ISOLATION, MOLECULAR CHARACTERIZATION OF FOOD-BORNE DRUG RESISTANT *SALMONELLA* spp. AND DETECTION OF CLASS 1 INTEGRONS

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ISOLATION, MOLECULAR CHARACTERIZATION OF FOOD-BORNE DRUG RESISTANT *SALMONELLA* SPP. AND DETECTION OF CLASS 1 INTEGRONS

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ABSTRACT

ISOLATION, MOLECULAR CHARACTERIZATION OF FOOD-BORNE DRUG RESISTANT *SALMONELLA* spp. AND DETECTION OF CLASS 1 INTEGRONS

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In this study, 59 epidemiologically unrelated *Salmonella* strains isolated from foods in Türkiye and 49 *Salmonella* strains obtained from National *Salmonella* Reference Laboratories of Germany were analysed. For the characterization of strains, analyses such as serotyping, phage typing, antibiotyping and molecular biological characterization were done. The strains exhibited 17 different serotypes with *S.* Enteritidis serotype and PT21 phage type being the most prevalent in Turkish isolates. The highest antimicrobial resistance was observed against NAL for Turkish strains, whereas it was against SUL for strains from German origin. Molecular typing of all strains exhibited different plasmid profiles and PFGE patterns. There were 1-4 plasmids/profile for Turkish strains and 1-7 plasmids/profile for German strains. The PFGE patterns revealed 42 different subgroups, having two major clusters with 44,3% arbitrary homology. Among 72 resistant strains, the most prevalent resistance genotypes were observed as bla_{tem-1} (%56, AMP resistance); *floR* (%100, CHL and FFC resistance); *aphA1* (%100, KAN and NEO resistance); *tet*(A) (%53, TET resistance); *aadA1* (%82, SPE and STR resistance); *sulI* (%78, SUL resistance). The class I integron variable region analyses exhibited 700 bp (1 strain), 1000 bp (37 strain), 1200 bp (16 strain) and 1600 bp (3 strain) integrons.

Keywords: *Salmonella*, antimicrobial resistance, lateral gene transfer

ÖZ

İLAÇ DİRENÇLİLİĞİ GÖSTEREN GIDA KAYNAKLI *SALMONELLA* spp. SUŞLARININ İZOLASYONU, MOLEKÜLER KARAKTERİZASYONU VE SINIF 1 İNTEGRONLARIN ARAŞTIRILMASI

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Bu çalışmada, Türkiye'de gıdalardan izole edilen 59 adet epidemiyolojik açıdan farklı *Salmonella* suşu ve Almanya Ulusal *Salmonella* Referans Laboratuvarlarından elde edilen 49 adet *Salmonella* suşu analiz edilmiştir. Suşların karakterizasyonu için serotiplendirme, faj tiplendirmesi, antibiyotiplendirme ve moleküler biyolojik karakterizasyon gibi analizler yapılmıştır. Türk izolatları en yaygını *S.* Enteritidis serotipi ve PT21 faj tipi olmak üzere 17 farklı serotip göstermiştir. En yüksek dirençlilik Türk suşları için nalidiksik asite karşı gözlenirken Alman suşları için sulfonamid bileşenlerine karşı olmuştur. Suşların moleküler tiplendirmesi farklı plazmid ve PFGE profilleri göstermiştir. Türk suşları için 1-4 adet plazmid/profil ve Alman suşları için ise 1-7 adet plazmid/profil elde edilmiştir. PFGE profilleri 42 ayrı altgrup oluşturarak 44,3% homoloji ile iki ana grupta toplanmıştır. 72 adet dirençli suşta en yaygın olarak bla_{tem-1} (%56, AMP direnç); *floR* (%100, CHL ve FFC direnç); *aphA1* (%100, KAN ve NEO direnç); *tet*(A) (%53, TET direnç); *aadA1* (%82, SPE ve STR direnç); *sulI* (%78, SUL direnç) antibiyotik direnç genleri gözlenmiştir. Çalışmada 700 bç (1 suş), 1000 bç (37 suş), 1200 bç (16 suş) ve1600 bç (3 suş)'lik sınıf I integronlara ait farklılaşmış bölgeler elde edilmiştir.

