)	-	-	-	1	-	-
III	C	1 (3)	1 (5)	-	-	-	-	-	-
	Sf	28 (78)	16 (76)	5	2	3	-	1	1
	T	21 (58)	18 (86)	-	-	2	1	-	-

The prevalence of antimicrobial resistance profiles of the present studies' isolates with different sources are given in Table 23-25. Resistance to category I antimicrobials are not observed in food origin and clinical human isolates whereas amoxicillin-clavulanic acid, ertapenem antimicrobials which are necessary for human treatment were not effective on some isolates obtained from animal sources such as sheep and cattle.

Among category II antimicrobials, amikacin and cefoxitin resistance were not observed in food isolates, but in animal-origin isolates 11% and 17% of them were resistant, respectively. And one and two clinical-human isolate was found to be resistant to amikacin and cefoxitin. Ampicillin resistance was observed in all sources, but gentamicin resistance was not seen. Kanamycin, nalidixic acid and streptomycin resistance was very high compared to other antimicrobials. In food-origin isolates, especially the ones isolated from chicken meat harbored a high resistance rate to kanamycin (52%), nalidixic acid (100%), and streptomycin (86%). Cephalothin resistance was high in animal-origin isolates compared to other isolates.

For the category III antimicrobials, it is obvious that the prevalence rate of resistance is higher with respect to other categories. Sulfonamide resistance is mostly detected in *Salmonella* isolates from every class of sources. And tetracycline could not have an effect on food-origin isolates.

Table 24 Prevalence of antimicrobial resistance in *Salmonella* isolates recovered from animal sources

Antimicrobial category	Antimicrobials	Overall n= 18 (%)	Cattle n= 8 (%)	Chicken n=1 (%)	Sheep n=9 (%)
I	Amc	4 (22)	-	-	4
	Eft	-	-	-	1
	Cro	-	-	-	-
	Cip	-	-	-	-
	Imp	-	-	-	-
	Etp	4 (22)	2	-	2
II	Ak	2 (11)	1	-	1
	Fox	3 (17)	2	-	1
	Amp	4 (22)	1	-	3
	Cn	-	-	-	-
	K	-	-	-	-
	N	2 (11)	1	-	1
	S	4 (22)	1	-	3
	Sxt	-	-	-	-
	Kf	7 (39)	3	-	4
III	C	1 (6)	-	-	1
	Sf	9 (50)	4	1	4
	T	3 (17)	2	-	1

Table 25 Prevalence of antimicrobial resistance in *Salmonella* isolates recovered from clinical human sources

Antimicrobial category	Antimicrobials	Overall n= 36 (%)	Age 0-10 n= 2 (%)	Age 20-30 n=4 (%)	Age 30-50 n=20 (%)	Age ≥50 n=10 (%)
I	Amc	-	-	-	-	-
	Eft	-	-	-	-	-
	Cro	-	-	-	-	-
	Cip	-	-	-	-	-
	Imp	-	-	-	-	-
	Etp	-	-	-	-	-
II	Ak	1 (3)	-	-	-	1
	Fox	2 (6)	-	1	1	-
	Amp	2 (6)	1	-	1	-
	Cn	-	-	-	-	-
	K	2 (6)	-	-	1	1
	N	2 (6)	-	1	1	-
	S	4 (11)	-	1	2	1
	Sxt	2 (6)	-	-	1	1
	Kf	1 (3)	-	-	1	-
III	C	2 (6)	-	-	1	1
	Sf	33 (92)	1	3	19	10
	T	2 (6)	1	-	1	-

3.4. Genotypic antimicrobial resistance profile results

3.4.1. Presence of antimicrobial resistance genes in the genomes of food-related resistant *Salmonella* isolates

Among 36 phenotypically resistant *Salmonella* isolates, 61% of them harbored an aminoglycoside resistance-related gene and 86% of them are associated with *aadA1* gene. Among aminoglycoside resistance genes that are analyzed in this study, no phenotypically resistant isolate had *aada2* or *aacC2* genes which are related with

streptomycin and kanamycin resistance. These genes can be transferred through microorganisms by plasmid and integrons. The presence of *strA* (8%) and *strB* (3%) genes was low compared to other genes (Table 26). Strong association (100%) is observed between $apha_{1\text{-}iab}$ gene presence and kanamycin resistance. Among 23 streptomycin resistant isolates, 19 of them (83%) are found to have *aada1* gene. 3 Infantis isolates have both *aadA1* and *strA* genes. *strB* gene is detected only from a Hadar serotype.

Tetracycline resistance is found to be related with *tetA* gene in food-origin *Salmonella* isolates. Every phenotypically resistant isolate have *tetA gene* but no *tetB* and *tetG* genes are detected. 5 ampicillin resistant isolates are seen to have $bla_{TEM\text{-}1}$ gene. However, none of the *Salmonella* isolate obtained from food sources have $bla_{PS13E\text{-}1}$, $bla_{CMY\text{-}2}$ or *ampC.*

Sulfonamide resistance was high at phenotypic resistance profiles and among 30 of the sulfonamide resistant isolate, 21 of them had *sul1* gene. While there was 2 trimethoprim resistant isolate (Salford and Infantis), trimethoprim resistance related genes (*dhfrI* and *dhfrXII*) are not observed.

According to disk diffusion results, one Infantis isolate was found to be resistant to chloramphenicol and *cmlA* gene was detected in this isolate.

Table 26 Distribution of antimicrobial resistance genes in resistant *Salmonella* isolates from food sources

Resistance genes	Overall n= 36 (%)	Chicken meat n=21 (%)	Sheep ground meat n=5 (%)	Cow ground meat n=2 (%)	Offal n=5 (%)	Cheese n=1 (%)	Parsley n=1 (%)	Pistachio n=1 (%)
aadA1	19 (53)	18 (86)	-	-	1 (20)	-	-	-
aadA2	-	-	-	-	-	-	-	-
strA	3 (8)	3 (14)	-	-	-	-	-	-
strB	1 (3)	-	-	-	-	1 (100)	-	-
aacC2	-	-	-	-	-	-	-	-
apha$_{1\text{-}iab}$	14 (39)	13 (62)	-	1 (50)	-	-	-	-
tetA	23 (64)	20 (95)	-	-	2 (40)	1 (100)	-	-
tetB	-	-	-	-	-	-	-	-
tetG	-	-	-	-	-	-	-	-
bla$_{TEM\text{-}1}$	5 (14)	3 (14)	-	-	1 (20)	1 (100)	-	-
bla$_{PS13E\text{-}1}$	-	-	-	-	-	-	-	-
bla$_{CMY\text{-}2}$	-	-	-	-	-	-	-	-
ampC	-	-	-	-	-	-	-	-
sul1	21 (58)	17 (81)	1 (20)	-	3 (60)	-	-	-
sul2	-	-	-	-	-	-	-	-
dhfrI	-	-	-	-	-	-	-	-
dhfrXII	-	-	-	-	-	-	-	-
cat1	-	-	-	-	-	-	-	-
cat2	-	-	-	-	-	-	-	-
flo	-	-	-	-	-	-	-	-
cmlA	1 (3)	1 (5)	-	-	-	-	-	-

3.4.2. Presence of antimicrobial resistance genes in the genomes of animal-related resistant *Salmonella* isolates

Aminoglycoside resistance was not as predominant as in the case of food-origin isolates, there were 7 phenotypically aminoglycoside-resistant isolates. Differently from food-origin isolates, in animal-origin isolates, no *aadA1* gene was detected; adversely *aadA2* gene was detected in one isolate (Typhimurium) that had been isolated from sheep (Table 27). 4 *strB* gene was found in which all isolates have streptomycin resistance; these are 2 Typhimurium, and 1 Hadar isolates.

Although there were 5 phenotypically tetracycline-resistant isolates, only two of them (Typhimurium and Hadar) were detected to have *tetA* gene. Similarly to the food-origin isolates, no *tetB* and *tetG* genes were found.

Beta-lactam resistance had a wide spectrum in animal-origin *Salmonella* isolates compared to other sources but molecular detection results have shown that only two beta-lactam resistance genes (bla_{TEM-1} and $bla_{PS13E-1}$) were present in these isolates.

Among 9 sulfonamide resistant isolates, only 1 of them was found to have *sul1* gene, and similar to food-origin sulfonamide-resistant isolates, *sul2*, *dhfrI* and *dhfrXII* genes were not seen. Although there was one chloramphenicol resistant isolate, the related genes were not observed in the genotyping results.

Table 27 Distribution of antimicrobial resistance genes in resistant *Salmonella* isolates from animal sources

Resistance genes	Overall n= 18 (%)	Cattle n= 8 (%)	Chicken n=1 (%)	Sheep n=9 (%)
aadA1	-	-	-	-
aadA2	1 (6)	-	-	1 (11)
strA	-	-	-	-
strB	4 (22)	1 (13)	-	3 (33)
aacC2	-	-	-	-
$apha_{1\text{-}iab}$	2 (11)	1 (13)	-	1 (11)
tetA	2 (11)	1 (13)	-	1 (11)
tetB	-	-	-	-
tetG	-	-	-	-
$bla_{TEM\text{-}1}$	5 (28)	2 (25)	-	3 (33)
$bla_{PS13E\text{-}1}$	1 (6)	-	-	1 (11)
$bla_{CMY\text{-}2}$	-	-	-	-
ampC	-	-	-	-
sul1	1 (6)	-	-	1 (11)
sul2	-	-	-	-
dhfrI	-	-	-	-
dhfrXII	-	-	-	-
cat1	-	-	-	-
cat2	-	-	-	-
flo	-	-	-	-
cmlA	-	-	-	-

3.4.3. Presence of antimicrobial resistance genes in the genomes of clinical human-related resistant *Salmonella* isolates

The prevalence of antimicrobial resistance genes in clinical-human related *Salmonella* isolates was found to be very low compared to other source groups. Most of the resistance was observed to sulfonamides, and 67% of the resistant isolates were Paratyphi B. Only 3 *sul1* resistance genes were detected whereas the number of phenotypically sulfonamide-resistant isolates was 33 (Table 28).

Table 28 Distribution of antimicrobial resistance genes in resistant *Salmonella* isolates from clinical human sources

Resistance genes	Overall n= 36 (%)	Age 0-10 n= 2 (%)	Age 20-30 n=4 (%)	Age 30-50 n= 20 (%)	Age ≥50 n= 10 (%)
aadA1	-	-	-	-	-
aadA2	-	-	-	-	-
strA	-	-	-	-	-
strB	-	-	-	-	-
aacC2	-	-	-	-	-
$apha_{1\text{-}iab}$	2 (6)	-	1 (25)	-	1 (10)
tetA	1 (3)	-	-	1 (5)	-
tetB	-	-	-	-	-
tetG	-	-	-	-	-
$bla_{TEM\text{-}1}$	4 (11)	1 (50)	1 (25)	2 (10)	-
$bla_{PS13E\text{-}1}$	-	-	-	-	-
$bla_{CMY\text{-}2}$	-	-	-	-	-
ampC	-	-	-	-	-
sul1	3 (8)	1 (50)	-	2 (10)	-
sul2	-	-	-	-	-
dhfrI	-	-	-	-	-
dhfrXII	-	-	-	-	-
cat1	-	-	-	-	-
cat2	-	-	-	-	-
flo	-	-	-	-	-
cmlA	-	-	-	-	-

All beta-lactam resistant isolates which have shown resistance to ampicillin (2), cefoxitin (2) and cephalothin (1) have found to have only $bla_{TEM\text{-}1}$ gene. Among 4 aminoglycoside resistant isolates, two of them had $apha_{1\text{-}iab}$ gene. And no chloramphenicol related genes were detected in two phenotypically resistant isolates.

3.5. The correlation of phenotypic and genotypic antimicrobial profiles of *Salmonella* isolates

Kappa statistics were measured to evaluate the agreement between phenotypic and genotypic data within each antimicrobial group (Table 29). Aminoglycoside, beta-lactam, and sulfonamides had shown very good correlation (kappa ≥ 0.9). The results indicated that the common genes that gave rise to the resistance phenotype had been included on the antimicrobial resistance tests. However, chloramphenicols and sulfonamides showed poor correlation (kappa ≤0.4) between phenotypic and genotypic data since only *cmlA* and *sul1* genes were detected in few isolates. Although there were 4 phenotypically trimethoprim-resistant isolates, *dhfrI* and *dhfrXII* genes were found to be not associated with the isolates in our study.

In general, it was observed that none of the resistant isolates had *aacC2, tetB, tetG,* bla_{CMY-2}*, ampC, sul2, dhfrI, dhfrXII, cat1, cat2,* and *flo* genes (Table 26). These results showed that there was a geographical difference between antimicrobial genotypic resistance profiles because the genes had been selected according to their prevalence in literature and phenotypic-association proven (Soyer et al., 2013). The selected genes in our study were also listed in National Antimicrobial Resistance Monitoring System (NARMS). In a study performed in U.S. in 2004, human and bovine-origin *Salmonella* isolates had been analyzed for antimicrobial resistance, and it was observed that in total 50% of them have bla_{CMY-2} or *ampC* but in our study we did not find any isolate having these genes. Also, in that study, 56 % of the isolates had *flo* gene, most of the aminoglycoside resistance had been related with *strA* and *strB* genes, however the findings of our study do not agree with this study (Soyer et al., 2013).

Table 29 Genotypic and phenotypic correlation found in resistant strains for given antimicrobial groups

Antimicrobial group	Food (Kappa[1])	Animal (Kappa)		Human Kappa)		Total (Kappa)	
Aminoglycoside genotype[2]	22 (0.93)	4	(0.73)	2	(0.79)	28	(0.90)
Aminoglycoside phenotype	23	6		3		32	
β-lactam genotype	5 (1.00)	6	(0.67)	4	(1.00)	15	(0.89)
β-lactam phenotype	5	9		4		18	
Tetracycline genotype	23 (1.00)	2	(0.49)	1	(0.65)	26	(0.90)
Tetracycline phenotype	23	5		2		30	
Sulfonamide genotype	21 (0.44)	1	(0.11)	3	(0.00)	25	(0.14)
Sulfonamide phenotype	30	9		36		75	
Trimethoprim genotype	0 (0.00)	0	(0.00)	0	(0.00)	0	(0.00)
Trimethoprim phenotype	2	0		2		4	
Chloramphenicol genotype	1 (1.00)	0	(0.00)	0	(0.00)	1	(0.39)
Chloramphenicol phenotype	1	1		2		4	
Quinolone genotype [3]	-	-		-		-	
Quinolone phenotype	23	3		2		28	

[1] The Cohen's Kappa statistic is a measure of the agreement above that expected by chance, a kappa of 0 indicates that there is no agreement and a value of 1 indicates a complete agreement.

[2] The resistance phenotype was to streptomycin, kanamycin or amikacin, and the resistance genotype was *aadA1/2*, *strA/B*, or $aphA_{1\text{-}iab}$

[3] Quinolone genotype resistance analysis was not involved in the study.

Another study comparing the antimicrobial resistance profiles of *Salmonella* isolates obtained from retail meats in U.S. and China, had shown that the resistance profiles change geographically. While U.S. isolates had mostly bla_{CMY-2} gene for resistance to beta-lactamase group of antimicrobial drugs (especially ceftriaxone resistance), it was not observed in Chinese isolates, bla_{TEM-1} gene was present in the isolates obtained from China. And no *flo* gene is detected in Chinese isolates while phenotypically chloramphenicol resistance is found (Chen et al. 2004). In a Danish study, β-lactamase resistance in multiresistant *Salmonella* Typhimurium DT104 was related with a different gene; *pse-1* (Sandvang et al., 2006).

In our study, the antimicrobial genes were chosen for nontyphoidal *Salmonella* isolates and this may be the reason of the lack of association between the genotypic and phenotypic profiles of human-origin *Salmonella* isolates, especially the serovar Paratyphi B. While sulfonamide resistance was found to be high by disk diffusion method, the number of resistance genes was very low.

3.6. Multi-drug resistance (MDR) among the isolates

MDR was defined as having resistance to two or more antimicrobial resistance agent. In total there were 41 phenotypically MDR *Salmonella* isolates, but the molecular characterization results had shown that 68% of them had MDR genotype (Table 30) which emphasizes that there may be a lack of genes that are associated with phenotypic profile. But in general, we observed that the prevalence of antimicrobial resistance genes was related with geographical region and also the source and serovar of the isolate.

The most prevalent MDR profile in food isolates were KSNTSf (8/35) (kanamycin, streptomycin, nalidixic acid, tetracycline and sulfisoxazole) and SNTSf (6/35) (streptomycin, nalidixic acid, tetracycline and sulfisoxazole); and they were almost all

seen in Infantis isolates. In all Infantis isolates NT (nalidixic acid, and tetracycline) resistance was observed. In Germany, an antimicrobial susceptibility study was performed on food materials and it was shown that the main three antimicrobial agents that have been observed to be not effective on the food isolates are streptomycin (93.7%), sulfamethaxazole (92.5%), tetracycline (80.9%) (Miko, Pries et al. 2005). In another study, tetracycline (80.0%), streptomycin (73.0%) and sulfamethaxazole (60.0%) resistance were displayed on USA retail meat samples such as chicken, beef, pork and turkey (White, Zhao et al. 2001). These three antimicrobials were also observed to be not efficient on food-origin *Salmonella* isolated from Turkey in our study.

Table 30 MDR *Salmonella* isolates

Strain	Source	Subsource	Serovar	Phenotype	Genotype
MET-S1-030	Food	Pistachio shell	Salford	SfSxt	-
MET-S1-050	Food	Chicken meat	Infantis	KSTAmpSfN	*aadA1 aphA1-iab tet*A *bla*$_{TEM-1}$*sul1*
MET-S1-056	Food	Chicken meat	Infantis	KSTAmpKfSfSxtCN	*aadA1 aphA1-iab tet*A *bla*$_{TEM-1}$ *sul1 cmlA*
MET-S1-088	Food	Chicken meat	Infantis	KSTSfN	*aphA1-iab tet*A *sul1*
MET-S1-092	Food	Chicken meat	Infantis	STSfN	*aadA1 tet*A *sul1*
MET-S1-103	Food	Chicken meat	Infantis	KSTSfN	*aadA1 aphA1-iab tet*A *sul1*
MET-S1-142	Food	Chicken meat	Infantis	STSfN	*aadA1 strA tetA sul1*
MET-S1-150	Food	Offal	Infantis	STSfN	*aadA1 tetA sul1*
MET-S1-163	Food	Urfa cheese	Hadar	STAmpKfN	*strB tetA bla*$_{TEM-1}$
MET-S1-197	Clinical human	Man/Young adult	Paratyphi B	FoxSf	*bla*$_{TEM-1}$
MET-S1-198	Clinical human	Man/Adult	Paratyphi B	FoxKfSf	*bla*$_{TEM-1}$
MET-S1-204	Clinical human	Woman/Adult	Typhimurium	KSSfSxtC	-
MET-S1-205	Clinical human	Woman/Adult	Paratyphi B	SfN	-

Table 30 Continued

Strain	Source	Subsource	Serovar	Phenotype	Genotype
MET-S1-211	Clinical human	Man/Adult	Paratyphi B	TAmp	*tetA* bla_{TEM-1}
MET-S1-218	Clinical human	Woman/Elder	Paratyphi B	AkKSSfSxtC	*aphA1-iab*
MET-S1-223	Clinical human	Woman/Kid	Typhimurium	TAmp	bla_{TEM-1}
MET-S1-235	Clinical human	Man/Adult	Paratyphi B	SSf	-
MET-S1-329	Food	Chicken meat	Infantis	STSfN	*aadA1 strA tetA sul1*
MET-S1-345	Food	Chicken meat	Infantis	KSTSfN	*aadA1 aphA1-iab tetA sul1*
MET-S1-351	Food	Chicken meat	Infantis	STSfN	*aadA1 strA tetA sul1*
MET-S1-492	Food	Chicken meat	Infantis	STN	*aadA1 tetA*
MET-S1-498	Food	Chicken meat	Infantis	KSTSfN	*aadA1 aphA1-iab tetA sul1*
MET-S1-510	Food	Chicken meat	Infantis	KSTSfN	*aadA1 aphA1-iab tetA sul1*
MET-S1-542	Animal	Sheep	Kentucky	KS	-
MET-S1-579	Food	Cow ground meat	Anatum	SSf	*aphA1-iab*
MET-S1-597	Food	Chicken meat	Infantis	KSTSfN	*aadA1 aphA1-iab tetA sul1*
MET-S1-606	Food	Chicken meat	Infantis	STSfN	*aadA1 tetA sul1*
MET-S1-625	Food	Offal	Newport	TAmp	*tetA* bla_{TEM-1}
MET-S1-653	Animal	Bull	Typhimurium	AkSTAmpKfN	*strB tetA* bla_{TEM-1}
MET-S1-654	Animal	Sheep	Anatum	AkSf	-
MET-S1-657	Animal	Sheep	Typhimurium	STAmpAmcSfCN	*aadA2 strB* $bla_{PS13E-1}$ *sul1*
MET-S1-663	Animal	Sheep	Typhimurium	TAmpKf	bla_{TEM-1}
MET-S1-668	Food	Chicken breast	Infantis	SSfN	*aadA1 sul1*

Table 30 Continued

Strain	Source	Subsource	Serovar	Phenotype	Genotype
MET-S1-669	Food	Chicken wing	Infantis	SAmpKfN	*aadA1* *bla*$_{TEM-1}$ *sul1*
MET-S1-671	Food	Chicken breast	Infantis	KSTSfN	*aadA1* *aphA1-iab* *tetA* *sul1*
MET-S1-672	Food	Chicken skin	Infantis	KSTSfN	*aadA1* *aphA1-iab* *tetA* *sul1*
MET-S1-673	Food	Chicken wing	Infantis	TN	*tetA*
MET-S1-674	Food	Chicken wing	Infantis	KSTSfN	*aadA1* *aphA1-iab* *tetA* *sul1*
MET-S1-703	Animal	Sheep	Hadar	STAmpAmcFoxKfErtN	*strB* *tetA* *bla*$_{TEM-1}$
MET-S1-704	Animal	Sheep	Chester	AmcFoxKfErt	-
MET-S1-706	Animal	Cattle	Montevideo	TErt	-
MET-S1-707	Animal	Cattle	Montevideo	FoxKfErt	*bla*$_{TEM-1}$
MET-S1-708	Animal	Cattle	Montevideo	FoxKf	-

3.7. Geographical clustering, as well as host clustering of AR genes

Presence of antimicrobial resistance genes, investigated in our study varied also with host species. The majority of resistant food isolates carried the AR genes, picked in this study. However the correlation of genotype and phenotype in animal and human isolates were lower.

Among 22 resistant food *Salmonella* isolates, which were phenotypically resistant to at least one antimicrobial agent, 65 % of them harbored an aminoglycoside gene and 93 % of these isolates were associated with *aadA1* gene. Furthermore, among 24 streptomycin resistant food isolates, 14 of them (58 %) had *aadA1* gene and none of the isolates with streptomycin resistance carried *aadA2* or *aacC2* genes. But, for animal isolates, differently than food-origin isolates, no *aadA1* gene was detected; adversely *aadA2* gene was detected in one isolate (*S.* serovar Typhimurium) that was obtained from sheep (Table 32). The frequency of *strA* (8 %) and *strB* (3 %) genes in aminoglycoside resistant isolates was lower than that of other antimicrobial resistance genes (Table 31). *strB* gene was only detected from two *S.* serovar Hadar isolates, which were obtained from cheese and ovine fecal samples. Strong association (100 %) was observed between $aphA_{1\text{-}iab}$ gene presence and kanamycin resistance. Tetracycline resistance was related with *tetA* gene in all *Salmonella* isolates.

Beta-lactam resistance in food-origin *Salmonella* isolates was related with only $bla_{TEM\text{-}1}$ gene (Table 32). Although beta-lactam resistance had a wide spectrum in animal-origin *Salmonella* isolates compared to other sources, according to the molecular detection results, only two beta-lactam resistance genes ($bla_{TEM\text{-}1}$ and $bla_{PS13E\text{-}1}$) were detected among them. Here, it was concluded that the prevalence of AR genes were related with geography and also the source and serovar of the isolate according to the AR profile comparisons.

Table 31 The distribution of antimicrobial resistance genes associated with phenotypic serovars detected in *Salmonella* isolates

Antimicrobial agent group	**Genes**	**Serovars (number)**		
		Food isolates	Animal isolates	Clinical human isolates
Aminoglycoside	*aadA1*	*S.* Infantis (14)	ND	ND
	aadA2	ND	*S.* Typhimurium (1)	ND
	strA	*S.* Infantis (3)	ND	ND
	strB	*S.* Hadar (1)	*S.* Hadar (1), *S.* Typhimurium (2)	ND
	$aphA_{1\text{-}iab}$	*S.* Infantis (9)	-	*S.* Paratyphi B (1)
Tetracycline	*tetA*	*S.* Infantis (15), *S.* Hadar (1), *S.* Typhimurium (1)	*S.* Hadar (1), Typhimurium (1)	*S.* Typhimurium (1)
Beta-lactam	$bla_{TEM\text{-}1}$	*S.* Infantis (2), *S.* Hadar (1), *S.* Typhimurium (1)	*S.* Montevideo (1), *S.* Hadar (1), *S.* Typhimurium (2)	*S.* Typhimurium (2), *S.* Paratyphi B (2)
	$bla_{PSE\text{-}13}$	ND	*S.* Typhimurium (1)	ND
Sulfonamide	*sul1*	*S.* Infantis (14)	*S.* Typhimurium (1)	Kentucky (2), Typhi (1)
Phenicol	*cmlA*	*S.* Infantis (1)	ND	ND

ND: Not detected

3.8. Coselection of AR among *Salmonella* serovar Infantis isolates

Half of the MDR isolates representing *S.* serovar Infantis were collected from chicken samples (n=15), which highlighted that a great effort should be taken to investigate the

reasons of contamination in chicken farms and consequences of this case. Also, possible unconditional statistical associations between the seven serovars (*S.* serovar Infantis, *S.* serovar Typhimurium, *S.* serovar Hadar, *S.* serovar Paratyphi B, *S.* serovar Kentucky, *S.* serovar Typhi and *S.* serovar Montevideo) and the resistance genes had resulted in the odds of identifying *aadA1, tetA, aphA1-IAB, sul1,* genes in *S.* serovar Infantis were 7.4, 5.7, 4.8 and 3.7 times higher (95% CI) than *Salmonella* isolates that were not *S.* Infantis (Table 31). The unconditional association found between the resistance genes detected in *Salmonella* of chicken meat origin proposed that there might be a likelihood of coselection of resistance to different classes of antimicrobial agents through mobile genetic elements. In a related manner, the emergence of *S.* Infantis in Israel (Gal-Mor, Valinsky et al. 2010, Aviv, Tsyba et al. 2014), which had been associated with a megaplasmid found on the emerging isolates, also demonstrated that there has been an increase of *S.* Infantis cases in Israel. Furthermore, the antimicrobial resistance profiles of broiler chickens in Hungary (Nógrády, Tóth et al. 2007) harboring MDR *S.* Infantis clones were similar to that of our isolates; and it has been reported that the possibility of spread of these isolates to individuals through chicken meat may result in a significant threat to public health.

The association of presence of different AR genes was analyzed by comparing odds ratios (Table 32) and numerous significant associations ($p < 0.00185$) were detected. The strongest associations, organized by their degree of log ODs, involved those between the following genes: *aadA1 and tetA, aadA1 and sul1, aph*$A_{1\text{-}IAB}$ and *sul1, tetA* and *aph*$A_{1\text{-}IAB}$*, aadA1* and *aph*$A_{1\text{-}IAB,}$ and *tetA* and *sul1* (Table 32). Since all the genes, especially *aadA1* and *aph*$A_{1\text{-}IAB,}$ were found in food- and specifically in chicken meat-related *S.* Infantis isolates, the presence of mobile genetic elements on these serovars may have enhanced the possibility of co-existence of these AR genes.

To investigate the presence of mobile genetic elements on *S.* serovar Infantis isolates, the number of the isolates were increased to 56 for the following studies.

Table 32 Association of antimicrobial resistance genes recovered from phenotypically resistant food, animal and human isolates

Outcome gene	**Predictor gene**	**Log odds ratio [1]**	**95 % CI**	**P value**
aadA1	*tetA*	5.51	13.17 - 4655.60	0.0002
tetA	*aphA1-IAB*	3.99	6.21 - 469.90	0.0006
aadA1	*aphA1-IAB*	3.97	8.92 - 315.99	p < 0.0001
aadA1	*sul1*	3.96	10.24 - 266.72	p < 0.0001
tetA	*sul1*	2.75	4.06 - 59.87	p < 0.0001
aphA1-IAB	*sul1*	2.51	2.92 -51.42	0.0003
Outcome gene	**Predictor serovar**	**Log odds ratio [2]**	**95 % CI**	**P value**
aadA1	*S.* Infantis	7.39	54.57- 48173.43	p < 0.0001
tetA	*S.* Infantis	5.71	15.83- 5787.99	0.0001
aphA1-IAB	*S.* Infantis	4.77	12.45- 1118.69	0.0001
sul1	*S.* Infantis	3.65	8.34 - 177.66	p < 0.0001

[1] The statistically significant unconditional associations from a logistic regression model are listed (p value of 0.05/27 comparisons; p < 0.00185).

[2] The statistically significant unconditional associations from a logistic regression model are listed (p value of 0.05/20 comparisons; p < 0.0025)

3.9. Antimicrobial resistance profile results according to the minimal inhibition concentration method

Minimal inhibitory concentration (MIC) method was done by commercial E-test, which is a well-developed method for antimicrobial susceptibility testing in laboratories in the world. Considering the importance of antibiotics in case of public health and the frequency of clinical usage; ertapenem (Type 1), amoxicillin-clavulanic acid (Type 1), trimethoprim-sulfamethoxasol (Type 2), amikacin (Type 2), ampicillin (Type 2) and tetracycline (Type 3) antibiotics were studied on *Salmonella* isolates that had resistance profile determined by disc diffusion method (Table 33). The study showed that tetracycline, amoxicillin-clavulanic acid, and ampicillin results are comparable with disk diffusion results; their MIC values were below the limits of resistances.

Table 33 Minimal inhibition concentration (MIC) values for selective isolates and antimicrobial agents

Isolate code	Source	ERT	AMC	SXT	AK	AMP	T
MET-S1-50	Food, chicken meat	S	S	S	S	≥ 256 mg/L	≥ 128 mg/L
MET-S1-56	Food, chicken meat	S	S	≥ 32/128 mg/L	S	≥ 256 mg/L	≥ 192 mg/L
MET-S1-88	Food, chicken meat	S	S	S	S	S	≥ 64 mg/L
MET-S1-92	Food, chicken meat	S	S	S	S	S	≥ 32 mg/L

S: Susceptible

Table 33 Continued

Isolate code	Source	ERT	AMC	SXT	AK	AMP	T
MET-S1-103	Food, chicken meat	S	S	S	S	S	≥ 64 mg/L
MET-S1-142	Food, chicken meat	S	S	S	S	S	≥ 128 mg/L
MET-S1-150	Food, offal	S	S	S	S	S	≥ 192 mg/L
MET-S1-163	Food, Urfa peyniri	S	S	S	S	≥ 256 mg/L	≥ 32 mg/L
MET-S1-204	Clinical human, age:45	S	S	≥ 0.032/0.6 mg/L	S	S	S
MET-S1-211	Clinical human, age:34	S	S	S	S	≥ 256 mg/L	≥ 32 mg/L
MET-S1-218	Clinical human, age: 57	S	S	≥ 0.064/1.2 mg/L	≥ 2 mg/L	S	S
MET-S1-223	Clinical human, age: 2	S	S	S	S	≥ 256 mg/L	≥ 48 mg/L
MET-S1-329	Food, chicken meat	S	S	S	S	S	≥ 192 mg/L
MET-S1-345	Food, chicken meat	S	S	S	S	S	≥ 96 mg/L
MET-S1-351	Food, chicken meat	S	S	S	S	S	≥ 128 mg/L
MET-S1-492	Food, chicken meat	S	S	S	S	S	≥ 192 mg/L

S: Susceptible

Table 33 Continued

Isolate code	Source	ERT	AMC	SXT	AK	AMP	T
MET-S1-498	Food, chicken meat	S	S	S	S	S	≥ 128 mg/L
MET-S1-510	Food, chicken meat	S	S	S	S	S	≥ 192 mg/L
MET-S1-597	Food, chicken meat	S	S	S	S	S	≥ 128 mg/L
MET-S1-606	Food, chicken meat	S	S	S	S	S	≥ 128 mg/L
MET-S1-625	Food, offal	S	S	S	S	≥ 256 mg/L	≥ 24 mg/L
MET-S1-653	Animal, cow	S	S	S	≥ 1 mg/L	≥ 256 mg/L	≥ 24 mg/L
MET-S1-654	Animal, sheep	S	S	S	≥ 1 mg/L	S	S
MET-S1-657	Animal, sheep	S	≥ 48/24 mg/L	S	S	≥ 256 mg/L	≥ 16 mg/L
MET-S1-663	Animal, sheep	S	S	S	S	≥ 256 mg/L	≥ 32 mg/L
MET-S1-703	Animal, sheep	≥ 0.016 mg/L	≥ 64/32 mg/L	S	S	≥ 256 mg/L	≥ -
MET-S1-704	Animal, sheep	≥ 0.032 mg/L	≥ 48/24 mg/L	S	S	S	S
MET-S1-706	Animal, cow	≥ 0.006 mg/L	S	S	S	S	≥ 0.75 mg/L
MET-S1-707	Animal, cow	≥ 0.047 mg/L	S	S	S	S	S

S: Susceptible

The MIC results for trimethoprim-sulfamethoxasol resistance in food isolates were also identical with disk diffusion method but the results for clinical human samples did not match. *Salmonella* isolates did not show resistance to ertapenem and amikacin according to MIC values.

3.10. Plasmid characterization of *Salmonella* isolates

In our first results we observed a chromosomal DNA fragments in agarose gels, which was observed to be common in plasmid DNA visualization. This was because of the moderately purified plasmid DNA, which was produced by ethanol precipitation of isopropanol cleared lysates. These unpurified plasmid DNA moved on agarose gels usually as single bands and result in an undefined plasmid band (Meyers *et al.,* 1976). The solutions may also have changing amounts of fragmented chromosomal DNA, and theymay not have been removed in the production of clear plasmid-carrying strains, and this banded may occur as a broad diffuse band (Figure 15). This region and band might be very close to plasmid DNA bands over a noteworthy variety of molecular size and thus affect the plasmid detection of uncharacterized strains in an unwanted way. Therefore, a great attention should be taken at determination of the plasmid size.

Strain comparison can be also performed by plasmid profiling; searching the presence of plasmids or the restricted profiles of plasmid when the bacterium has plasmids. The plasmid profiling can also be used for finding the outbreak related strain in epidemiological studies for various species such as *Escherichia, Klebsiella, Staphylococcus,* and *Salmonella.* For instance, plasmid profiling was found to be very effective for *Salmonella* serovar Typhimurium; and it gave similar results with phagotypization, and better results compared to resistotypization in case of discrimination power (Threlfall et al., 1986). It has been also used for detecting the source of infection among multi-drug resistant (MDR) *Salmonella* Typhimurium

strains in Sao Paolo (Brazil). It was founded that infections linked with strains having the same plasmid profile arised among children hospitalized in the same hospital.

Plasmid profile analysis can also be found to be effective on finding the foodborne outbreak causing strain in other serovars of *Salmonella*. To exemplify, a beef from a farm was detected to be the origin of an outbreak in U.S., and the food was harboring *Salmonella* serovar Newport and many people were observed to be infected due to this serovar. But, interestingly, only the people, who had plasmid-harboring strain had became ill. This was a result of a specific R plasmid found on the strain, and it had given ampicillin, carbencillin, and tetracycline resistance to the strain investigated (Holmberg et al., 1984).

As a second case, plasmid profiling of *Salmonella* serovar Enteritidis isolates obtained from poultry during 1989 to 1990 in Canada had shown that plasmid profiling has a better discrimination power compared to phagetyping (Dorn et al., 1992). In another study, 105 strains of *S.* serovar Enteritidis, in which most of them were human-related, were studied and seven plasmid profilies were obtained and most of the plasmids had a size about 36 MDa (Fernandes et al., 2003). And lastly, in Ankara, Turkey, 64 *Salmonella* serovar Enteritidis isolates were studied from a laboratory collection of University of Medical Science in Ankara. 88% of them had from 1 to 4 plasmids and the size of the plasmid changed from 2.5 to 100 kb. It was noteworthy to observe most of strains having plasmid 57 kb in size (Tekeli et al., 2006).

Heretofore, 83 *Salmonella* isolates (1 Corvallis, 3 Enteritidis, 2 Hadar, 54 Infantis, 5 Kentucky, 3 Othmarschen, 6 Paratyphi B, 1 Salford, 2 Typhi, 6 Typhimurium) were examined for plasmid analysis and 13 of them (2 Enteritidis, 2 Hadar, 3 Infantis, 1 Kentucky, 1 Othmarschen, 1 Paratyphi B, 3 Typhimurium) had shown positive results. In Figure 15, 3 Infantis, 1 Hadar and 3 Typhimurium plasmids were shown.

Except the plasmids found in Typhimurium (≈100 kb), the plasmid sizes were all different. *Salmonella* serovars Hadar (MET S1-163 and MET S1-703) had been

determined to have more than 6 plasmids (Table 36) whereas all Infantis serovar harbored only 1 plasmid. Although there have been many MDR Infantis isolates, only 3 of them had plasmid by that time and interestingly all of the three plasmid sizes were quite different from each other (≈40, 45 and 47 kb).

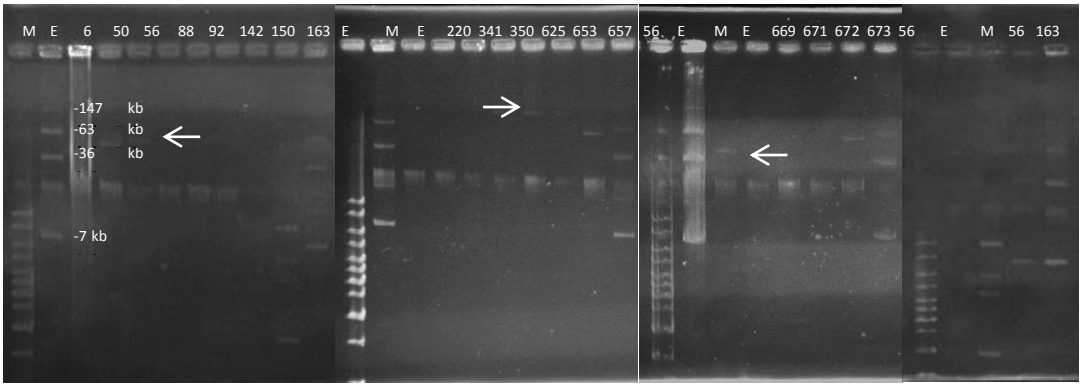


Figure 15 Gel photographs for plasmid profiling (M) Gene ruler 1kb marker, (E) E.coli 39R861 with 7, 36, 63, 147 kb bands

In a study conducted in Japan, researchers investigated cephalosporin resistance in plasmids of 10 Infantis serovars obtained from poultry flocks, the size of the plasmids were 95 kb with *aphA1, aadA1, tetA, sul1* antimicrobial resistance genotype and 140 kb with *blaCTX-M-14, aphA1, aadA1, tetA, sul1* genotype (Kameyama et al. 2012). And in Colombia and Argentina, 2.7 kb plasmids were found in Infantis isolates which were related with quinolone resistance (Karczmarczyk et al., 2010). And a recent study, that is performed in Turkey with 42 clinical non-related *Salmonella* isolates (Enteritidis, n = 23; Infantis, n = 14; Munchen, n = 2; Typhi, n = 3), only four of them (9.3%) had plasmid. 1 of the plasmid belonged to the *S.* Enteritidis serotype, one belonged to *S.* serovar Munchen, and two were from *S.* serovar Typhi isolates. None

of the Infantis (n=14) were found to have plasmid. Isolates carrying plasmid had 1–4 plasmids whose size ranged between 5.0 and 150 kb.