Anahtar Kelimeler: *Salmonella*, antimikribiyel dirençlilik, lateral gen transferi

To Whom I Love;

Annem, Babam ve Abime

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ABBREVIATIONS

°C	Degree Celcius
AFLP	Amplified Fragment Length Polymorphism
AMC	Amoxicillin/clavulanic acid
AMP	Ampicillin
ATM	Aztreonam
BPW	Buffered Peptone Water
BSA	Bismuth Sulfite Agar
CAZ	Ceftazidime
CDC	Centers for Disease Control and Prevention
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
COL	Colistin
CPD	Cefpodoxime
CRO	Ceftriaxone
CTX	Cefotaxime
CXM	Cefuroxime
DNA	Deoxyribonucleic Acid
DT	Definitive Type
EFT	Ceftiofur
EMB	Eosin Methylene Blue
ESBL	Extended Spectrum β-Lactamase
FFC	Florfenicol
FOX	Cefoxitine
GEN	Gentamicin
h	Hour
IMP	Imipenem
Inc	Incompatibility

IS	Insertion Sequence
KAN	Kanamycin
kb	Kilobase
LB	Luria Bertani
MIC	Minimum Inhibitory Concentration
min	Minute
MKKTTn	Müller Kauffmann Thetrathionate Novobiocin Broth
MLST	Multilocus Sequence Typing
NAL	Nalidixic acid
NEO	Neomycin
NT	Non-Typable
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PT	Phage Type
QRDR	Quinolone Resistance Determining Region
RAPD	Randomly Amplified Polymorphic DNA
RDNC	**R**eacting with the typing phage, but lytic pattern **D**id **N**ot **C**orrespond to any recognized phage types
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RVB	Rappaport Vassiliadis Broth
SGI1	*Salmonella* Genomic Island 1
SPT	Spectinomycin
STR	Streptomycin
SUL	Sulfonamide compounds
SXT	Trimethoprim/sulfamethoksazol
TET	Tetracycline
TMP	Trimethoprim
Tn	Transposon
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

CHAPTER 1

INTRODUCTION

Salmonella infections are one of the most prevalent food-borne infections both in Türkiye and worldwide. Infections via animal origin foods especially poultry, meat, egg, and raw milk are the sources of the illness that *Salmonella* cause so called "salmonellosis". The patient has the symptoms stomachache, fever, diarrhoea, nausea and vomiting whereas life-threatening for the infants, elderly and immunocompromised patients.

There are over 2500 serotypes among *Salmonella* and to track the epidemics, it is needed to perform detailed characterization by different typing methods. Among *Salmonella* serotypes *S.* Enteritidis, *S.* Typhimurium and *S.* Virchow share the first three places in the Top10 list of *Salmonella* infections in Europe.

Since 1950s antimicrobial resistance among *Salmonella* serotypes has became prevalent. Due to the common use of antimicrobials not only for treatment of infections but also for using as animal growth promoters, selective pressure is established where antimicrobial resistant strains overcome and maintain growth. Acquired resistance causes serious problems in the treatment of severe cases of salmonellosis.

Either by mutations or by lateral gene transfer, bacteria acquire resistance. Especially through plasmids, transposons and integrons lateral gene transfer maintains the quick spread of resistance. One of these mobile genetic elements, the integrons, is the main subject of the research on antimicrobial resistance, since they maintain a platform for gene cassettes encoding antimicrobial resistance. In *Salmonella* genus, multidrug resistance is related to class I integrons.

The thesis concerns characterization of antimicrobial resistant serotypes of *Salmonella* and the prevalence of class I integrons. For this aim, food samples of animal origin were collected from several different markets and from one dairy factory from which *Salmonella* strains were isolated and characterized. In addition to this isolates, a number of other strains added from the culture collection of *Salmonella* Reference Laboratories in Germany. Strains were characterized by serotyping, phage typing, plasmid profiling and pulsed field gel electrophoresis. The antimicrobial susceptibilities were analysed by both disk diffusion and minimal inhibitory concentration studies. The genotypic properties of the resistance obtained were detected by PCR amplification and DNA sequence analysis. To reveal the relation of resistance with mobile genetic elements and lateral gene transfer, conjugation, transformation and class I integron detection was performed. This study exhibited the prevalent serotypes and their antimicrobial resistance of Turkish food origin *Salmonella* strains and their similarities with German *Salmonella* isolates.