According to the plasmid profiles, it was visualized that AR was not always related with plasmids. Antimicrobial susceptible isolates such as *S.* serovar Enteritidis, Othmarschen; were found to have plasmids. Although, 2 other human-related *S.* serovar Othmarschen were not having plasmids, the food-related one was found to have multiple plasmids. But, on the other hand, it was interesting to observe two isolates from different sources (food and animal), harboring similar AR profile and also similar plasmid profile (Table 34, *S.* serovar Hadar). *S.* serovar Hadar, is also an emerging foodborne serovar in Europe since 1995s. For instance, in 1996, 9 *S.* serovar Hadar isolated were reported to the Spanish National Reference Laboratory, and 6 of them were related with poultry. Also, in 1998, five *S.* serovar Hadar outbreaks were from a cream-cake. The plasmid profiling of these isolates had resulted in plasmids from 1.3 kb to 66 kb in size, with all having multiple plasmids like the ones we observed in our isolates (Valdezate, Echeita et al. 2000).

The MDR *S.* serovar Typhimurium isolates were positive in terms of plasmid presence, and the human-related one had shown a different plasmid profile with multiple plasmid sizes. The phenomenon of having different plasmid profiles with different sizes of plasmids for this serovar, Typhimurium, is also common in literature (Li, Liao et al. 2013, Hooton, Timms et al. 2014, Wong, Yan et al. 2014).

Table 34 Plasmid profile of genetically antimicrobial resistant *Salmonella* isolates

MET ID Code	Serovar	Source	Phenotypic AR	Genotypic AR	Plasmid profile
MET-S1-221	Enteritidis	Human	Susceptible	ND	5-5.5-20-25 kb
MET-S1-660	Enteritidis	Animal	Susceptible	ND	55 kb
MET S1-163	Hadar	Food	S-T-Amp-Kf-N	*strB tetA* bla_{TEM-1}	4.5-5-7-8-20-22-30-55 kb
MET S1-703	Hadar	Animal	S-T-Amp-Amc-Fox-Kf-Ert-N	*strB tetA* bla_{TEM-1}	4.5-5-7-20-22-30-55 kb
MET S1-050	Infantis	Food	K-S-T-Amp-Sf-N	*aadA1 aphA1-iab tet*A bla_{TEM-1} *sul1*	45 kb
MET S1-056	Infantis	Food	K-S-T-Amp-Kf-Sf-Sxt-C-N	*aadA1 aphA1-iab tet*A bla_{TEM-1} *sul1 cmlA*	47 kb
MET S1-669	Infantis	Food	S-Amp-Kf-N	*aadA1* bla_{TEM-1} *sul1*	40 kb
MET S1-542	Kentucky	Animal	Sf	ND	90 kb
MET S1-87	Othmarschen	Food	Susceptible	ND	30-50-95-97 kb
MET S1-197	Paratyphi B	Human	Fox-Sf	bla_{TEM-1}	2.5-3-6.5-100 kb
MET S1-204	Typhimurium	Human	K-S-Sf-Sxt-C	ND	3-4-7-23-30-35-50-70-105 kb
MET S1-653	Typhimurium	Animal	Ak-S-T-Amp-Kf-N	*strB tetA* bla_{TEM-1}	95 kb
MET S1-657	Typhimurium	Animal	S-T-Amp-Amc-Sf-C-N	*aadA2 strB* $bla_{PS13E-1}$ *sul1*	97 kb

ND: Not detected

3.11. Association of antimicrobial resistance genes with chromosome or plasmid

Most common antimicrobial resistance genes (*aadA1, tetA, bla$_{TEM1}$, aphA$_{1\text{-}iab}$, sul1)* were identified whether they are plasmid-mediated or chromosome-associated.

Firstly, three *Salmonella* serovar Infantis isolates (MET S1-50, MET S1-56, and MET S1-669) and 1 Hadar isolate (MET S1-163) that have been to harbor plasmids were examined for the presence of antimicrobial resistance gene. *bla$_{TEM1}$* gene was searched in these isolates and all of the plasmids were found to have *bla$_{TEM1}$* resistance gene (Figure 14). It was interesting to note all the *S.* serovar Infantis isolates that have *bla$_{TEM1}$* gene, had one plasmid around 50 kb in size and the previous studies identifying *bla$_{TEM1}$* gene also agrees with our findings (Soto, González-Hevia et al. 2003, Huang, Dai et al. 2009, Dionisi, Lucarelli et al. 2011)

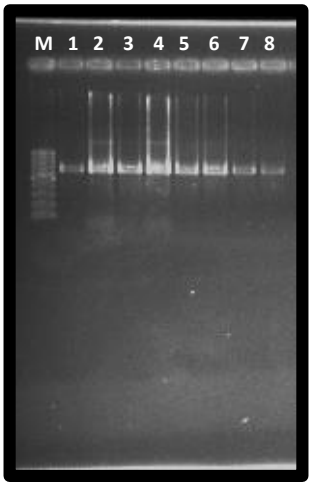


Figure 16 Gel photograph for *blaTEM1* presence in (1) MET S1-50 plasmid, (2) MET S1-50 chromosome, (3) MET S1-56 plasmid, (4) MET S1-56 chromosome, (5) MET S1-163 plasmid, (6) MET S1-163 chromosome, (7) MET S1-669 plasmid, (8) MET S1-669 chromosome and (M) Gene ruler, 100 bp (from 1000 bp to 100 bp) as a marker

Although *bla*$_{TEM-1}$ harboring plasmids were detected on PFGE and conventional gel electrophoresis, some probably smaller plasmids, which contain *aadA1* and *sul1* genes could not be visualized, which may be due low number of plasmids. Also since genomic DNA contamination during plasmid isolation may cause inaccurate results, this may have been the reason for not observing any plasmid by PFGE or gel electrophoresis for those genes (Figure 17-18).

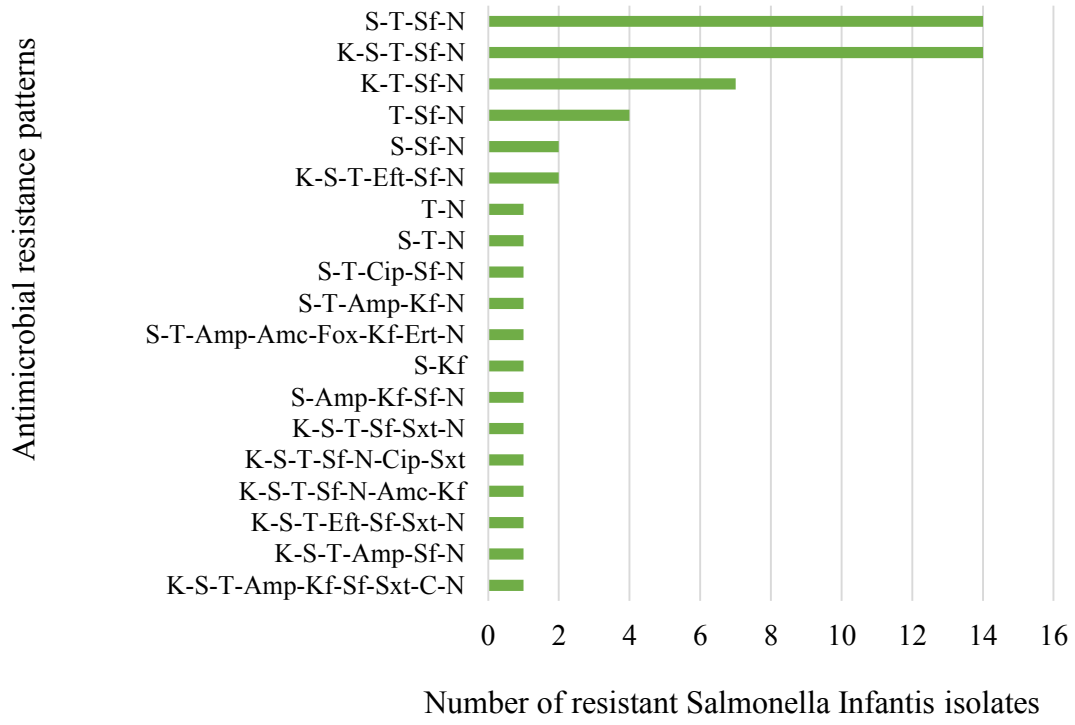


Figure 17 The distribution of phenotypic antimicrobial resistance patterns of 50 *Salmonella* Infantis isolates

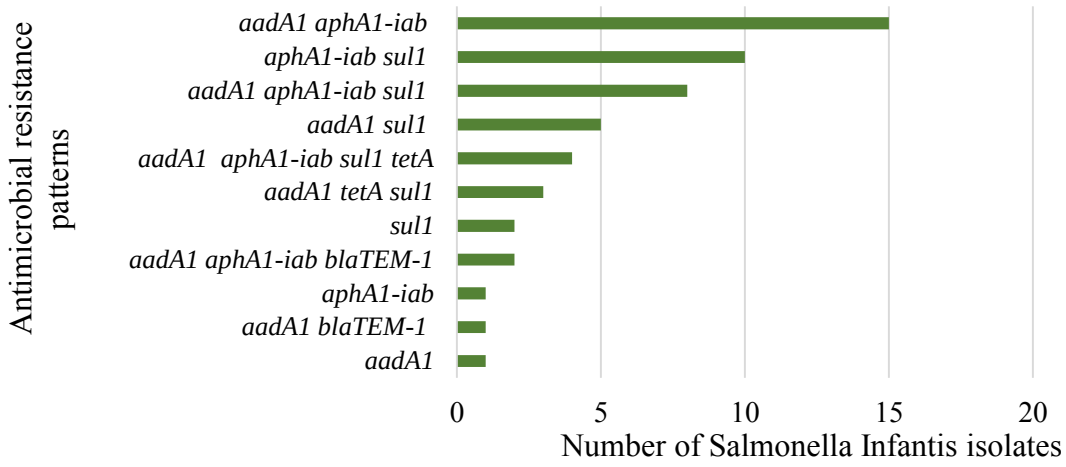


Figure 18 The distribution of genetic antimicrobial resistance patterns of 50 *Salmonella* Infantis plasmids

At the end, *aphA-1iab* and *bla*$_{TEM-1}$ genes were found to be 100 % plasmid-mediated (Table 35), whereas the other common AR genes could be found on chromosome and plasmid depending on the serovar. For instance 71 % of *aadA1* genes were plasmid-mediated, but 85 % of *tetA* genes were chromosome-mediated.

Table 35 AR genes found after plasmid isolation of *Salmonella* isolates

MET ID Code	Serovar	Source	Phenotypic AR profile	AR genes found on whole genome	AR genes found on plasmids
MET S1-163	Hadar	Food	S-T-Amp-Kf-N	*strB tetA* bla_{TEM-1}	bla_{TEM1}
MET S1-703	Hadar	Animal	S-T-Amp-Amc-Fox-Kf-Ert-N	*strB tetA* bla_{TEM-1}	*tetA sul1*
MET S1-050	Infantis	Food	K-S-T-Amp-Sf-N	*aadA1 aphA1-iab tetA* bla_{TEM-1}*sul1*	*aadA1 aphA1-iab* $bla_{\text{TEM-1}}$
MET S1-056	Infantis	Food	K-S-T-Amp-Kf-Sf-Sxt-C-N	*aadA1 aphA1-iab tetA* bla_{TEM-1} *sul1 cmlA*	*aadA1 aphA1-iab* $bla_{\text{TEM-1}}$
MET S1-088	Infantis	Food	K-S-T-Sf-N	*aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-092	Infantis	Food	S-T-Sf-N	*aadA1 tetA sul1*	*aadA1*
MET S1-103	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-142	Infantis	Food	S-T-Sf-N	*aadA1 strA aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-150	Infantis	Food	S-T-Sf-N	*aadA1 tetA sul1*	*aphA1-iab aadA1*
MET S1-329	Infantis	Food	S-T-Sf-N	*aadA1 strA tetA sul1*	*aphA1-iab aadA1*
MET S1-345	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-492	Infantis	Food	S-T-N	*aadA1 tetA*	*aphA1-iab aadA1*
MET S1-498	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-510	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-597	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-606	Infantis	Food	S-T-Sf-N	*aadA1 tetA sul1*	*aadA1*
MET S1-668	Infantis	Food	S-Sf-N	*aadA1 sul1*	*sul1 aadA1*

Table 35 Continued

MET ID Code	Serovar	Source	Phenotypic AR profile	AR genes found on whole genome	AR genes found on plasmids
MET S1-669	Infantis	Food	S-Amp-Kf-N	*aadA1* bla_{TEM-1} *sul1*	*aadA1 blaTEM-1*
MET S1-671	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-672	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-673	Infantis	Food	T-N	*tetA*	*sul1 aphA1-iab aadA1*
MET S1-674	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab tetA sul1*	*aphA1-iab aadA1*
MET S1-676	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab sul1*	*sul1 aphA1-iab aadA1*
MET S1-677	Infantis	Food	K-S-T-Sf-Sxt-Cip	*aadA1 aphA1-iab sul1*	Negative
MET S1-678	Infantis	Food	K-S-T-Sf-N	*aadA1 tetA aphA1-iab sul1*	*sul1 aphA1-iab*
MET S1-679	Infantis	Food	T-Sf-N	*aadA1 tetA sul1*	*sul1 aphA1-iab*
MET S1-680	Infantis	Food	K-S-T-Amc-Kf-Sf-N	*aadA1 aphA1-iab sul1*	*aphA1-iab*
MET S1-682	Infantis	Food	K-S-T-Sf-Sxt-N	*aadA1 aphA1-iab sul1*	*sul1 aphA1-iab*
MET S1-683	Infantis	Food	T-Sf-N	*aadA1 tetA sul1*	*aphA1-iab aadA1 sul1*
MET S1-684	Infantis	Food	K-S-T-Sf-Eft-N	*aadA1 tetA aphA1-iab sul1*	*aphA1-iab sul1*
MET S1-685	Infantis	Food	S-T-Sf-N	*aadA1 sul1*	*aadA1 tetA sul1*
MET S1-686	Infantis	Food	K-S-T-Sf-N	*aadA1 tetA aphA1-iab sul1*	*aadA1 aphA1-iab sul1*
MET S1-687	Infantis	Food	K-T-Sf-N	*aadA1 tetA aphA1-iab sul1*	*aphA1-iab sul1*
MET S1-688	Infantis	Food	T-Sf-N	*tetA sul1*	*aadA1 sul1*
MET S1-689	Infantis	Food	T-Sf-N	*aadA1 sul1 tetA*	*aadA1 tetA*

Table 35 Continued

MET ID Code	Serovar	Source	Phenotypic AR profile	AR genes found on whole genome	AR genes found on plasmids
MET S1-690	Infantis	Food	S-Sf-N	*aadA1 sul1*	*aadA1 sul1*
MET S1-691	Infantis	Food	K-S-T-Eft-Sf-Sxt-N	*tetA aadA1 aphA1-iab sul1*	*aadA1 aphA1-iab sul1*
MET S1-692	Infantis	Food	K-S-T-Sf-N	*aadA1 aphA1-iab sul1*	*aadA1 tetA aphA1-iab sul1 tetA*
MET S1-693	Infantis	Food	K-S-T-Sf-Eft-N	*tetA aadA1 aphA1-iab sul1*	*aadA1 tetA aphA1-iab sul1 tetA*
MET S1-694	Infantis	Food	K-S-T-Sf-N	*tetA aadA1 aphA1-iab*	*aadA1 aphA1-iab sul1*
MET S1-695	Infantis	Food	S-T-Sf-N	*aadA1*	*aadA1 sul1*
MET S1-696	Infantis	Food	T-Sf-N	*tetA aadA1 aphA1-iab sul1*	*aadA1 tetA aphA1-iab sul1*
MET S1-697	Infantis	Food	S-Kf	*tetA aadA1 sul1*	*aadA1 tetA sul1*
MET S1-698	Infantis	Food	S-T-Cip-Sf-N	*tetA aadA1 sul1*	*aadA1 tetA sul1*
MET S1-699	Infantis	Food	S-T-Sf-N	*aadA1 sul1*	*sul1 aadA1*
MET S1-700	Infantis	Food	K-S-T-Sf-N	*tetA aadA1 aphA1-iab sul1*	*aphA1-iab sul1 aadA1*
MET S1-701	Infantis	Food	K-T-Sf-N	*aadA1 aphA1-iab sul1*	*aphA1-iab sul1*
MET-S1-737	Infantis	Food	K-T-Sf-N	*aadA1 aphA1-iab sul1*	*aphA1-iab sul1 aadA1*
MET-S1-738	Infantis	Food	S-T-Sf-N	*aadA1 sul1*	*aphA1-iab sul1 tetA*
MET-S1-739	Infantis	Food	S-T-Sf-N	*sul1*	*aphA1-iab sul1*
MET-S1-741	Infantis	Food	S-T-Sf-N	*aadA1 sul1*	*sul1*

Table 35 Continued

MET ID Code	Serovar	Source	Phenotypic AR profile	AR genes found on whole genome	AR genes found on plasmids
MET-S1-745	Infantis	Food	S-T-Sf-N	*aadA1 sul1*	*sul1*
MET-S1-746	Infantis	Food	K-T-Sf-N	*aadA1 aphA1-iab sul1*	*aphA1-iab sul1*
MET-S1-747	Infantis	Food	K-T-Sf-N	*aphA1-iab sul1*	*aphA1-iab sul1*
MET-S1-749	Infantis	Food	K-T-Sf-N	*aadA1 aphA1-iab sul1*	*aphA1-iab sul1*

3.12. Class-1 integrons of *Salmonella* isolates

Class 1 integrons are the most frequently found integrons that are considered to be the major contributors to multidrug resistance in Gram-negative bacteria (Fluit and Schmitz 2004). The integrons contain two conserved segments (5'CS and 3'CS) divided by a variable region that usually holds one or more gene cassettes. The 5'CS contains the integrase gene (*intI1*). The 3'CS generally has of *qacEΔ1*, and *sul1* that encodes sulfonamide resistance. The gene cassettes found in the variable regions are mobile and normally encode for antibiotic resistance. *qacEΔ1* is known to function as a multidrug transporter (Kazama, Hamashima et al. 1999, Chuanchuen, Khemtong et al. 2007) and since it is found on a conserved location on 3' region of class 1 integrons, it is broadly spread among Gram-negative bacteria (Paulsen, Littlejohn et al. 1993).

In our isolates, nearly half of the *S.* serovar Infantis (52.4 %) isolates had presented Class-1 integron related with integrase gene (Table 36). And three food-originated serovars Hadar, Salford and Corvallis, one animal-origin serovar Kentucky, and Enteritidis, and lastly one human-origin Typhimurium isolates were also found to

comprise Class-1 integron integrase gene. Remarkably, the two integrons that were from isolates obtained from animal sources, had a 200 bp Class-1 integrons, while the other isolates had 1 kb or larger integrons.

The size of the class 1 integrons of *S.* serovar Infantis isolates was all the same, nearly 1 kb. The size of the class 1 integrons of the same serovar isolates were also nearly same, 1.8 kb in an Ireland study, where the isolates were gathered from pigs (O'Mahony, Saugy et al. 2005).

qacEΔ1 gene was detected only at *S.* serovar Infantis isolates, 76.2 % of them had this antimicrobial resistance transporter gene. *qacEΔ1* gene is mostly associated with *S.* serovar Typhimurium DT 104 (Guerra, Junker et al. 2004), but can also be found on *S.* serovar Infantis (O'Mahony, Saugy et al. 2005).

At antimicrobial resistance gene screening, *sul1* gene was found to be very frequent on *S.* serovar Infantis isolates, but here, we did not found *sul1* gene often (42.9 %). On the other hand, it was important to observe *sul1* gene on class 1 integrons containing isolates, which do not have sulfonamide resistance gene on their plasmids.

The presence of class 1 integrons in *Salmonella* spp. in foods, animal or clinical human samples is very important when these zoonotic pathogens share their antimicrobial resistance profiles and have also virulence characteristics, which may result in severe outbreaks.

Table 36 Class-1 integrons of *Salmonella* isolates in our study

METU ID Code	Serovar	Source	Class 1 integron genes		
			5CS-3CS int1 (product size)	***sul1***	***qacEΔ1***
MET S1-024	Corvallis	Food	+ (1 kb)	-	-
MET-S1-217	Enteritidis	Human	-	-	-
MET-S1-221	Enteritidis	Human	-	-	-
MET-S1-660	Enteritidis	Animal	+ (200 bp)	-	-
MET S1-163	Hadar	Food	+ (>1 kb)	-	-
MET S1-050	Infantis	Food	-	-	-
MET S1-056	Infantis	Food	-	-	-
MET S1-088	Infantis	Food	-	-	+
MET S1-092	Infantis	Food	+ (1 kb)	-	+
MET S1-103	Infantis	Food	-	-	-
MET S1-142	Infantis	Food	+ (1 kb)	+	+
MET S1-150	Infantis	Food	+ (1 Kb)	-	+
MET S1-329	Infantis	Food	+ (1 kb)	-	+
MET S1-345	Infantis	Food	-	-	-
MET S1-351	Infantis	Food	-	-	-
MET S1-492	Infantis	Food	-	+	+
MET S1-498	Infantis	Food	-	+	+
MET S1-510	Infantis	Food	-	+	+
MET S1-597	Infantis	Food	+ (1 kb)	+	+
MET S1-606	Infantis	Food	+ (1 kb)	+	+
MET S1-668	Infantis	Food	-	-	+
MET S1-669	Infantis	Food	+ (1 kb)	+	+
MET S1-671	Infantis	Food	+ (1 kb)	+	+
MET S1-672	Infantis	Food	+ (1 kb)	-	+
MET S1-673	Infantis	Food	+ (1 kb)	-	+
MET S1-674	Infantis	Food	+ (1 kb)	+	+
MET S1-219	Kentucky	Human	-	-	-
MET S1-228	Kentucky	Human	-	-	-
MET S1-313	Kentucky	Food	-	-	-
MET S1-405	Kentucky	Animal	+ (200 bp)	-	-
MET S1-542	Kentucky	Animal	-	-	-

Table 36 Continued

METU ID Code	Serovar	Source	Class 1 integron genes		
			5CS-3CS int1 (product size)	***sul1***	***qacEΔ1***
MET S1-227	Othmarschen	Human	-	-	-
MET S1-237	Othmarschen	Human	-	-	-
MET S1-87	Othmarschen	Food	-	-	-
MET S1-195	Paratyphi B	Human	-	-	-
MET S1-197	Paratyphi B	Human	-	-	-
MET S1-198	Paratyphi B	Human	-	-	-
MET S1-201	Paratyphi B	Human	-	-	-
MET S1-205	Paratyphi B	Human	-	-	-
MET S1-218	Paratyphi B	Human	-	-	-
MET S1-031	Salford	Food	+ (1 kb)	+	-
MET S1-220	Typhi	Human	-	-	-
MET S1-234	Typhi	Human	-	-	-
MET S1-204	Typhimurium	Human	+ (1 kb)	-	-
MET S1-211	Typhimurium	Human	-	-	-
MET S1-625	Typhimurium	Food	-	-	-
MET S1-653	Typhimurium	Animal	-	-	-
MET S1-657	Typhimurium	Animal	-	-	-
MET S1-663	Typhimurium	Animal	-	-	-

3.13. Virulence characteristics of *Salmonella* isolates

Here, the virulence of the *Salmonella* isolates that were important, in terms of antimicrobial resistance profiles, and being presence in all types of sources, were studied. Our data demonstrated a common core of virulence genes specific to serovar and source of the isolates, and these virulence characteristics might be required for invasive salmonellosis (Table 37). Typhoid *Salmonella* isolates that were all from human sources had shown significantly different virulence gene profiles. 7 virulence-associated genes (i.e. *ctdB, gatC, hlyE, pefA, sseI, sopE and tcfA)* were all observed in *S.* serovar Typhi isolates.

On the other hand, interestingly, food-related *Salmonella* isolates were also found to have chromosome-associated virulence genes *gatC* and *tcfA* in *S.* serovar Infantis and plasmid-associated virulence gene *pefA* in *S.* serovar Hadar. The results demonstrated that virulence characteristics of *Salmonella* isolates were not specific to only human.

Gifsy-1 and Gifsy-3 associated virulence genes (*gogB* and *sspH*) were not detected in our isolates but Gifsy-2 associated *sseI* gene was found on human-origin *S.* serovar Enteritidis, Paratyphi B, Typhi, and Typhimurium; and also on animal-origin *S.* serovar Typhimurium and remarkably on food-origin *S.* serovar Salford. It is well-known that the *sseI* gene is related with typhoid or human-related virulence characteristics (Huehn, et al., 2010), thus it was interesting to detect the gene on animal and also food-related isolates, probably due to its mobility due to being on bacteriophages.

The chromosome-associated, *sodC* gene, was only detected on human-origin *S.* serovar Enteritidis, Typhimurium and animal-origin *S.* serovar Typhimurium again. Virulent *S.* serovar Typhimurium was previously found to have periplasmic Cu-Zn superoxide dismutase gene (Fang, et al., 1999), *sodC;* thus it can be concluded that there was an agreement between with our isolates and literature.

76.2 % of *S.* serovar Infantis isolates had harbored *tcfA* gene and also the gene was detected on the serovars; Corvallis, Typhi and Typhimurium. It was noteworthy to observe this chromosome-associated, fimbriae-related gene on many Infantis isolates. But Huehn and his colleagues had also found that 11 Infantis isolates, which were isolated from poultry and human sources, had 100 % of *tcfA* gene (Huehn, et al., 2010).

gatC gene was observed nearly at all (68 %) isolates from human-origin to food-origin. A little is known about the galactitol transporter gene in literature but it was interesting to notice the gene in all *S.* serovar Infantis isolates.

Cytolethal distending toxin gene, *ctdB,* which is found on chromosome, was identified in *S.* serovar Typhi (n=2) and also in 1 food-origin *S.* serovar Infantis and 1 *S.* serovar Kentucky isolates. Up to now, according to literature search, *cdtB* gene was not detected in any isolate obtained from food sources. This toxin can cause a variety of mammalian cells to become irreversibly blocked in the pre-mitotic phase of the cell cycle (Pickett and Whitehouse 1999). In addition, a common virulent associated hemolysin gene, *hlyE,* was also detected on the same isolates (MET S1-92/Infantis, MET S1-313/Kentucky) together with typhoid isolates. Thus, our findings has shown that there is a high possibility of these two food-originated *Salmonella* isolates may cause severe illness if they are transmitted to humans.

Table 37 Virulence characteristics of *Salmonella* isolates found by Real-time PCR (Ct value <25)

MET ID Code	Serovar	Source	Virulence genes											
			ctdB	***gatC***	***gogB***	***hlyE***	***pefA***	***ssek3***	***sseI***	***sspH***	***sodC***	***sopE***	***STM 2759***	***tcfA***
MET S1-024	Corvallis	Food	-	+ (13.5)	-	-	-	-	-	-	-	-	-	+ (13.3)
MET-S1-217	Enteritidis	Human	-	+ (21.9)	-	-	-	-	+ (14.0)	-	+ (14.1)	-	-	-
MET-S1-221	Enteritidis	Human	-	+ (15.8)	-	-	-	-	+ (14.3)	-	+ (17.6)	-	-	-
MET-S1-660	Enteritidis	Animal	-	+ (13.0)	-	-	-	-	+ (13.3)	-	+ (13.0)	-	-	-
MET S1-163	Hadar	Food	-	-	-	-	+ (17.1)	-	-	-	-	-	-	-
MET S1-050	Infantis	Food	-	-	-	-	-	-	-	-	-	-	-	-
MET S1-056	Infantis	Food	-	+ (21.2)	-	-	-	-	-	-	-	-	-	-
MET S1-088	Infantis	Food	-	+ (14.0)	-	-	-	-	-	-	-	-	-	+ (15.0)
MET S1-092	Infantis	Food	+ (24.4)	+ (14.0)	-	+ (25.3)	-	-	-	-	-	-	-	+ (14.3)
MET S1-103	Infantis	Food	-	+ (17.5)	-	-	-	-	-	-	-	-	-	-
MET S1-142	Infantis	Food	-	+ (14.0)	-	-	-	-	-	-	-	-	-	+ (15.5)
MET S1-150	Infantis	Food	-	+ (14.4)	-	-	-	-	-	-	-	-	-	+ (17.6)
MET S1-329	Infantis	Food	-	+ (14.3)	-	-	-	-	-	-	-	-	-	+ (17.3)
MET S1-345	Infantis	Food	-	+ (14.0)	-	-	-	-	-	-	-	-	-	+ (21.2)
MET S1-351	Infantis	Food	-	+ (22.6)	-	-	-	-	-	-	-	-	-	-
MET S1-492	Infantis	Food	-	+ (14.5)	-	-	-	-	-	-	-	-	-	+ (18.3)
MET S1-498	Infantis	Food	-	+ (13.7)	-	-	-	-	-	-	-	-	-	+ (14.0)
MET S1-510	Infantis	Food	-	+ (13.7)	-	-	-	-	-	-	-	-	-	+ (15.0)
MET S1-597	Infantis	Food	-	+ (13.5)	-	-	-	-	-	-	-	-	-	+ (14.5)
MET S1-606	Infantis	Food	-	+ (13.3)	-	-	-	-	-	-	-	-	-	+ (14.0)
MET S1-668	Infantis	Food	-	+ (14.0)	-	-	-	-	-	-	-	-	-	+ (15.6)
MET S1-669	Infantis	Food	-	+ (13.5)	-	-	-	-	-	-	-	-	-	+ (14.5)
MET S1-671	Infantis	Food	-	+ (13.2)	-	-	-	-	-	-	-	-	-	-

- : Not detected
+ : Positive

Table 37 Continued

MET ID Code	Serovar	Source	Virulence genes											
			ctdB	*gatC*	*gogB*	*hlyE*	*pefA*	*ssek3*	*sseI*	*sspH*	*sodC*	*sopE*	*STM 2759*	*tcfA*
MET S1-672	Infantis	Food	-	+ (14.3)	-	-	-	-	-	-	-	-	-	+ (14.4)
MET S1-673	Infantis	Food	-	+ (14.0)	-	-	-	-	-	-	-	-	-	+ (15.1)
MET S1-674	Infantis	Food	-	+ (13.3)	-	-	-	-	-	-	-	-	-	+ (15.6)
MET S1-219	Kentucky	Human	-	+ (14.2)	-	-	-	-	-	-	-	-	-	-
MET S1-228	Kentucky	Human	-	-	-	-	-	-	-	-	-	-	-	-
MET S1-313	Kentucky	Food	+ (24.1)	+ (13.3)	-	+ (26.5)	-	-	-	-	-	-	-	+ (13.2)
MET S1-405	Kentucky	Animal	-	+ (13.5)	-	-	-	-	-	-	-	-	-	+ (13.4)
MET S1-542	Kentucky	Animal	-	+ (13.7)	-	-	-	-	-	-	-	-	-	-
MET S1-227	Othmarschen	Human	-	-	+ (25.4)	-	-	-	-	-	-	-	-	-
MET S1-87	Othmarschen	Food	-	-	-	-	-	-	-	-	-	-	-	+ (15.0)
MET S1-195	Paratyphi B	Human	-	-	-	-	-	-	+ (26.3)	-	-	-	-	-
MET S1-197	Paratyphi B	Human	-	-	-	-	-	-	-	-	-	-	-	-
MET S1-198	Paratyphi B	Human	-	-	-	-	-	-	-	-	-	-	-	-
MET S1-201	Paratyphi B	Human	-	+ (13.5)	-	-	-	-	+ (23.7)	-	-	+ (28.2)	-	-
MET S1-205	Paratyphi B	Human	-	+ (12.9)	-	-	-	-	+ (13.7)	-	-	+ (27.1)	-	-
MET S1-218	Paratyphi B	Human	-	-	-	-	-	-	+ (25.3)	-	-	-	-	-
MET S1-031	Salford	Food	-	+ (13.0)	-	-	-	-	+ (14.0)	-	-	-	-	+ (13.0)
MET S1-220	Typhi	Human	+ (13.3)	+ (13.0)	-	+ (8.4)	+ (27.0)	-	+ (22.2)	-	-	+ (13.4)	-	+ (13.4)
MET S1-234	Typhi	Human	+ (14.1)	+ (13.5)	-	+ (11.0)	+ (27.1)	-	+ (23.6)	-	-	+ (13.6)	-	+ (14.1)
MET S1-204	Typhimurium	Human	-	+ (13.5)	-	-	+ (13.6)	-	+ (13.7)	-	+ (12.8)	-	+ (13.7)	-
MET S1-211	Typhimurium	Human	-	+ (16.1)	-	-	+ (21.4)	-	+ (13.9)	-	+ (15.8)	-	-	-
MET S1-625	Typhimurium	Food	-	-	-	-	+ (17.1)	-	-	-	-	-	-	-
MET S1-653	Typhimurium	Animal	-	+ (14.9)	-	-	-	-	+ (14.1)	-	+ (21.6)	-	-	-
MET S1-657	Typhimurium	Animal	-	+ (15.3)	-	-	-	-	+ (15.0)	-	+ (21.7)	-	-	-
MET S1-663	Typhimurium	Animal	-	+ (17.3)	-	-	+ (21.0)	-	+ (16.8)	-	+ (15.6)	-	-	-

CHAPTER 4

CONCLUSION

Characterization of *Salmonella* isolates collected from animal and human, as well as foods in Sanliurfa region provided better understanding of transmission (i.e. transmission of *Salmonella* to humans) and ecology of *Salmonella* in that region.

From our knowledge, this study is the first study in Turkey that analyzes the phenotypic features of *Salmonella* isolates, as well as genetic subtypes through farm to fork chain. Antimicrobial resistance had differed according to source of isolate; such as aminoglycoside resistance was predominant in food isolates, however beta-lactam resistance was higher in animal isolates.

Presence of resistance to high-risk Category I antimicrobials such as amoxicillin-clavulanic acid and ertapenem at animal isolates (*S.* serovar Montevideo, *S.* serovar Hadar and *S.* serovar Typhimurium, and *S.* serovar Chester), which were collected from cattle and sheep feces, has indicated the importance of the possibility of transmission of resistance to food and also to human; since the same serovars were also observed in their food products such as cow ground meat and sheep ground meat.

Occurrence of different AR gene profiles designated a potential association of isolates between source, serovar and geography. The reason of not observing a possible local serotypes in food samples, *S.* serovar Telaviv and persistent and MDR *S.* serovar Infantis, in human cases may be related to their low virulence capacities. Unlikely, a rare serovar, *S.* serovar Othmarschen, was collected from both food and human sources, but they had carried two different virulence genes; *tcfA* and *gogB*. And a MDR *S.* serovar Infantis and Kentucky were detected to have two important virulence genes; *ctdB* and *hlyE*. Presence

of such serovars, especially MDR ones, has potential to cause severe cases in humans in future, and it underlines the importance of food safety from "farm-to-fork chain".

Our work entitles the sequence subtypes possible endemic to Turkey and submits the diversity of *Salmonella* in this region by subtyping and antimicrobial susceptibility methods. By establishing a web-based databank (foodmicrobetracker.com; Pathogen Detector: pathogendetector-metu.rhcloud.com) it was ensured to build a permanent and solid *Salmonella* archive for the future studies in Turkey.

CHAPTER 5

RECOMMENDATIONS

Salmonella causes significant problem globally. Although there have been several limitations in this study, these data provide important information for the phenotypic and genetic characterization of *Salmonella* isolates from food to animal and to human in Turkey.

For further studies, the number of the isolates, especially for MDR *S.* serovar Infantis, could be increased and thus the reason of the resistance in those serovars can be identified by additional methods such as detection of other integrons, SGIs, and resistance genes.

Searching the mechanism behind the possible local serovar of Turkey, *S.* serovar Telaviv, could be interesting in future.

A unique serovar, *S.* serovar Othmarschen, was observed in food and clinical human sources; and it would be remarkable to analyze the similarities among different isolates by increasing their sample size.

The initial isolates, which were used to see the differences/similarities among food, animal and human sources in this study, were from Sanliurfa region. Getting samples from all over the regions of Turkey will bring out a better picture of the antimicrobial resistance characterization specific to our country.

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APPENDIX A

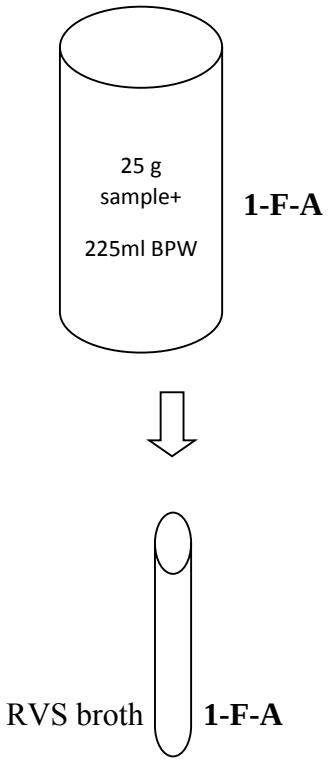
DOCUMENTATION SCHEME USED IN *SALMONELLA* ISOLATION

Seasons:

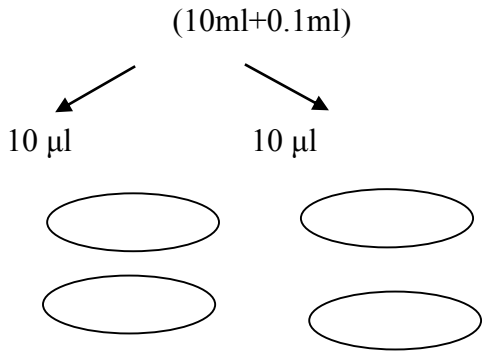
Season	Code
Spring	I
Summer	Y
Fall	S
Winter	K

Food Type:

Food Type	Code
Sheep Ground Meat	1
Cow Ground Meat	2
Chicken Meat	3
Offal	4
Unripened Cheese	5
Urfa Cheese	6
Pistachio	7
Isot	8



Location:



Location	Code
First Location	F
Second Location	S

BGA-1-F-A-a BGA-1-F-A-b
XLD-1-F-A-a XLD-1-F-A-b

Quality Categories:

Quality	Code
High	A
Medium	B
Low	C

Documentation Format:

I –

1-F-A 1-S-A

1-F-B 1-S-B

1-F-C 1-S-C (Total 48 samples per month)

APPENDIX B

MULTIDRUG RESISTANT *SALMONELLA* ISOLATES

Table 38 Multidrug resistance (MDR) profiles of the *Salmonella* isolates found in three different sources (Food, animal and clinical human)

Isolate code	Serotype	Source of isolate	Antimicrobial agents
MET-S1-579	Anatum	Food	K, S, SF
MET-S1-654	Anatum	Animal	AK, SF
MET-S1-163	Hadar	Food	S, N, AMP, T, KF
MET-S1-703	Hadar	Animal	S, N, AMP, AMC, T, FOX, KF, ETP
MET-S1-050	Infantis	Food	K, S, N, AMP, T, SF
MET-S1-056	Infantis	Food	K, S, N, AMP, T, SF, KF, SXT, C
MET-S1-088	Infantis	Food	K, S, N, T, SF
MET-S1-092	Infantis	Food	S, N, T, SF
MET-S1-142	Infantis	Food	S, N, T, SF
MET-S1-150	Infantis	Food	S, N, T, SF
MET-S1-329	Infantis	Food	S, N, T, SF
MET-S1-345	Infantis	Food	K, S, N, T, SF
MET-S1-351	Infantis	Food	S, N, T, SF
MET-S1-492	Infantis	Food	S, N, T
MET-S1-498	Infantis	Food	K, S, N, T, SF
MET-S1-510	Infantis	Food	K, S, N, T, SF
MET-S1-597	Infantis	Food	S, N, T, SF
MET-S1-606	Infantis	Food	S, N, T, SF
MET-S1-668	Infantis	Food	S, N, T SF
MET-S1-669	Infantis	Food	S, N, AMP, T, KF, SF

Table 38 Continued

Isolate code	Serotype	Source of isolate	Antimicrobial agents
MET-S1-671	Infantis	Food	K, S, N, T, SF
MET-S1-672	Infantis	Food	K, S, N, T, SF
MET-S1-673	Infantis	Food	N, T, SF
MET-S1-674	Infantis	Food	K, S, N, T, SF
MET-S1-542	Kentucky	Animal	K, S
MET-S1-706	Montevideo	Animal	T, KF
MET-S1-707	Montevideo	Animal	FOX, KF, ETP
MET-S1-708	Montevideo	Animal	FOX, KF
MET-S1-625	Newport	Food	AMP, T,
MET-S1-198	Paratyphi B	Human	FOX, KF, SF
MET-S1-204	Paratyphi B	Human	K, S, SF, SXT, C
MET-S1-211	Paratyphi B	Human	AMP, T
MET-S1-218	Paratyphi B	Human	AK, K, S, SF, SXT, C
MET-S1-235	Paratyphi B	Human	S, SF
MET-S1-704	Saintpaul	Animal	AMC, FOX, KF, ETP
MET-S1-030	Salford	Food	SF, SXT
MET-S1-223	Typhimurium	Human	AMP, T
MET-S1-653	Typhimurium	Animal	AK, S, N, AMP, T, KF
MET-S1-657	Typhimurium	Animal	S, N, AMP, AMC, T, SF, C
MET-S1-663	Typhimurium	Animal	AMP, T, KF
MET-S1-103	Virchow	Food	K, S, N, T, SF

*The isolates that have shown antimicrobial resistance to 2 or more than 2 antimicrobial agents are defined as MDR

.