CHAPTER 2

LITERATURE SURVEY

In this chapter, a review of current knowledge in the literature about *Salmonella* and antimicrobial resistance is given. The genus *Salmonella* is discussed by its genus properties, infections and epidemiology. To provide an overview of characterization of *Salmonella*, typing techniques are described. Thereafter, the dimensions of *Salmonella* infections both worldwide and in Türkiye are argued. Subsequently, antimicrobial resistance of *Salmonella* is discussed. The most common antimicrobial groups are presented and resistance mechanisms reported for *Salmonella* against those groups of antimicrobials are explained. Later, the spread of antimicrobial resistance are detailed with a special focus on horizontal gene transfer. Finally, the aim of the study is explained.

2.1. General Properties of the Genus *Salmonella*

The genus *Salmonella* belongs to the family *Enterobacteriaceae* whose members are Gram-negative, nonspore-forming, facultatively anaerobic, glucose fermenting, nitrate reducing, oxidase-negative, and straight rods of about 0.7-1.5 X 2.0-5.0 μm. Most *Salmonella* are motile via peritrichous flagellae except *S.* Gallinarum and *S.* Pullorum. The chromosome of type strain *S.* Typhimurium LT2 is 4857 kilobases (kb) with the G+C% content of 53%. Genetic relatedness of the genus is 85-100%. Based on their biochemical capacities; members of the genus *Salmonella* can be differentiated from other genera within the family *Enterobacteriaceae* (Le Minor, 1984; McClelland *et al.*, 2001).

Salmonella nomenclature has changed many times and still is not stable. The genus *Salmonella* was previously differentiated into two species: *Salmonella enterica* and *Salmonella bongori*. However, a new species, *S. subterranea* was identified and validated (Shelobolina *et al.*, 2004; Validation List No: 102, 2005). Among them, the species *Salmonella enterica* (*S. enterica*) is further divided into the six subspecies *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI). Formerly, *S. bongori* was the subspecies V, but later considered as a separate species (Fluit, 2005; Tindall *et al.*, 2005).

Fermentation of selected substances, such as dulcitol, malonate, sorbitol, d-tartrate, galacturonate, mucate, salicine, ONPG, and lactose, as well as production of enzymes such as gelatinase, γ-glutamyl-transferase or β-glucuronidase, but also lysis by phage O1 allow a differentiation between the different species and subspecies (Le Minor 1984).

Furthermore, the genus composed of over 2500 serotypes differentiated according to three different types of surface antigens discussed in section 2.2.1 in more detail. 99% of these serotypes belong to *S. enterica* and nearly 60% of them are in *S. enterica* subsp. *enterica*. The average DNA sequence similarity between *Salmonella* serotypes is 96-99% (Edwards *et al.*, 2002).

2.2. *Salmonella* Typing

Salmonella outbreaks often result from the spread of a strain whose progeny are genetically closely related. Thus, a better understanding of *Salmonella* epidemiology to control *Salmonella* infections is essential. The determination of the relatedness of strains within a *Salmonella* serotype is a prerequisite for the identification of the sources of infection and for tracing the routes of *Salmonella* dissemination in outbreaks. Since biochemical analysis did not further differentiate between the bacteria assigned to the same *S. enterica* subspecies, other methods have been used including serotyping, phage typing, and molecular analyses (Olsen, 2000; Riley, 2004).

For the purpose of strain differentiation a number of typing methods have been improved. In general, they are classified into two major groups that are conventional (=phenotypic) methods, such as serotyping and phage typing (Table 2.1) and molecular (=genotypic) methods, such as plasmid profiling, insertion sequence (IS) typing, ribotyping, randomly amplified polymorphic DNA analysis (RAPD), amplified fragment length polymorphism (AFLP), pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) (Table 2.2). An ideal typing method should fulfil the following six criteria: typeability, reproducibility, discriminatory power, and ease of interpretation, easy to use, and low cost. Any method used currently for typing of *Salmonella* strains is an ideal method alone in terms of these criteria, but all methods exhibit benefits and also limitations (Olive and Bean, 1999; Aarts *et al.*, 2001; Yan *et al.*, 2003; Lukinmaa *et al.*, 2004). In the choice of the most suitable methods for a typing system, besides considering the limitations, the aims of the studies (e.g. identification of the sources of infections, reconstruction of the chains of infection, distinguishing between an outbreak-related and –unrelated strain, global epidemiological studies) can play an important role (Riley, 2004).