APPENDIX C

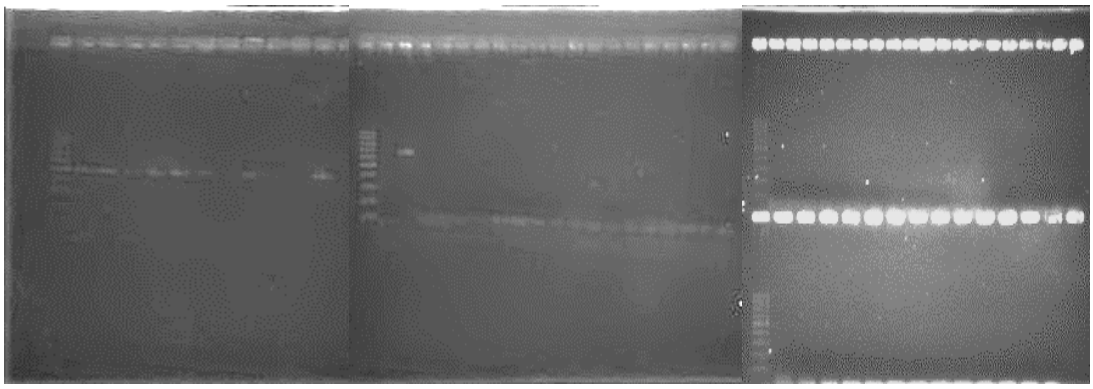
THE DISTRIBUTION OF ANTIMICROBIAL RESISTANCE AMONG *SALMONELLA* ISOLATES

Table 38 The distribution of resistant *Salmonella* isolates according to the source (food, animal and clinical human) and antimicrobial agents

Antimicrobial agent	**Number of food-origin resistant isolates**	**Number of animal-origin resistant isolates**	**Number of clinical human-origin resistant isolates**
Amikacin	0	2	1
Streptomycin	22	5	4
Kanamycin	11	1	2
Aminoglycosides	**33**	**8**	**7**
Nalidixic acid	20	3	2
Quinolones	**20**	**2**	**2**
Tetracycline	23	5	2
Tetracyclines	**23**	**5**	**2**
Cephalothin	1	9	1
Ceftriaxone	0	1	0
Ceftiofur	0	1	0
Ceftriaxone	0	5	2
Ampicillin	6	5	2
Amoxicillin-clavulanic acid	0	4	0
Ertapenem	0	4	0
Beta-lactams	**7**	**29**	**5**
Chloramphenicol	1	1	2
Phenicols	**1**	**1**	**2**
Sulfisoxazole	17	9	33
Trimethoprim-sulfamethoxazole	1	0	2
Sulfonamides and trimethoprims	**18**	**9**	**35**

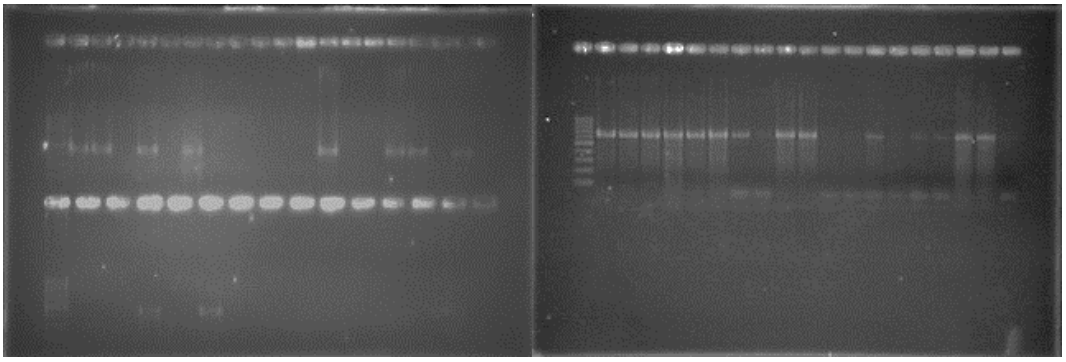
APPENDIX D

ANTIMICROBIAL GENOTYPING RESULTS VISUALIZED FROM GEL PHOTOGRAPHS



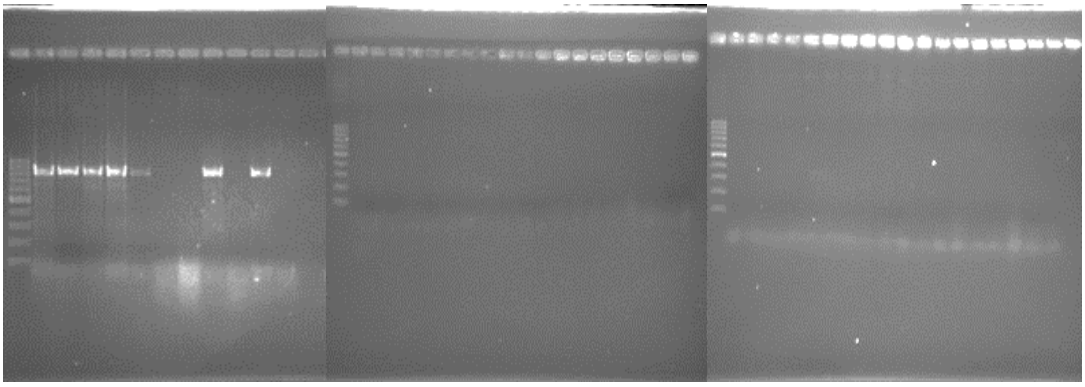
(a) (b) (c)

Figure 19 Gel photograph for (a) *aadA1* gene with MET S1-50 (+), MET S1-329 (+), MET S1-345 (+), MET S1-351 (+), MET S1-492 (+), MET S1-498 (+), MET S1-668 (+), MET S1-669 (+), MET S1-671 (+), MET S1-672 (-), MET S1-674 (+) in order.(b) *aadA2* gene with MET S1-655(-), MET S1-657(+), MET S1-668(-), MET S1-669(-), MET S1-671(-), MET S1-672(-), MET S1-674(-), MET S1-703(-), MET S1-674(-), negative control and (c) *aacC2* gene all isolates (-)



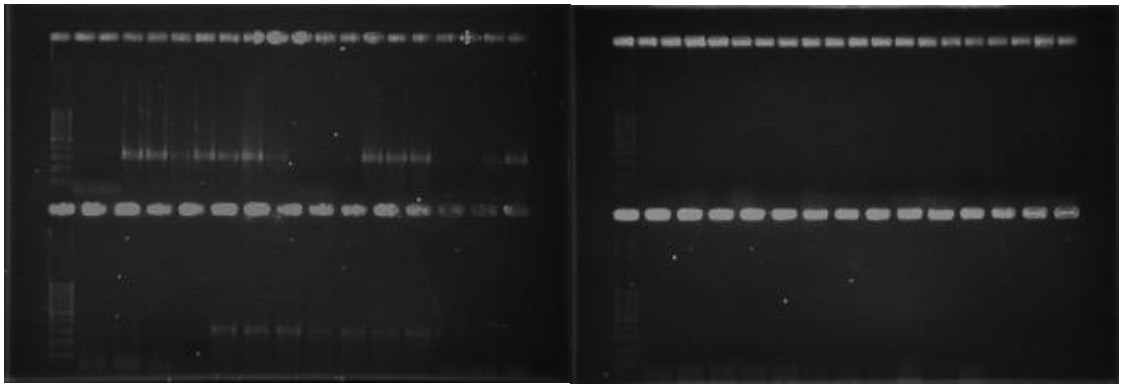
(a) (b)

Figure 20 Gel photograph for (a) *aphA-iab* gene with MET S1-579(+), MET S1-597(+), MET S1-542(-), MET S1-142(+), MET S1-150(-), MET S1-172(+), MET S1-421(-), MET S1-492(-), MET S1-498(-), MET S1-510(-), MET S1-512(-), MET S1-655(+), MET S1-517(-), MET S1-625(-), MET S1-195(+), MET S1-204(+), MET S1-218(-), MET S1-235(-), MET S1-671(+), negative control, MET S1-668(-), MET S1-669(-), MET S1-671(+),MET S1-397(-),MET S1-674(+), negative control in order and, (b) *blaTEM1* gene with MET S1-50(+), MET S1-56(+), MET S1-163(+), MET S1-625(+), MET S1-669(+), MET S1-653(+), MET S1-655(+), MET S1-657(-), MET S1-663(+), MET S1-703(+), MET S1-704(-), MET S1-706(-), MET S1-707(+), MET S1-707(-), MET S1-708(+), MET S1-197(+), MET S1-198(+), MET S1-211(+), MET S1-223(+) in order.



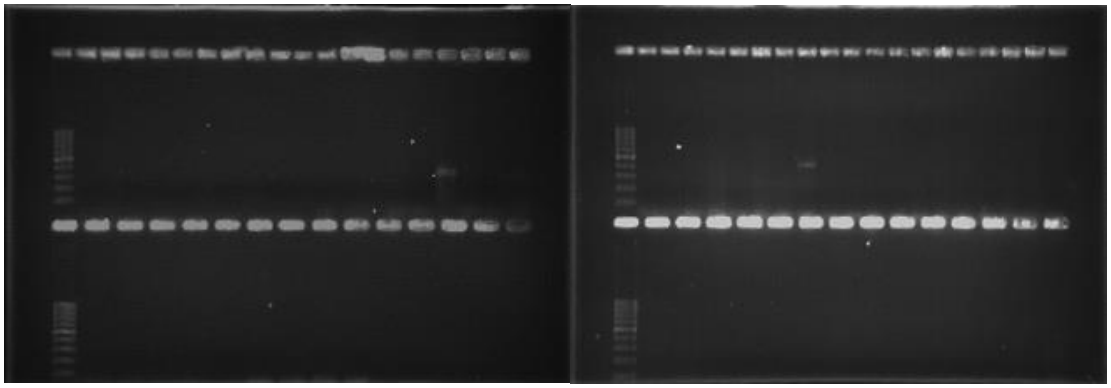
(a) (b) (c)

Figure 21 Gel photograph for (a) *tetA* gene with MET S1-671(+), MET S1-672(+), MET S1-673(+), MET S1-674(+), MET S1-653(+), MET S1-657(-), MET S1-663(-), MET S1-703(+), MET S1-706(-), MET S1-211(+), negative control in order; and (b) *tetB* gene all isolates (-), and (c) *tetG* gene all isolates (-)



(a) (b)

Figure 22 Gel photograph for (a) *sul1* gene with MET S1-30(-), MET S1-410(-), MET S1-50(+), MET S1-56(+), MET S1-88(+), MET S1-92(+), MET S1-103(+), MET S1-142(+), MET S1-150(+), MET S1-248(-),MET S1-258(-), MET S1-313(-), MET S1-329(+),MET S1-345(+), MET S1-351(+), MET S1-421(-), MET S1-439(-),MET S1-498(+), MET S1-510(+), MET S1-512(+), MET S1-517(+), MET S1-557(-), MET S1-579(-), MET S1-597(+), MET S1-606(+), MET S1-668(+), MET S1-669(+), MET S1-671(+), MET S1-672(+), MET S1-674(+), negative control in order, and (b) *sul2* gene all isolates (-)



(a) (b)

Figure 23 Gel photograph for (a) *cat1, cat2, flo* and *cmlA* genes with MET S1-56 (+) for *cmlA* gene and (b) bla_{PSE13} and bla_{CMY} genes with MET S1-657 (+) for bla_{PSE13} gene

APPENDIX E

PLASMID SIZE VISUALIZATION ON PFGE GEL PHOTOGRAPHS

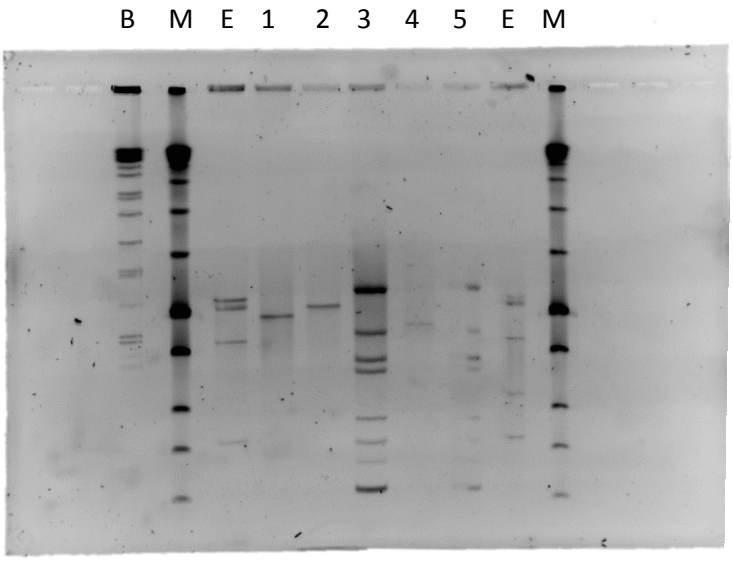


Figure 24 *Salmonella* plasmid size determination by S1 nuclease on PFGE (B: *Salmonella* Braenderup, M: PFG marker, E: *E.coli* control strain, 1: MET S1-50, 2: MET S1-56, 3: MET S1-163, 4: MET S1-669, 5: MET S1-703)

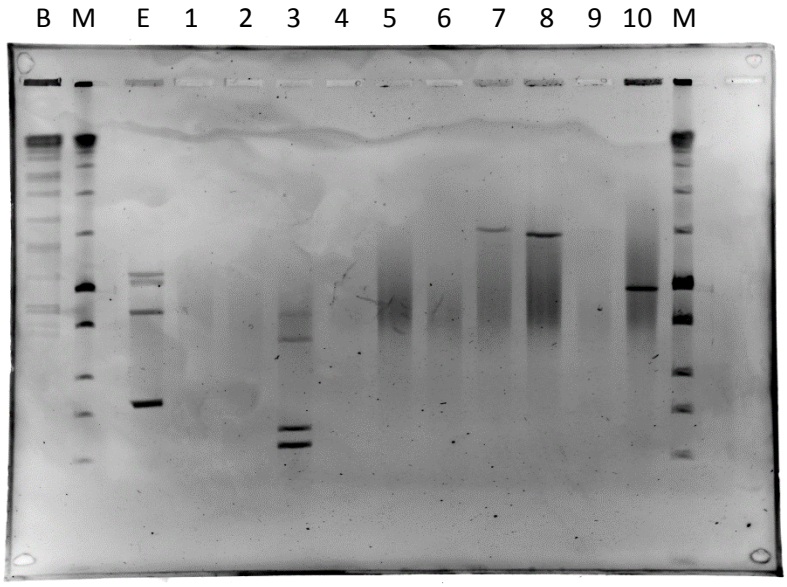


Figure 25 *Salmonella* plasmid size determination by S1 nuclease on PFGE (B: *Salmonella* Braenderup, M: PFG marker, E: *E.coli* control strain, 1: MET S1-218, 2: MET S1-219, 3: MET S1-221, 4: MET S1-228, 5: MET S1-237, 6: MET S1-625, 7: MET S1-653, 8: MET S1-657, 9: MET S1-663, 10: MET S1-50)

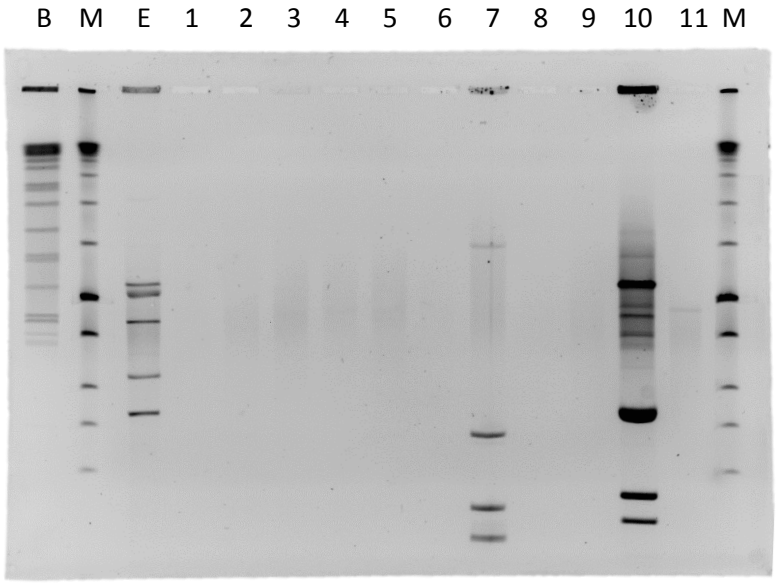


Figure 26 *Salmonella* plasmid size determination by S1 nuclease on PFGE (B: *Salmonella* Braenderup, M: PFG marker, E: *E.coli* control strain, 1: MET S1-220, 2: MET S1-234, 3: MET S1-195, 4: MET S1-197, 5: MET S1-198, 6: MET S1-201, 7: MET S1-204, 8: MET S1-205, 9: MET S1-211, 10: MET S1-217, 11: MET S1-669)

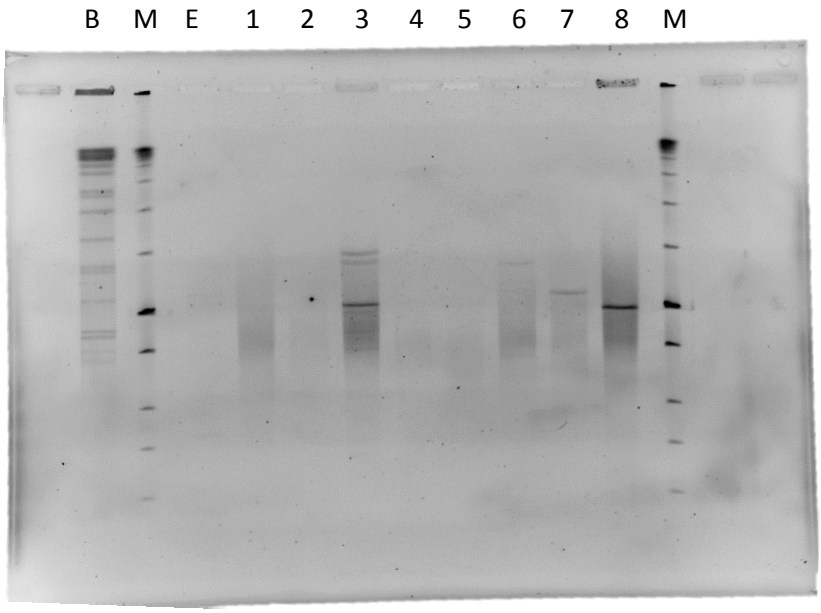


Figure 27 *Salmonella* plasmid size determination by S1 nuclease on PFGE (B: *Salmonella* Braenderup, M: PFG marker, E: *E.coli* control strain, 1: MET S1-674, 2: MET S1-227, 3: MET S1-87, 4: MET S1-313, 5: MET S1-405, 6: MET S1-542, 7: MET S1-660, 8: MET S1-50)