It is obvious that it is difficult to find a single method, which is most suitable for typing of *Salmonella* strains. As a consequence, typing systems – consisting of several different methods – are preferentially used. In such complex typing systems, individual methods may serve to answer different questions and limitations of one method may be compensated by other methods.

Table 2.1. Phenotypic methods for typing of *Salmonella* isolates (modified from Yan *et al.*, 2003).

Method	Typeability*	Reproducibility	Discrimination	Comments
Biotyping	All	Very good	Poor	Relatively cost-effective, easy and fast to perform highly discriminative systems are not available for all different types of bacterial pathogens
Serotyping	Variable	Good – very good	Good	Easy and fast to perform (slide agglutination), standardised antisera are only commercially available for a subset of bacterial pathogens
Phage typing	Variable	Good	Good	Results are difficult to interpret, performance of the tests can be only carried out in the National Reference Laboratories, international standardised typing phages are only available for few bacterial pathogens
Antimicrobial resistance pattern	All	Good – very good	Good	Easy to perform according to standardised protocols; results are easy to interpret, large variation in the discriminatory power
Whole cell protein profiles	All	Poor – good	Poor	Difficult interpretation of the mostly very complex fragment patterns, patterns can vary according to the cultivation conditions
Multilocus-Enzyme-Electrophoresis	All	Good	Good	Difficult to standardise

***** All organisms within a species must be typeable by the method used.

Table 2.2. Molecular methods for differentiation of *Salmonella* isolates (modified from Yan *et al.*, 2003).

Method	Typeability*	Reproducibility	Discrimination	Comments
Plasmid analysis	Variable	Poor – good	Poor – good	Relatively cost-effective, easy and fast to perform instability of plasmid profiles
Ribotyping	All	Good – very good	Good	Time-consuming, no specific gene probes commercially available
IS typing	Variable	Poor – very good	Poor – good	Time-consuming, no specific gene probes commercially available
PFGE	Almost all	Good – very good	Good – very good	Time-consuming, expensive equipment and chemicals; considerable experimental experience needed
RAPD	All	Poor	Very good	Difficult to standardise; interpretation of the results often highly problematic, reproducibility is influenced by many different factors
AFLP	All	Good – very good	Very good	Time-consuming, expensive equipment and chemicals; considerable experimental experience needed
MLST	All	Good – very good	Good – very good	Time-consuming, expensive

***** All organisms within a species must be typeable by the method used.

2.2.1. Serotyping

Serotyping is the initial step for routine diagnostics of *Salmonella* strains and performed with commercially available omni-, poly- and monovalent antisera. Up to date, over 2500 serotypes of *Salmonella* has been identified and classified in the Kaufmann-White scheme. This scheme differentiates between O (=somatic) antigens of the cell surface, H1 and H2 (=flagellar) antigens of the phase 1 or phase 2, respectively (Selander *et al.*, 1996) and the Vi (=capsular) antigens which, however, may only be present in very few serotype, such as Typhi, Paratyphi C or Dublin. Each *Salmonella* serogroup has a group specific O-antigen. Within each O-group, different serovars are distinguished by the combination of O- and H-antigens that are present. Each serotype has a specific antigenic formula where the O-antigens are indicated by Arabic numbers, the H1-antigens by lower case letters and the H2-antigens again by Arabic numbers. Examples for the antigenic formulas of some few *Salmonella enterica* subsp. *enterica* serotypes are given in Table 2.3. In these formulas, underlined antigens may only be expressed once the culture is lysogenised by the corresponding converting phage whereas letters or numbers in brackets indicate antigens which may be present or absent without relation to phage conversion (Le Minor, 1984). For most of the isolates assigned to *S. enterica* and the subspecies I, antigenic formula corresponds to a serotype name. In contrast, serotypes identified after 1996 in the subspecies *salamae*, *houtenae* and *indica* and in the subspecies *bongori* are designated only by antigenic formula (Brenner *et al.*, 2000).