APPENDIX F

VISUALIZATION OF ANTIMICROBIAL RESISTANCE GENES ON PLASMIDS OF *SALMONELLA* ISOLATES

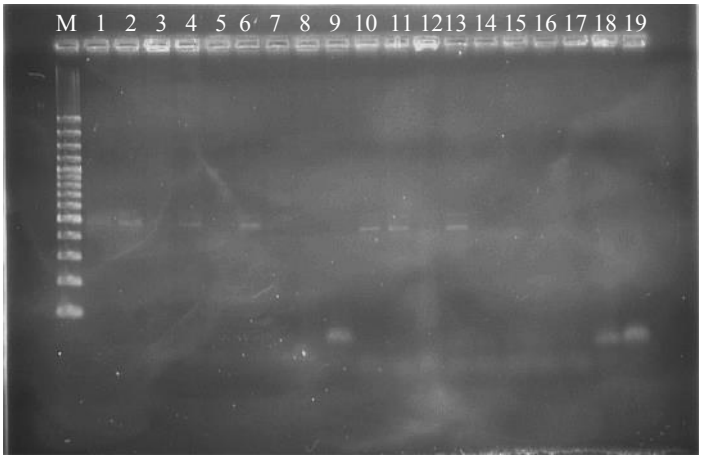


Figure 28 Gel photograph for *aadA1* (1-9) and *aphA* (10-19) genes in plasmids of 1: MET S1-50 plasmid (+), 2: MET S1-56 plasmid (+), 3: MET S1-50 cell (+), 4: MET S1-56 cell (+), 5: MET S1-163 plasmid (+), 6: MET S1-669 plasmid (+), 7: *E. coli* control (-), 8: *E. coli* control (-), 9(N): Negative control, 10: MET S1-50 plasmid (+), 11: MET S1-56 plasmid (+), 12: MET S1-50 cell (+), 13: MET S1-56 cell (+), 14: MET S1-163 plasmid (-), 15: MET S1-669 plasmid (-), 16: *E. coli* control (-), 17: *E. coli* control (-),18: *E. coli* cell control (-), 19 (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

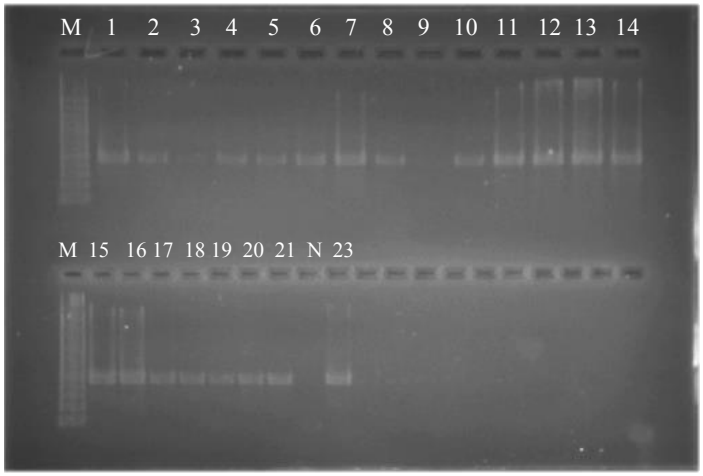


Figure 29 Gel photograph for *aadA1* gene in plasmids of 1: MET S1-6 (+), 2: MET S1-88 (+), 3: MET S1-92 (+), 4: MET S1-103 (+), 5: MET S1-142 (+), 6: MET S1-150 (+), 7: MET S1-329 (+), 8: MET S1-345 (+), 9: MET S1-351 (-), 10: MET S1-492 (+), 11: MET S1-498 (+), 12: MET S1-510 (+), 13: MET S1-597 (+), 14: MET S1-606 (+), 15: MET S1-668 (+), 16: MET S1-669 (+), 17: MET S1-671 (+), 18: MET S1-672 (+), 19: MET S1-673 (+), 20: MET S1-676 (+), 21:MET S1-50 (+), 22 (N): Negative control, 23: MET S1-669 (+), M: GeneRuler 50 bp DNA ladder as marker

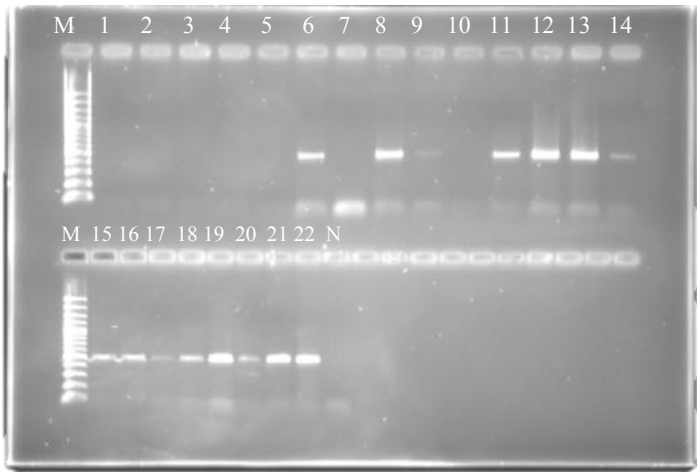


Figure 30 Gel photograph for *aadA1* gene in plasmids of 1: MET S1-677 (-), 2: MET S1-678 (-), 3: MET S1-679 (-), 4: MET S1-680 (-), 5: MET S1-682 (-), 6: MET S1-683 (+), 7: MET S1-684 (-), 8: MET S1-685 (+), 9: MET S1-686 (+), 10: MET S1-687 (-), 11: MET S1-688 (+), 12: MET S1-689 (+), 13: MET S1-690 (+), 14: MET S1-691 (+), 15: MET S1-692 (+), 16: MET S1-693 (+), 17: MET S1-694 (+), 18: MET S1-695 (+), 19: MET S1-696 (+), 20: MET S1-697(+), 21:MET S1-698 (+), 22: MET S1-50 (+), 23 (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 31 Gel photograph for *aadA1* gene in plasmids of 1: MET S1-698 (+), 2: MET S1-699 (+), 3: MET S1-700 (+), 4: MET S1-701 (-), 5: MET S1-737 (+), 6: MET S1-738 (-), 7: MET S1-739 (-), 8: MET S1-741 (-), 9: MET S1-745 (-), 10: MET S1-746 (-), 11: MET S1-747 (-), 12: MET S1-749 (-), 13(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

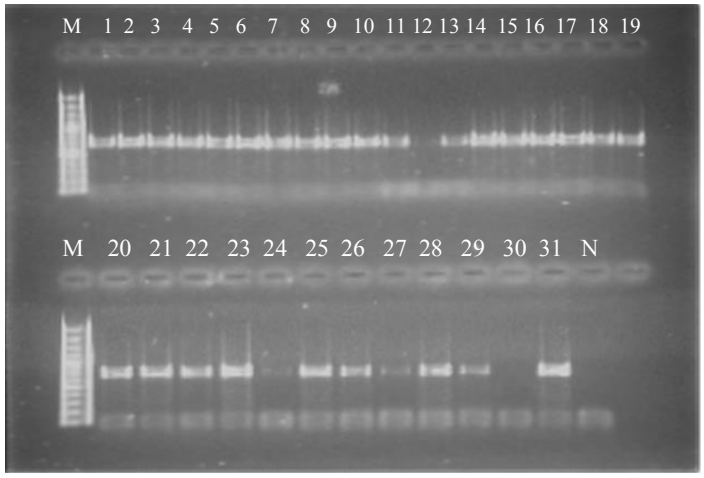


Figure 32 Gel photograph for *aphA* gene in plasmids of 1: MET S1-6 (+), 2: MET S1-50 (+), 3: MET S1-56 (+), 4: MET S1-88 (+), 5: MET S1-92 (+), 6: MET S1-103 (+), 7: MET S1-142 (+), 8: MET S1-150 (+), 9: MET S1-163 (-), 10: MET S1-329 (+), 11: MET S1-345 (+), 12: MET S1-351 (-), 13: MET S1-492 (+), 14: MET S1-498 (+), 15: MET S1-510 (+), 16: MET S1-597 (+), 17: MET S1-606 (+), 18: MET S1-668 (+), 19: MET S1-669 (+), 20: MET S1-671 (+), 21:MET S1-672 (+), 22:MET S1-673 (+), 23:MET S1-676 (+), 24:MET S1-677 (+), 25:MET S1-678 (+), 26:MET S1-679 (+), 27:MET S1-680 (+), 28:MET S1-682 (+), 29:MET S1-683 (+), 30:MET S1-684 (-), 31:MET S1-703 (+), 32 (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 33 Gel photograph for *aphA* gene in plasmids of 1: MET S1-682 (-), 2: MET S1-683 (-), 3: MET S1-684 (+), 4: MET S1-685 (-), 5: MET S1-686 (+), 6: MET S1-687 (+), 7: MET S1-688 (-), 8: MET S1-689 (-), 9: MET S1-690 (-), 10: MET S1-691 (+), 11: MET S1-692 (+), 12: MET S1-693 (+), 13: MET S1-694 (+), 14: MET S1-695 (-), 15: MET S1-696 (+), 16:MET S1-697 (+), 17: MET S1-698 (+), 18: MET S1-699 (-), 19: MET S1-700 (+), 20: MET S1-701 (+), 21: MET S1-737 (+), 22: MET S1-738 (+), 23: MET S1-739 (+), 24: MET S1-741 (-), 25: MET S1-745 (+), 26: MET S1-746 (+), 27: MET S1-747 (+), 28: MET S1-749 (+), 29: MET S1-703 (+), 30: MET S1-56 (+), 31(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 34 Gel photograph for *tetA* gene in plasmids of 1: MET S1-677 (-), 2:MET S1-678 (-), 3:MET S1-679 (-), 4:MET S1-680 (-), 5:MET S1-682 (-), 6:MET S1-683 (-), 7:MET S1-684 (-), 8: MET S1-685 (+), 9: MET S1-686 (-), 10: MET S1-687 (-), 11: MET S1-688 (-), 12: MET S1-689 (-), 13: MET S1-690 (-), 14: MET S1-691 (+), 15: MET S1-692 (+), 16: MET S1-693 (+), 17: MET S1-694 (-), 18: MET S1-695 (-), 19: MET S1-696 (+), 20:MET S1-697 (+), 21: MET S1-698 (+), 22: MET S1-699 (-), 23: MET S1-700 (-), 24(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

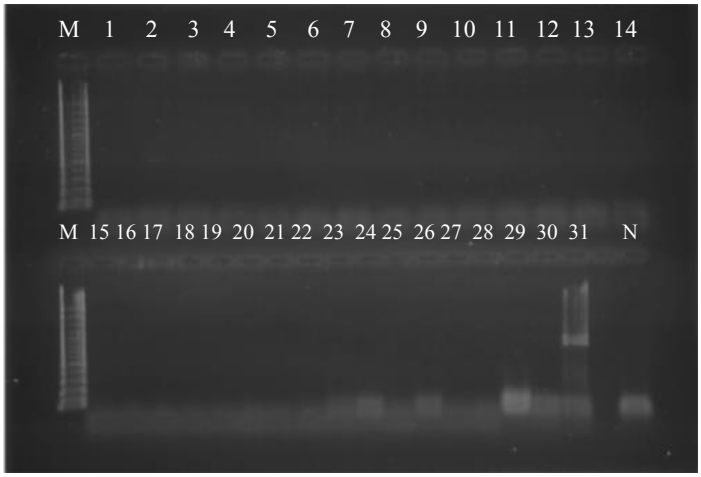


Figure 35 Gel photograph for *tetA* gene in plasmids of 1: MET S1-6 (-), 2: MET S1-50 (-), 3: MET S1-56 (-), 4: MET S1-88 (-), 5: MET S1-92 (-), 6: MET S1-103 (-), 7: MET S1-142 (-), 8: MET S1-150 (-), 9: MET S1-163 (-), 10: MET S1-329 (-), 11: MET S1-345 (-), 12: MET S1-351 (-), 13: MET S1-492 (-), 14: MET S1-498 (-), 15: MET S1-510 (-), 16: MET S1-597 (-), 17: MET S1-606 (-), 18: MET S1-668 (-), 19: MET S1-669 (-), 20: MET S1-671 (-), 21:MET S1-672 (-), 22:MET S1-673 (-), 23:MET S1-676 (-), 24:MET S1-677 (-), 25:MET S1-678 (-), 26:MET S1-679 (-), 27:MET S1-680 (-), 28:MET S1-682 (-), 29:MET S1-683 (-), 30:MET S1-684 (-), 31:MET S1-703 (+), 32 (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

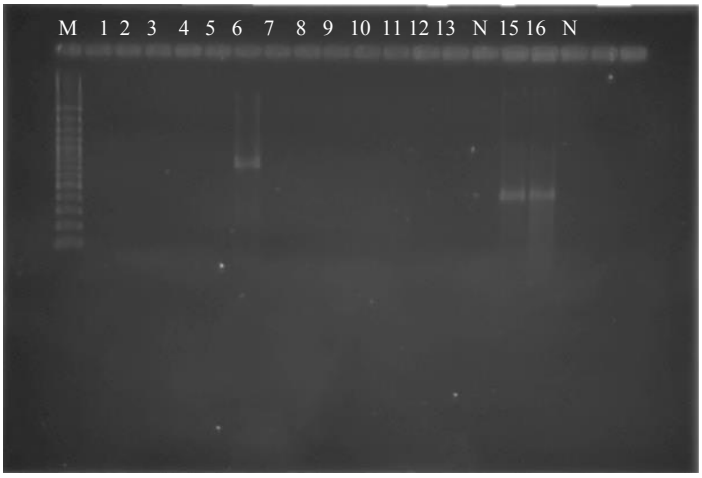


Figure 36 Gel photograph for *tetA* (1-14) and *aphA* (15-17) gene in plasmids of 1: MET S1-698 (-), 2: MET S1-699 (-), 3: MET S1-700 (-), 4: MET S1-701 (-), 5: MET S1-737 (-), 6: MET S1-738 (+), 7: MET S1-739 (-), 8: MET S1-741 (-), 9: MET S1-745 (-), 10: MET S1-746 (-), 11:MET S1-747 (-), 12: MET S1-749 (-), 13: MET S1-692 (+), 14(N): Negative control for *tetA*, 15: MET S1-747 (+), 16: MET S1-749 (+), 17(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 37 Gel photograph for *sul1* gene in plasmids of 1: MET S1-50 (-), 2: MET S1-56 (-), 3: MET S1-88 (-), 4: MET S1-92 (-), 5: MET S1-103 (-), 6: MET S1-142 (-), 7: MET S1-150 (-), 8: MET S1-163 (-), 9: MET S1-329 (-), 10: MET S1-345 (-), 11: MET S1-351 (-), 12: MET S1-492 (+), 13: MET S1-498 (-), 14: MET S1-510 (-), 15: MET S1-597 (-), 16: MET S1-606 (-), 17: MET S1-669 (-), 18: MET S1-56 (+), 19: MET S1-703 (+), M: GeneRuler 50 bp DNA ladder as marker

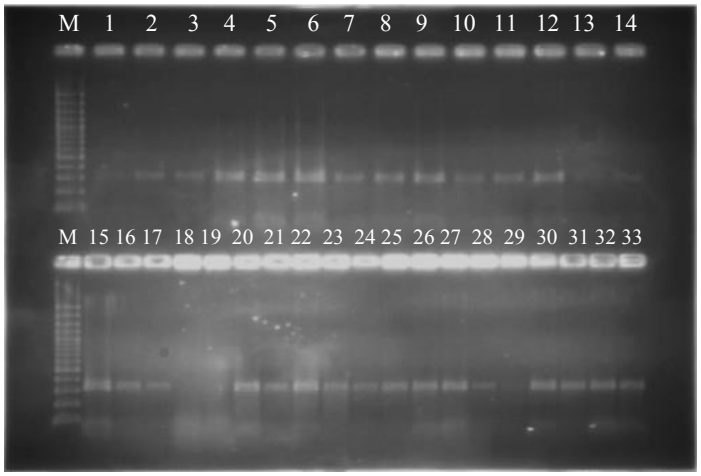


Figure 38 Gel photograph for *sul1* gene in plasmids of 1: MET S1-679 (-), 2:MET S1-680 (+), 3:MET S1-682 (+), 4:MET S1-683 (+), 5:MET S1-684 (+), 6: MET S1-685 (+), 7: MET S1-686 (+), 8: MET S1-687 (+), 9: MET S1-688 (+), 10: MET S1-689 (+), 11: MET S1-690 (+), 12: MET S1-691 (+), 13: MET S1-692 (+), 14: MET S1-693 (+), 15: MET S1-694 (+), 16: MET S1-695 (+), 17: MET S1-696 (+), 18:MET S1-697 (-), 19: MET S1-698 (-), 20: MET S1-699 (+), 21: MET S1-700 (+), 22: MET S1-701 (+), 23: MET S1-737 (-), 24: MET S1-738 (+), 25: MET S1-739 (+), 26: MET S1-741 (+), 27: MET S1-745 (+), 28: MET S1-746 (+), 29:MET S1-747 (+), 30: MET S1-749 (+), 31: MET S1-56 (+), 32: MET S1-163 (+), 33: MET S1-703(+), M: GeneRuler 50 bp DNA ladder as marker, M: GeneRuler 50 bp DNA ladder as marker

APPENDIX G

CLASS 1 INTEGRON ASSOCIATED GENES VISUALIZED ON GEL PHOTOGRAPHS OF *SALMONELLA* ISOLATES

Figure 39 Gel photograph for *int1* gene in 1: MET S1-88 (-), 2: MET S1-92 (+), 3: MET S1-103 (-), 4: MET S1-142 (+), 5: MET S1-329 (+), 6: MET S1-345 (-), 7: MET S1-351 (-), 8: MET S1-492 (-), 9: MET S1-498 (-), 10: MET S1-510 (-), 11: MET S1-597 (+), 12: MET S1-606 (+), 13:MET S1-668 (-), 14: MET S1-669 (+), 15: MET S1-671 (+), 16: MET S1-672 (+), 17: MET S1-673 (+), 18: MET S1-674 (+), 19: MET S1-87 (-), 20: MET S1-313 (-), 21: MET S1-405 (+), 22: MET S1-542 (-), 23: MET S1-660 (+), 24:MET S1-24 (+), 25: MET S1-31 (+), 26(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

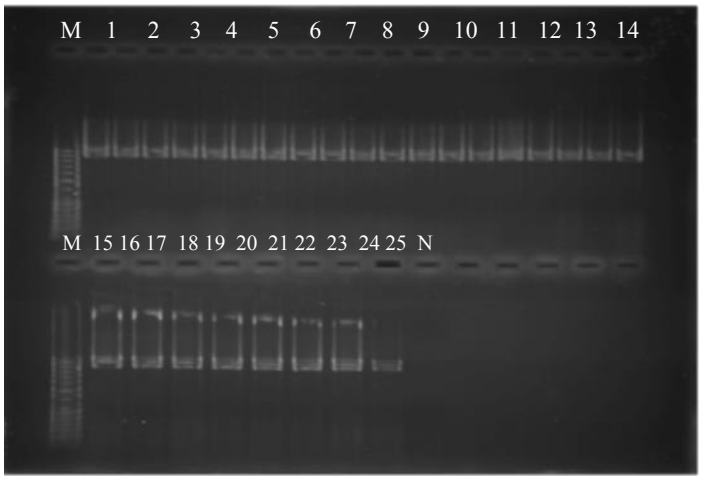


Figure 40 Gel photograph for *int1* gene in 1: MET S1-685 (+), 2: MET S1-686 (+), 3: MET S1-687 (+), 4: MET S1-688 (+), 5: MET S1-689 (+), 6: MET S1-690 (+), 7: MET S1-691 (+), 8: MET S1-692 (+), 9: MET S1-693 (+), 10: MET S1-694 (+), 11: MET S1-695 (+), 12: MET S1-696 (+), 13:MET S1-697 (+), 14: MET S1-698 (+), 15: MET S1-699 (+), 16: MET S1-700 (+), 17: MET S1-701 (+), 18: MET S1-737 (+), 19: MET S1-738 (+), 20: MET S1-739 (+), 21: MET S1-741 (+), 22: MET S1-745 (+), 23: MET S1-746 (+), 24:MET S1-747 (+), 25: MET S1-749 (+), 26(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