Table 2.3. Examples for the antigenic formulas of selected *Salmonella enterica* subsp. *enterica* serotypes according to Kaufmann-White scheme (Poppoff and Le Minor, 2001).

Serotype	**O-antigen(s)**	**H1-antigen(s)**	**H2-antigen(s)**
S. Enteritidis	1, 9, 12	[f], g, m, [p]	[1, 7]
S. Dublin	1, 9, 12, [Vi]	g, p	-
S. Gallinarum	1, 9, 12	-	-
S. Typhimurium	1, 4, 5, 12	i	1, 2
S. Virchow	6, 7	r	1, 2
S. Infantis	6, 7, 14	r	1, 5

Serotyping is easy to perform and standardized antisera are commercially available. However, it only allows the assignment of *Salmonella* strains to a specific serotype, and no further differentiation between strains of the same serotype is achieved. Besides, a small number of *S. enterica* serotypes are responsible for a majority of infections; hence subdivision of strains within a serotype is necessary.

Throughout the thesis the complete serotype designation name was replaced by an internationally accepted abbreviated designation for the ease of reading. As an example, instead of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (complete designation), only *S.* Typhimurium (abbreviated designation) was used.

2.2.2. Phage Typing

Phage typing reflects the different susceptibilities to a lytic pattern between two strains within a serotype. A *Salmonella* strain is subjected to a specified set of typing phages and the lytic pattern obtained commonly allows the assignment to a specific phage type. The strains exhibiting a lytic pattern that does not correspond to a known phage type are classified as RDNC (= **R**eacting with the typing phage, but lytic pattern **D**id **N**ot **C**orrespond to any recognized phage types). Phage typing is mostly performed for serotypes such as *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi or *S.* Paratyphi, although phage typing systems are also available for a number of additional serotypes, including *S.* Virchow. In general, phage typing is only performed by the National Reference Centers, since only these institutions have access to the defined sets of typing phages. The interpretation of the results requires considerable experience (Riley, 2004). Although, phage typing in *Salmonella* epidemiology has been used since the 1950s, the stability of phage types can be limited by phage type conversion (Rabsch *et al.*, 2002), even during an outbreak (Mmolawa *et al.*, 2002). This is due to the acquisition of a temperate phage or a plasmid. Besides, host-controlled phage defence mechanisms such as restriction/modification systems and phage adsorption inhibition are also responsible for the phage typing difficulties of a *Salmonella* strain.

2.2.3. Molecular Methods

The drawbacks of phenotypic typing methods have led to the development of typing methods based on genotypic information. Currently used molecular typing methods are based on restriction endonuclease digestion, nucleic acid amplification, or nucleotide sequencing techniques.

One of the molecular typing methods effectively in use is **plasmid analysis**. It is particularly important, since most of the plasmids harbour virulence and antimicrobial resistance properties in *Salmonella*. Plasmid content of the host within the same serotype reveals the differentiation according to the profile (the number and molecular sizes of plasmids) obtained. The different plasmid profiles within a serotype points the lateral transfer by gaining or loosing the plasmid(s). The plasmids found in *Salmonella* differ in size 2 – 200 kb with different functionalities (Aarts *et al.*, 2001; Porwollik and McClelland, 2003; Rychlik *et al.*, 2006).

Insertion sequence (IS) typing is based on the variation of insertion sequences from strain to strain. Insertion sequences are mobile DNA elements that are able to integrate within the bacterial genome. This diversity has been used for *Salmonella* with IS*200* fingerprinting (Aarts *et al.*, 2001). **Ribotyping** describes the hybridization of restriction-digested DNA fragments with probes specific for rDNA. It results in profiles only consisting of a small number of bands, which are easy to interpret. However, this feature also limits the ability to distinguish between closely related strains. Therefore, ribotyping is considered not suitable for local epidemiological studies or surveillance studies in a restricted region (Aarts *et al.*, 2001; Riley, 2004).

DNA amplification-based typing methods which use either specific or non-specific primers, yield amplicon patterns exhibiting discriminations. **RAPD (randomly amplified polymorphic DNA)** is performed with short (10 bases in length) random primers at low annealing temperatures to amplify multiple fragments of bacterial DNA. Due to the amplification conditions, this method is sensitive to slight changes within amplification parameters, thus it is hard to achieve reproducibility. However, ribotyping is a supplementary tool in conjunction with other typing methods (Olive

and Bean, 1999; Aarts *et al.*, 2001; Yan et al., 2003). Another method, the **AFLP (amplified fragment length polymorphism)** analysis combines digestion of whole cell DNA with two suitable restriction endonucleases, ligation of suitable adapters and subsequent PCR amplification. The resulting amplicons are labelled by fluorescent tags and analysed using an automated sequencer. It has been considered a highly discriminative, but labour- and cost-intensive method (Riley, 2004).

MLST (multilocus sequence typing) is a method based on the partial sequencing of a set of housekeeping genes, which varies due to mutation or recombination events. Nucleotide differences in the individual genes are combined and used to determine the differentiation of strains (Yan *et al.*, 2003). This method is extremely useful for long-term epidemiological studies or phylogenetic analyses; however, its discriminatory power is insufficient for short-term epidemiological studies or outbreak investigations. The reason for this is based on the relatively high stability of the sequenced housekeeping genes within short periods of time. Consequently, MLST does not allow a differentiation among such strains. Besides, the costs for MLST are still high (Enright and Spratt, 1999).

PFGE (pulsed field gel electrophoresis) has been considered as the "gold standard" among other molecular typing methods. It has a considerable discriminatory power, which also fulfils the criteria of typeability and reproducibility, since it is the best standardized method (Olive and Bean, 1999; Aarts *et al.*, 2001). By cutting the bacterial DNA with rare-cutting restriction endonucleases and running with special electrophoresis separation technique, it separates the large fragments of DNA up to 12000 kb) and yields strain specific patterns. It avoids the deficiency of common agarose-based DNA electrophoresis in separating large DNA fragments (>50 kb). However, this method requires the presence of expensive specialized equipment, high quality chemicals, and a considerable experience in the preparation of the DNA-containing agarose slices. Moreover, single genetic events, such as point mutations, integration, deletion or recombination events, can result in differences in the fragment patterns (Tenover *et al.*, 1995; Riley, 2004; Herschleb *et al.*, 2007).

Among the molecular typing methods, PFGE is usually considered as the method of choice to determine the molecular relatedness among *Salmonella* strains. It has also been recommended as one of the methods to be used to achieve global standardization and exchange of molecular typing data via the Internet (Lindsay *et al.*, 2002).

2.3. *Salmonella* Pathogenicity and Risk of Food Contaminations

Salmonella strains are zoonotic that infects a wide range of host organisms from reptiles to human (Selander *et al.*, 1996; Tükel *et al.*, 2005; Tükel *et al.*, 2006; Tükel *et al.*, 2007). Serotypes differ in their host adaptations and virulence that can be classified into different groups (Uzzau *et al.*, 2000). Isolates, which are pathogenic to man belong to subspecies I (Fluit, 2005). Human-adapted serotypes, such as *S.* Typhi and *S.* Paratyphi, cause severe typhoidal disease in humans, and not pathogenic to animals. Contrary to this, serotypes adapted to animals such as *S.* Gallinarum (poultry), *S.* Choleraesuis (swine), *S.* Abortusovis (sheep) or *S.* Dublin (cattle) are the ones that cause systemic illness in their primary hosts. Non-host adapted serotypes also differ in their virulence properties with high virulence, such as *S.* Enteritidis and *S.* Typhimurium, or low virulence, such as *S.* Agona, *S.* Derby and *S.* Hadar. Despite this classification, all animal origin serotypes are considered as potential pathogen in humans (Giannella, 1996).

The major sources of *Salmonella* are mainly beef, poultry and eggs, pork and dairy products. Once the contaminated food is ingested and *Salmonella* survive the acidic environment of stomach, it colonizes in the gastrointestinal tract. Adherence of intestinal epithelium is followed by entry to lamina propria. At this site, the bacteria may replicate and establish a local infection, or they may be ingested by macrophages, which may disseminate the *Salmonella* to deeper tissues such as regional lymph nodes, liver, and spleen to establish a systemic infection (Selander *et al.*, 1996). While thyphoidal diseases occur with the invasion to blood, gastroenteritis causing *Salmonella* rarely invade blood. However, this invasion also leads to a systemic disease, which requires antimicrobial treatment of the patient.