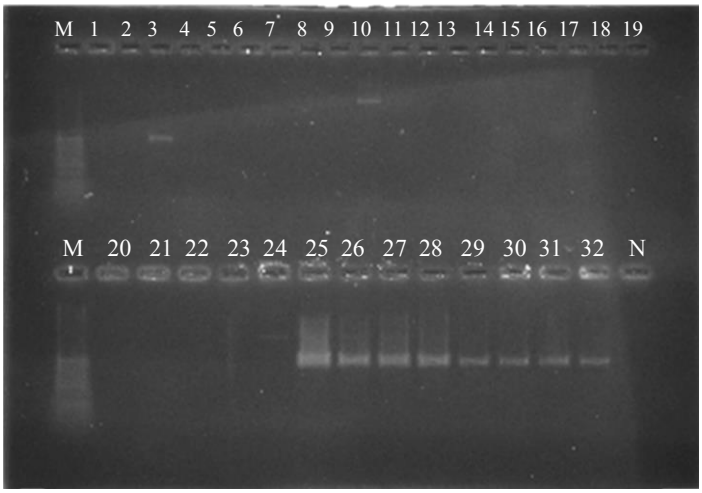


Figure 41 Gel photograph for *int1* gene in 1: MET S1-50 (-), 2: MET S1-56 (-), 3: MET S1-150 (+), 4: MET S1-220 (-), 5: MET S1-234 (-), 6: MET S1-195 (-), 7: MET S1-197 (-), 8: MET S1-198 (-), 9: MET S1-201 (-), 10: MET S1-204 (+), 11: MET S1-205 (-), 12: MET S1-211 (-), 13:MET S1-217 (-), 14: MET S1-218 (-), 15: MET S1-219 (-), 16: MET S1-221 (-), 17: MET S1-227 (-), 18: MET S1-228 (-), 19: MET S1-237 (-), 20: MET S1-625 (-), 21: MET S1-653 (-), 22: MET S1-657 (-), 23: MET S1-663 (-), 24:MET S1-163 (+), 25: MET S1-676 (+), 26: MET S1-677 (+), 27: MET S1-678 (+), 28: MET S1-679 (+), 29: MET S1-680 (+), 30: MET S1-682 (+), 31: MET S1-683 (+), 32: MET S1-684 (+), (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 42 Gel photograph for *qace*Δ1 gene in 1: MET S1-88 (+), 2: MET S1-92 (+), 3: MET S1-103 (-), 4: MET S1-142 (+), 5: MET S1-329 (+), 6: MET S1-345 (-), 7: MET S1-351 (-), 8: MET S1-492 (+), 9: MET S1-498 (+), 10: MET S1-510 (+), 11: MET S1-597 (+), 12: MET S1-606 (+), 13:MET S1-668 (+), 14: MET S1-669 (+), 15: MET S1-671 (+), 16: MET S1-672 (+), 17: MET S1-673 (+), 18: MET S1-674 (+), 19: MET S1-87 (-), 20: MET S1-313 (-), 21: MET S1-405 (-), 22: MET S1-542 (-), 23: MET S1-660 (+), 24:MET S1-24 (-), 25: MET S1-31 (-), 26(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

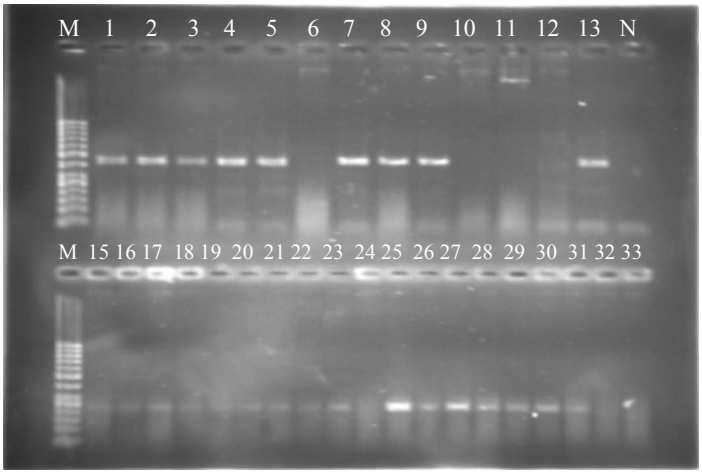
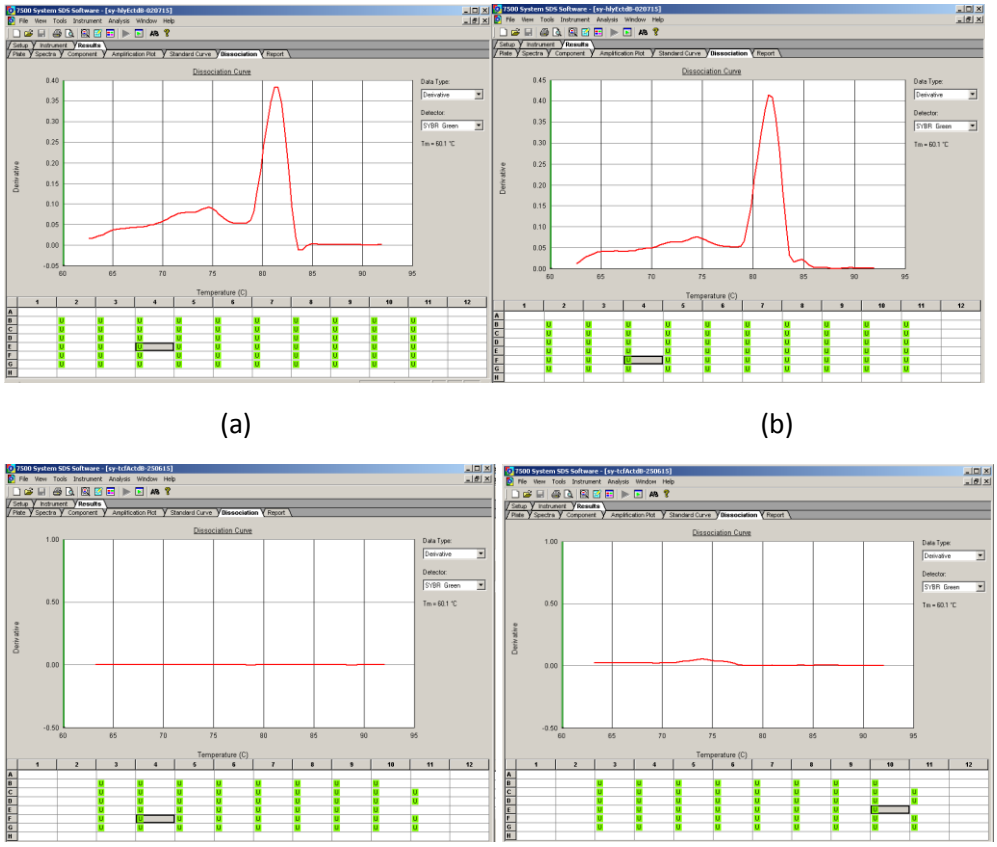


Figure 43 Gel photograph for *sul1* (1-14) and *qace*Δ1 (15-33) genes in 1: MET S1-701 (+), 2: MET S1-737 (+), 3: MET S1-738 (+), 4: MET S1-739 (+), 5: MET S1-741 (+), 6: MET S1-745 (-), 7: MET S1-746 (+), 8: MET S1-747 (+), 9: MET S1-749 (+), 10: MET S1-313 (-), 11: MET S1-204 (-), 12: MET S1-660 (-), 13: MET S1-684 (+), 14 (N): Negative control, 15: MET S1-676 (+), 16: MET S1-677 (+), 17: MET S1-678 (+), 18: MET S1-679 (+), 19: MET S1-680 (+), 20: MET S1-682 (+), 21: MET S1-683 (+), 22: MET S1-684 (+), 23: MET S1-685 (+), 24: MET S1-686 (-), 25: MET S1-687 (+), 26: MET S1-688 (+), 27: MET S1-689 (+), 28: MET S1-690 (+), 29: MET S1-691 (+), 30: MET S1-692 (+), 31: MET S1-693 (+), 32: MET S1-694 (-), 33 (N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

Figure 44 Gel photograph for *sul1* gene in 1: MET S1-88 (-), 2: MET S1-92 (-), 3: MET S1-103 (-), 4: MET S1-142 (+), 5: MET S1-329 (-), 6: MET S1-345 (-), 7: MET S1-351 (-), 8: MET S1-492 (+), 9: MET S1-498 (+), 10: MET S1-510 (+), 11: MET S1-597 (+), 12: MET S1-606 (+), 13:MET S1-668 (-), 14: MET S1-669 (+), 15: MET S1-671 (+), 16: MET S1-672 (-), 17: MET S1-673 (-), 18: MET S1-674 (+), 19: MET S1-87 (-), 20: MET S1-313 (-), 21: MET S1-405 (-), 22: MET S1-542 (-), 23: MET S1-660 (-), 24:MET S1-24 (-), 25: MET S1-31 (-), 26(N): Negative control, M: GeneRuler 50 bp DNA ladder as marker

APPENDIX H

REAL-TIME PCR DISSOCIATION CURVES AND CTS FOR VIRULENCE GENES ON *SALMONELLA* ISOLATES



(a) (b)

(c) (d)

Figure 45 Dissociation curves of (a) MET S1-92, (b) MET S1-313, (c) negative control, and (d) no template sam ple control for as an example for *cdtB* gene on real-time PCR

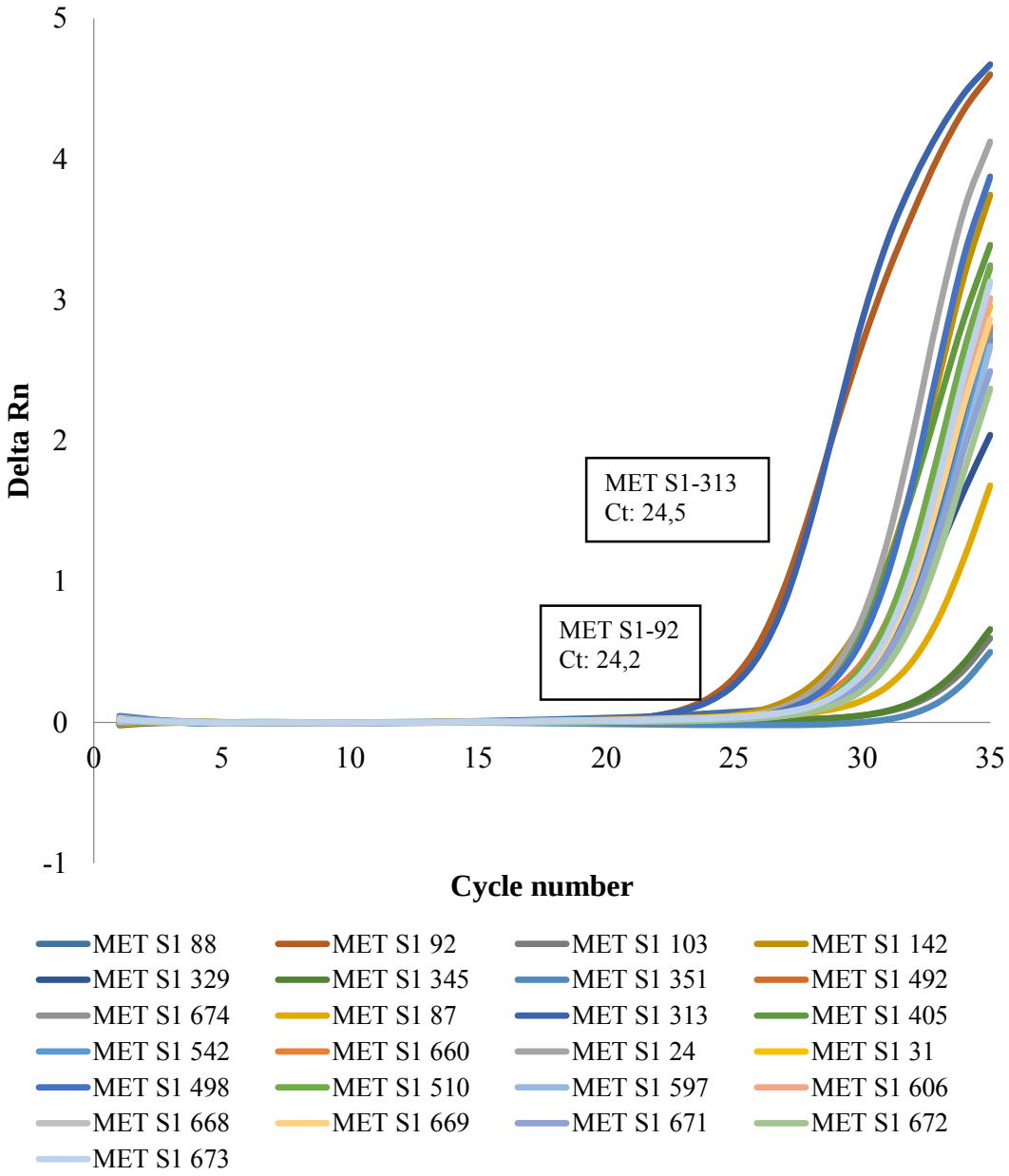


Figure 46 Amplification plot of *Salmonella* isolates for detection of the virulence gene, *ctdB* gene, as an example

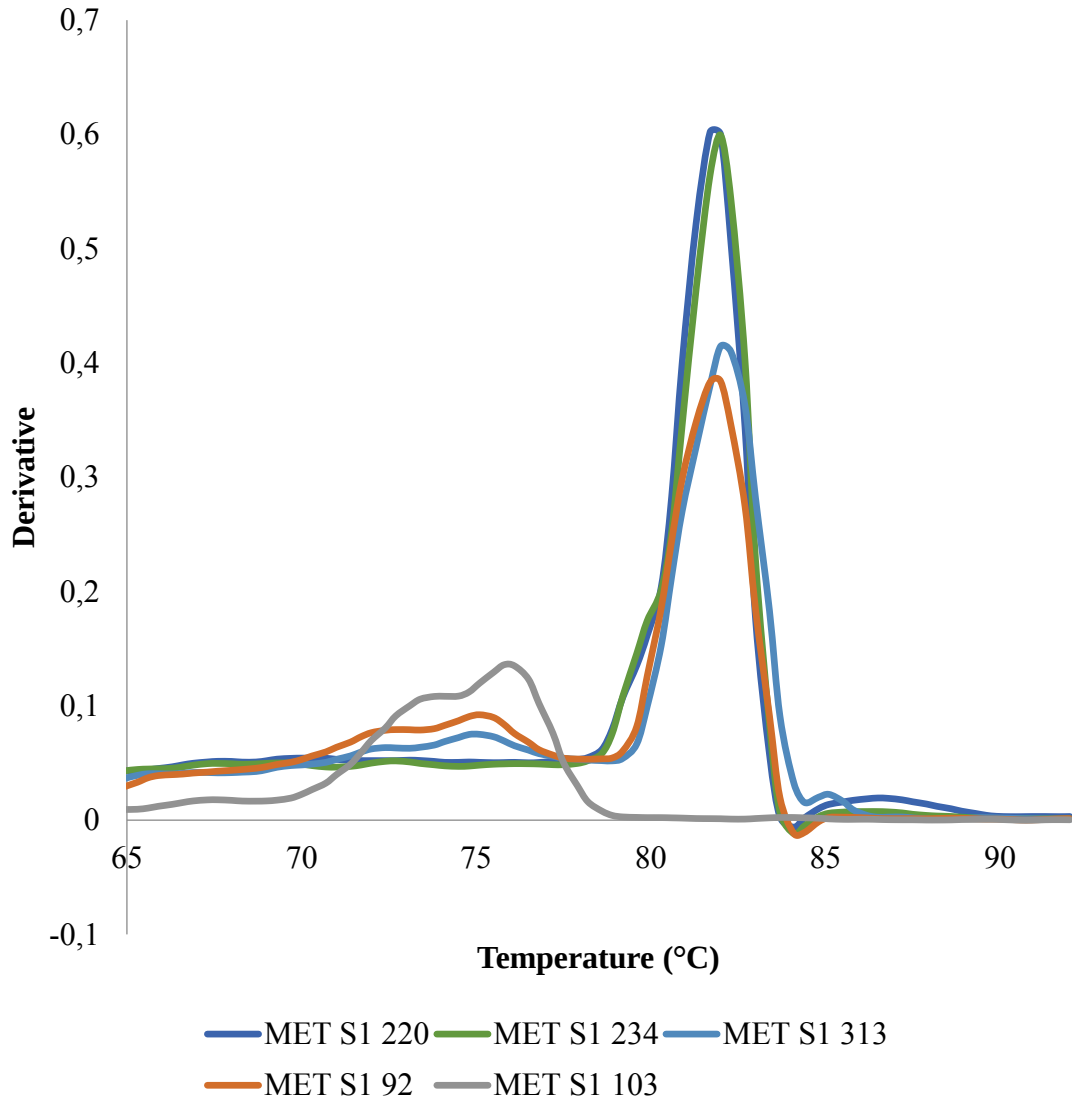


Figure 47 Dissociation curve of *Salmonella* isolates for detection of the virulence gene, *ctdB* gene, by real-time PCR

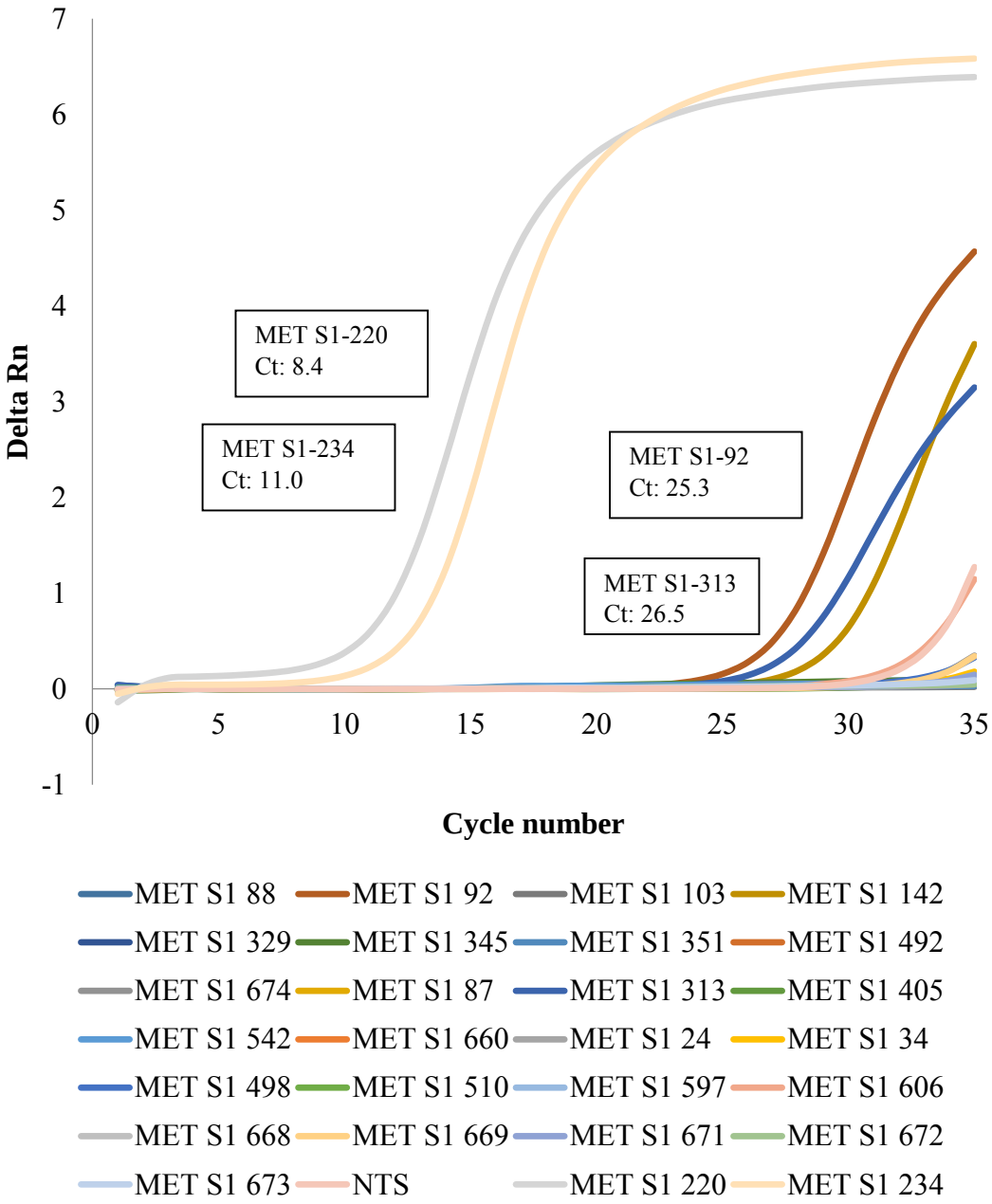


Figure 48 Amplification plot of *Salmonella* isolates for detection of the virulence gene, *hlyE* gene, as an example