The term “salmonellosis” comprises for the clinical disease gastroenteritis. *Salmonella* gastroenteritis is characterized by a sudden onset of diarrhoea, headache, abdominal pain, vomiting and fever. The incubation period is 6-72 h depending on host and inoculum. Certain serotypes, such as *S.* Typhi and *S.* Paratyphi are the causative agents of typhoid fever. Patients usually have fever as high as high as 40°C in addition those mentioned symptoms. Risk factors for salmonellosis include extremes of age, alteration of the endogenous flora of the intestine (e.g. as a result of antimicrobial therapy or surgery), diabetes, malignancy, rheumatologic disorders, infections like HIV, and therapeutic immunosuppressant of all types (Hohmann, 2001). The mild cases of gastroenteritis are treated by dehydration with commercial dehydration solutions, whereas in severe gastroenteritis, septicaemia or typhoid fever requires antimicrobial drug treatment where β-lactam and quinolone group of antimicrobials are used.

The surveillance studies are done worldwide to control the *Salmonella* epidemics. The most extensive global statistics are from World Health Organization (WHO) and the most prevalent serotype reported is *S.* Enteritidis (61%) from human and *S.* Typhimurium (23%) from non-human sources between the years 2000-2005 (http://www.who.int/salmsurv/links/GSSProgressReport2005.pdf). In the regional bases, Centres for Disease Control and Prevention (CDC) and Health Protection Agency (HPA) are the institutions conducting the survey of *Salmonella* epidemics in United States and Europe, respectively. According to CDC annual report on 2005, the number of cases of typhoid fever has been relatively small and constant, mostly associated with travel outside the United States. *S.* Typhi isolates are reported through the National Salmonellosis Surveillance System; 348 isolates were reported in 2005. On the other hand, a total of 35.836 non-typhoidal *Salmonella* isolates were reported in 2005. The national rate was 12.2 per 100.000 population. The thirty most common serotypes of *Salmonella* in 2005 represent 82% of all *Salmonella* isolates where *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg; (52% of all isolates) were the four most common serotypes (http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2005/SalmonellaAnnualSummary2005.pdf). On the other hand, HPA carry out a project leading by the European

Commission called Enter-Net, where the international surveillance network for human gastrointestinal infections is conducted (http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm). Enter-Net gives quarterly reports about *Salmonella* every year. According to the latest quarterly report from January-March 2007, the total number of human *Salmonella* isolates are 16.488 with 11.059 (67,1%) being *S.* Enteritidis, *S.* Typhimurium or *S.* Virchow (http://www.hpa.org.uk/hpa/inter/enter-net/07q1summ.pdf).

2.4 *Salmonella* Infections in Türkiye

As in whole world, *Salmonella* infections are also common in Türkiye. However, there is no national *Salmonella* reference centre to provide reliable statistical data neither for outbreaks nor for isolates. Thus, tracing of *Salmonella* infections, epidemics or serotype prevalence in clinical, animal or food isolates is not possible.

Attempts are made in the institute laboratories belonging to Turkish Ministry of Agriculture to establish the routine serotyping and antibiotyping of *Salmonella* isolates from foods. In a study performed by two Veterinary Research Institutes (in Ankara and Adana, respectively) of the ministry, in the isolates from neck and wing samples of poultry, the most prevalent serotypes were reported as *S.* Enteritidis (32%), *S.* Virchow (31%), and *S.* Typhimurium (Yazıcıoğlu *et al.*, 2005).

In the annual reports of the year 2005 of Turkish Ministry of Health, biological infections are given in groups where 1806 cases of *S.* Typhi with the morbidity level of 7,17 per 100.000 population are recorded. The other salmonellosis causative agents has been serogrouped but not serotyped and among them the highest cases observed is *Salmonella* serogroup O:9 (D1) with 100 cases. It must be noted that in this serogroup *S.* Enteritidis is the most prevalent one in worldwide.