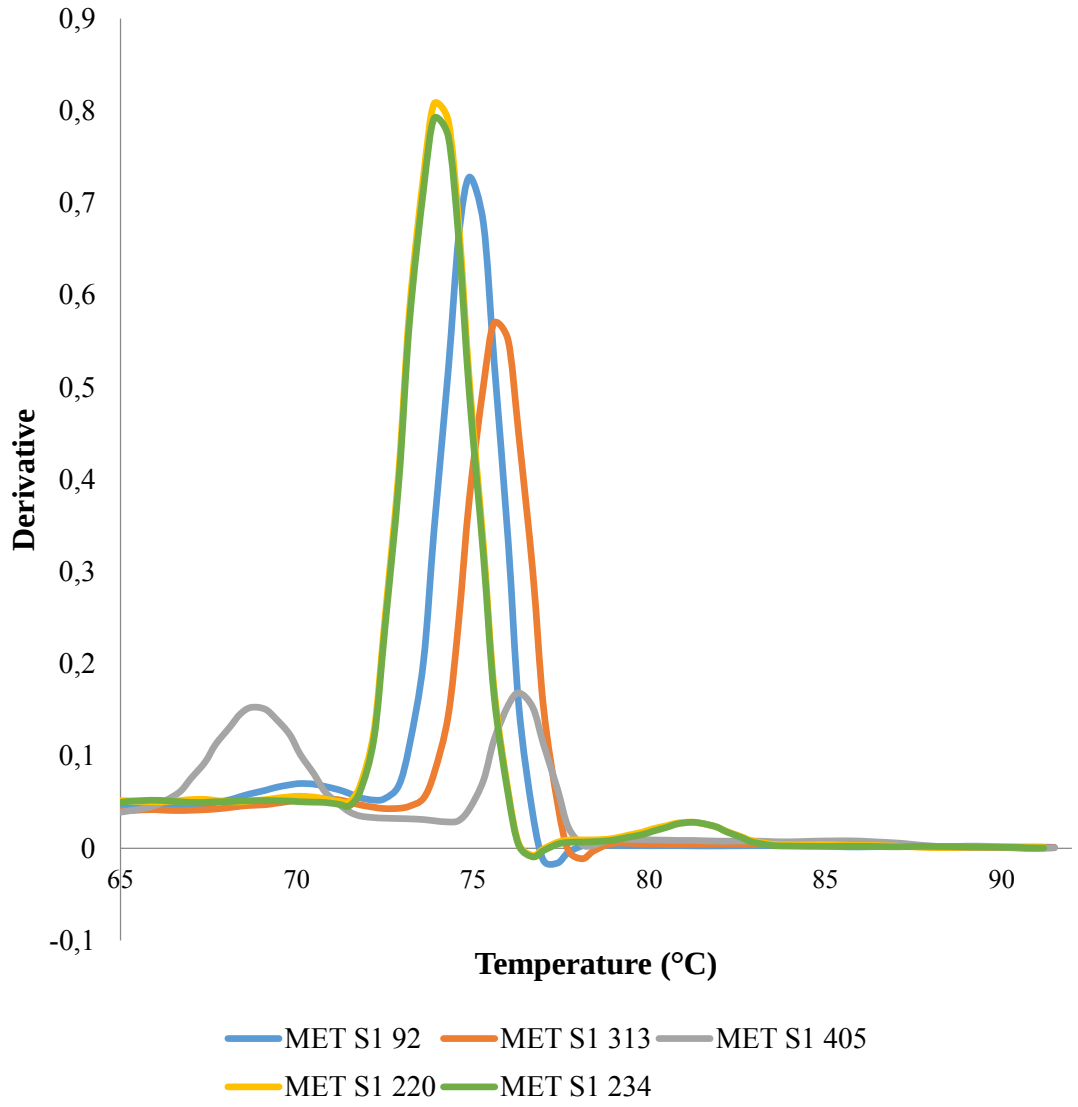


Figure 49 Dissociation curve of *Salmonella* isolates for detection of the virulence gene, *hlyE* gene, by real-time PCR

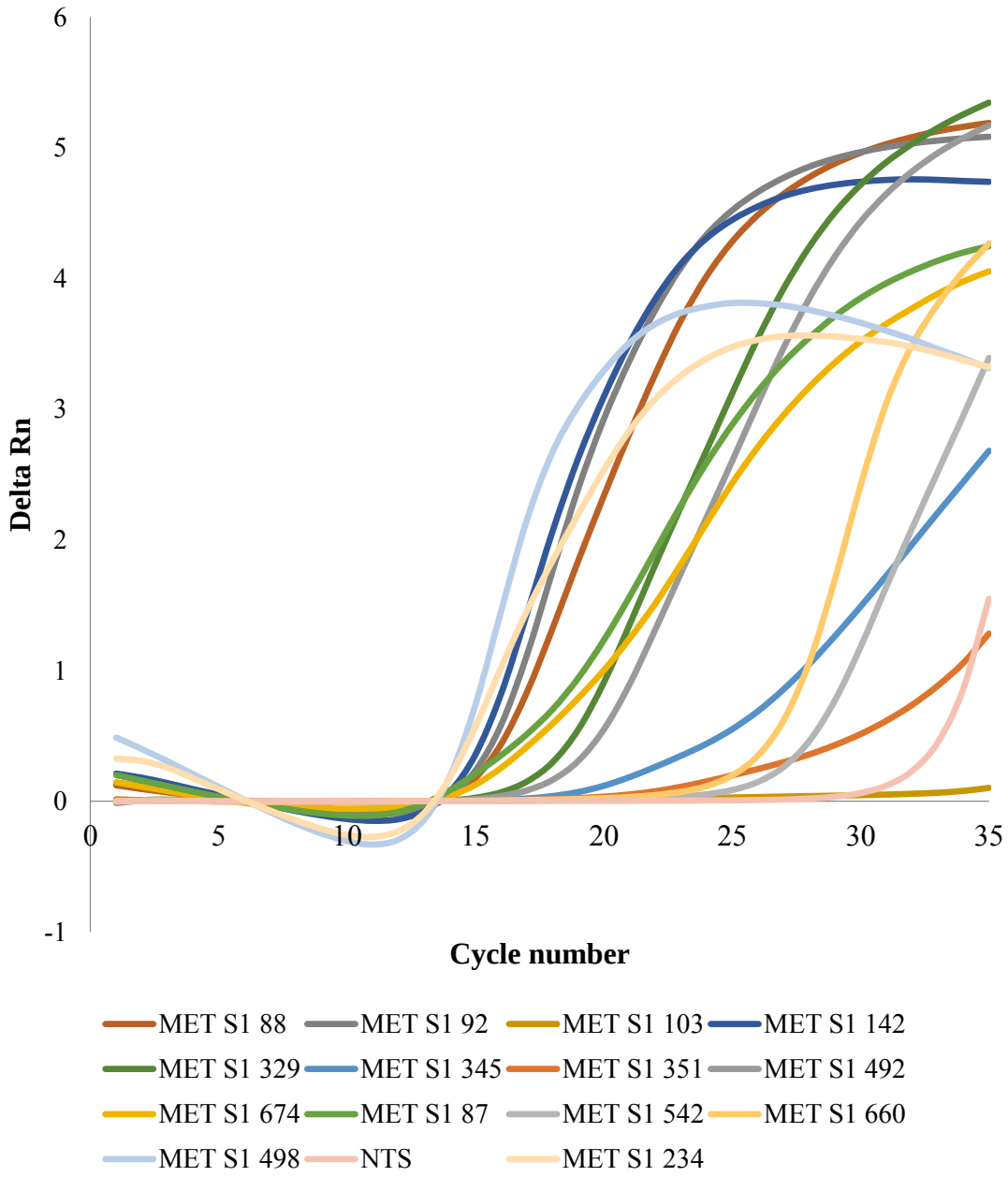


Figure 50 Amplification plot of *Salmonella* isolates for detection of the virulence gene, *tcfA* gene, as an example

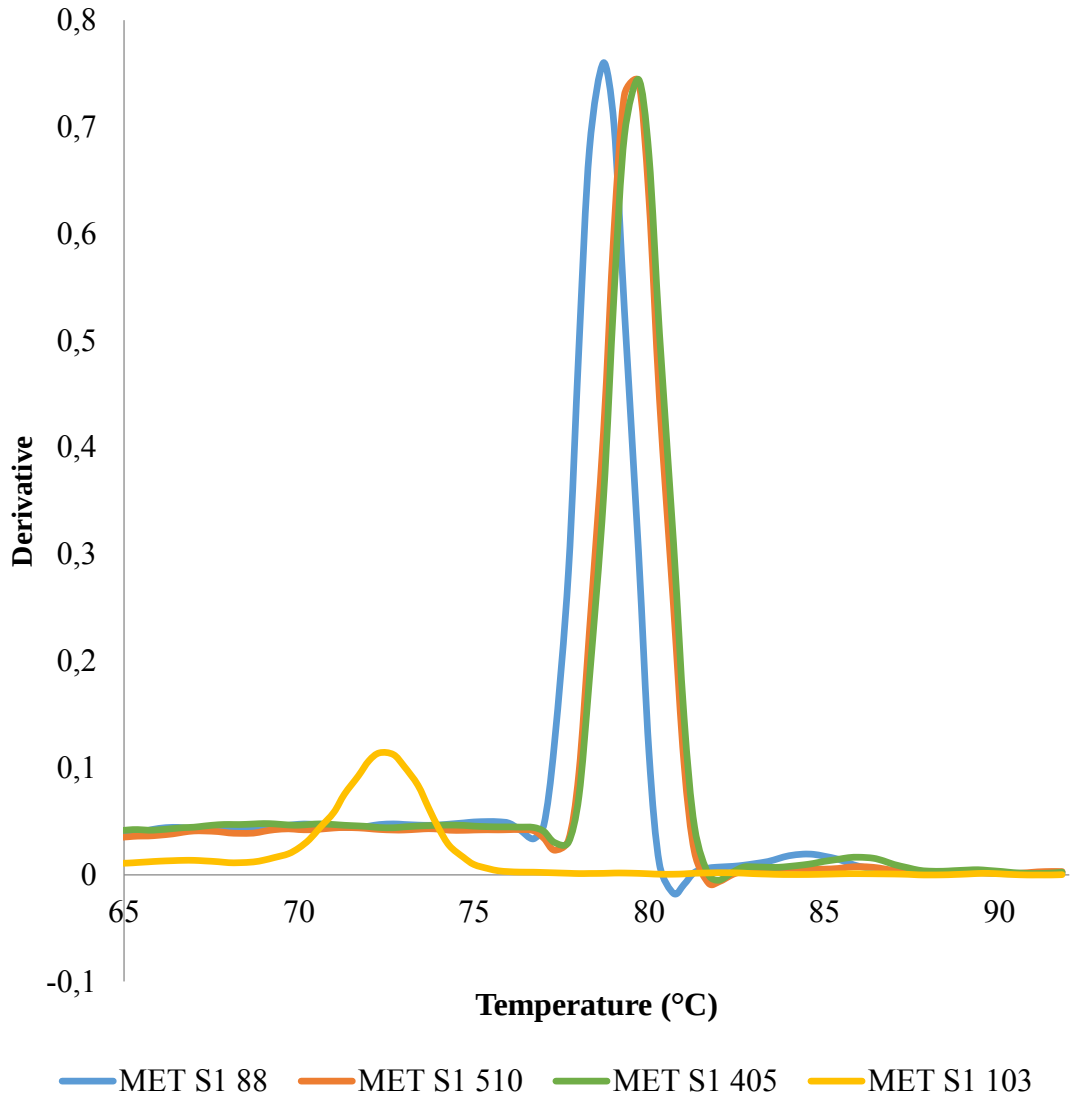


Figure 51 Dissociation curve of *Salmonella* isolates for detection of the virulence gene, *tcfA* gene, by real-time PCR

VITA

PERSONAL INFORMATION

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EDUCATION

Degree	Institution	Year of Graduation
M.Sc.	METU, Department of Food Engineering	2010
B.Sc.	METU, Department of Food Engineering	2008
Minor	METU, Department of Biological Sciences	2008
High School	İst.Köy Hizmetleri Anatolian High School, İstanbul	2004

WORK EXPERIENCE

Year	Place	Enrollment
2009-2015	METU, Department of Food Engineering, Ankara	Research Assist.
2007	Tat Konserve San. A.Ş. Maret Tuzla Factory, İstanbul	Intern
2007	Tat Konserve San. A.Ş. Mustafakemalpaşa Factory, Bursa	Intern
2006	Sütaş Karacabey Factory, Bursa	Intern
2006	Nestlé Türkiye Gıda San. A.Ş. Karacabey Factory, Bursa	Intern

FOREIGN LANGUAGES

English (fluent), German (basic), French (basic)

PUBLICATIONS

- Papers
    - **Sinem Acar**, Ece Bulut, Bora Durul, Ilhan Uner, Mehmet Kur, M. Dilek Avsaroglu, Hüseyin Avni Kirmaci, Yasar Osman Tel, Fadile Y. Zeyrek, Yesim Soyer. *Salmonella diversity from farm to fork in Turkey,* Plos One (Submitted)
    - F. Yeni, **S. Acar**, Ö.G. Polat, Y. Soyer, H. Alpas, 2014. *Rapid and standardized methods for detection of foodborne pathogens and mycotoxins on fresh produce*, Food Control, Volume 40, June 2014, Pages 359-367, ISSN 0956-7135, http://dx.doi.org/10.1016/j.foodcont.2013.12.020
    - F. Yeni, **S. Acar**, H Alpas, Y. Soyer, 2014. *Most Common Foodborne Pathogens and Mycotoxins on Fresh Produce: A review of Recent Outbreaks,* Manuscript ID BFSN-2013-0904, Critical Reviews in Food Science and Nutrition (Accepted)
    - Elif Gunel, Gozde Polat Kilic, Ece Bulut, Bora Durul, **Sinem Acar**, Hami Alpas, Yeşim Soyer, 2015.*Salmonella surveillance on fresh produce in retail in Turkey,* Internation Journal of Food Microbiology, Volume 199, January 2015, Pages 72-77, http://dx.doi.org/10.1016/j.ijfoodmicro.2015.01.010
    - Y. Soyer, A. Karaaslan, B. Durul, E. Bulut, **S. Acar**, I. Haydaroglu, and H. Vardin. *Molecular characterization of Salmonella in pistachio (Pistacia vera) samples from retail markets.* Journal of Food, Agriculture and Environment (Accepted)
    - Bora Durul, **Sinem Acar**, Ece Bulut, Emmanuel O. Kywere, Huseyin A. Kirmaci, and Yesim Soyer, 2014. *Subtyping of Salmonella food isolates suggests overrepresentation of serovar Telaviv in Turkey.* Foodborne Pathogens and Disease (In Press)
    - **Sinem Yavas**, Behic Mert, Zumrut B. Ogel, *Production of wheat straw nano-fibrils by high-pressure homogenization and its effect on enzymatic saccharification,* Manuscript ID: GHPR-2011-0129, High Pressure Research (Under Review)

- International Conference Papers
    - **S. Acar**, E. Bulut, S. Aydin, Y. Soyer. Characterization of plasmid mediated antimicrobial resistance patterns of poultry-related *Salmonella* Infantis isolates. 4th ASM Conference on Antimicrobial Resistance in Zoonatic Bacteria and Foodborne Pathogens (2015), Washington D.C., USA (Travel Grant)
    - **S. Acar**, E. Bulut, B. Durul, I. Uner, D. Avsaroglu, H.A. Kirmaci, O.Y. Tel, F.Y. Zeyrek, Y.Soyer. Antimicrobial Genotyping of *Salmonella*

isolates with a comparison of serotype and source (food, animal, and human) distribution. International Association for Food Protection (IAFP) General Meeting, (2014), Indianapolis, Indiana, USA (Technical-Oral Presentation)

- **S. Acar**, E. Bulut, B. Durul, I. Uner, D. Avsaroglu, H.A. Kirmaci, O.Y. Tel, F.Y. Zeyrek, Y.Soyer. Comparison of phenotypic and genotypic antimicrobial resistance profiles of *Salmonella* isolates from farm/field to fork in Turkey. 2nd International Food Technology Congress (2014), Kusadasi, İzmir
- **S.Acar**, E. Bulut, B. Durul, I. Uner, M. Kur, D. Avsaroglu, H.A. Kirmaci, O.Y. Tel, F.Y. Zeyrek, N. Dilsiz, Y.Soyer. Various antimicrobial susceptibility profiles obtained from *Salmonella* from farm/field to fork in Turkey. 4th ASM Conference on *Salmonella*: The Bacterium, the Host and the Environment, Boston, Massachusetts, USA (Travel Grant)
- B. Durul, E. Bulut, **S. Acar**, H.A. Kirmaci, Y.Soyer. Multiple *Salmonella* Serovars Collected from Street Foods in Turkey Present Same Allelic Profiles. 4th ASM Conference on *Salmonella*: The Bacterium, the Host and the Environment (2013), Boston, Massachusetts, USA
- E. Bulut, B. Durul**, S. Acar**, I. Uner, M. Kur, D. Avsaroglu, H. A. Kirmaci, O .Y. Tel, F.Y. Zeyrek, M. Wiedmann, and Y. Soyer. Pulsed Field Gel Electrophoresis (PFGE) analysis of temporally matched *Salmonella* isolates from human, food and animal sources in southern part of Turkey. 114th General Meeting, American Society for Microbiology (ASM) (2014), Boston, Massachusetts, USA
- B. Durul, **S. Yavas Acar**, E. Bulut, I. Uner, M. Kur, D. Avsaroglu, H. A. Kirmaci,Y. O. Tel, F. Y. Zeyrek, N. Dilsiz, Y. Soyer, Molecular Characterization of *Salmonella* isolates collected from different sources in Turkey, 113th General Meeting, American Society for Microbiology (2013), Denver, Colorado, USA

SCHOLARSHIPS/AWARDS

TUBITAK (The Scientific and Technological Research Council of Turkey) National Scholarship for PhD Students (2211)

Travel Grant, American Society for Microbiology, 4th ASM Conference on Salmonella: The Bacterium, the Host and the Environment, October 2013, Boston, USA

Travel Grant, American Society for Microbiology , 4th ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, May 2015, Washington D.C., USA

Middle East Technical University Doctorate Performance Award, 2010-2011

HOBIES

Oil painting, Planting, Photography, Travel, Doing experiment ☺