Other epidemiological data on *Salmonella* strains isolated in Türkiye can be obtained only through the collection of findings from individual studies. There is only one study in the literature found as having the most extensive scope of surveillance conducted by Erdem *et al.* (2005). The research covering clinical samples from 10

Turkish provinces collected over 2000-2002 years period. The most prevalent serotypes were found to be *S.* Enteritidis (47,7%) and *S.* Typhimurium (34,7%). On the other hand, the information on food isolates is poorer in comparison to clinical isolates. In a recent study, 75,4% of isolates from infected chickens was reported as seogroup D1 where 71,7% was typed *S.* Enteritidis and as 28,3% *S.* Gallinarum. The rest of the isolates were found to be *S.* Typhimurium (24,6%) (Kılınç and Aydın, 2006).

2.5. Antimicrobials and Antimicrobial Resistance

Antimicrobials used in the therapy of infectious diseases are the drugs that either kill or suppress microorganisms such as bacteria, viruses and parasites. Antibiotics are the subgroup of antimicrobials that act only against bacteria. The actions of antibiotics are (i) inhibition of cell wall synthesis, (ii) inhibition of protein synthesis, (iii) inhibition of DNA/RNA precursor (folate) synthesis, (iv) inhibition of DNA/RNA synthesis, and (v) disruption of membrane proteins (Walsh, 2003).

Antimicrobials have not only been used for therapy of diseases but also have been used in food animal production for prophylaxis, metaphylaxis and as growth promoters. Even though all growth promoters with antimicrobial activity have been banned since 1999 in the Europe – especially for prophylactic and metaphylactic applications – they still constitute a major selective pressure, which affects resistance development not only in *Salmonella*, but also in many other bacteria (Schwarz and Chaslus-Dancla, 2001). The selective pressure refers to the impact of antimicrobial use on a microbial population, in which resistant organisms gain a survival advantage over those susceptible ones (Furuya and Lowy, 2006). According to Clinical Laboratory Standards Institute (CLSI), resistance designates the isolates that are not inhibited by “usually achievable systemic concentration of the agent with normal dosage schedules and/or fall in the range where specific antimicrobial resistance mechanisms are likely (e.g. beta-lactamases) and clinical efficacy has not been reliable in treatment studies” (CLSI, 2006).

A constant increase in the numbers of resistant strains has been observed since the 1950s. This is based on the distinctly higher selective pressure as imposed by the use of antimicrobial agents for various purposes in human and veterinary medicine, aquaculture and horticulture during the last 60 years (Helmuth, 2000; Schwarz and Chaslus-Dancla, 2001). According to the bulletin of WHO in 2002, the mortality rate in outbreaks involving resistant strains of *Salmonella* spp. was found to be 3,4%, whereas it was only 0,2% in those sensitive strains (Smith and Coast, 2002).

Another concerning aspect of antimicrobial resistance is the multidrug resistance of pathogens, which makes the selection of antimicrobials more difficult in the clinical treatment of the disease. The most common *Salmonella* serotype having multidrug resistance is *S.* Typhimurium definitive phage type DT104. It acquired multiple drug resistance, with an isolate from the United Kingdom found to display a phenotype of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) so called penta-resistance. The zoonotic nature of DT104 may have provided the environment for the acquisition of the ACSSuT resistance phenotype since this resistance includes four of the five most common drug classes used in veterinary medicine (Mulwey *et al.*, 2006). Later, it was seen that multidrug resistance phenotype is emerging in other *Salmonella* serotypes too. In the recent studies, serotypes such as *S.* Typhi, *S.* Paratyphi, *S.* Infantis, *S.* Uganda, *S.* Agona, and *S.* Newport, *S.* Hadar, *S.* Heidelberg are exhibited multidrug resistance in addition to *S.* Typhimurium (Martinez *et al.*, 2005; Velge *et al.*, 2005; Pokharel *et al.*, 2006; Holt *et al.*, 2007; Nógrády *et al.*, 2007; Zhao *et al.*, 2007).

The resistance mechanisms can be classified as (i) destruction or modification of the antimicrobial agent, (ii) pumping the antimicrobial agent out from the cell by efflux pumps, (iii) replacement or modification of the antibiotic target, and (iv) reduction in cell membrane permeability (Fig. 2.1). Microorganisms are developing resistance mechanisms by accumulating mutations in the gene locations of target proteins or acquiring mobile genetic elements carrying resistance genes (Walsh, 2003).

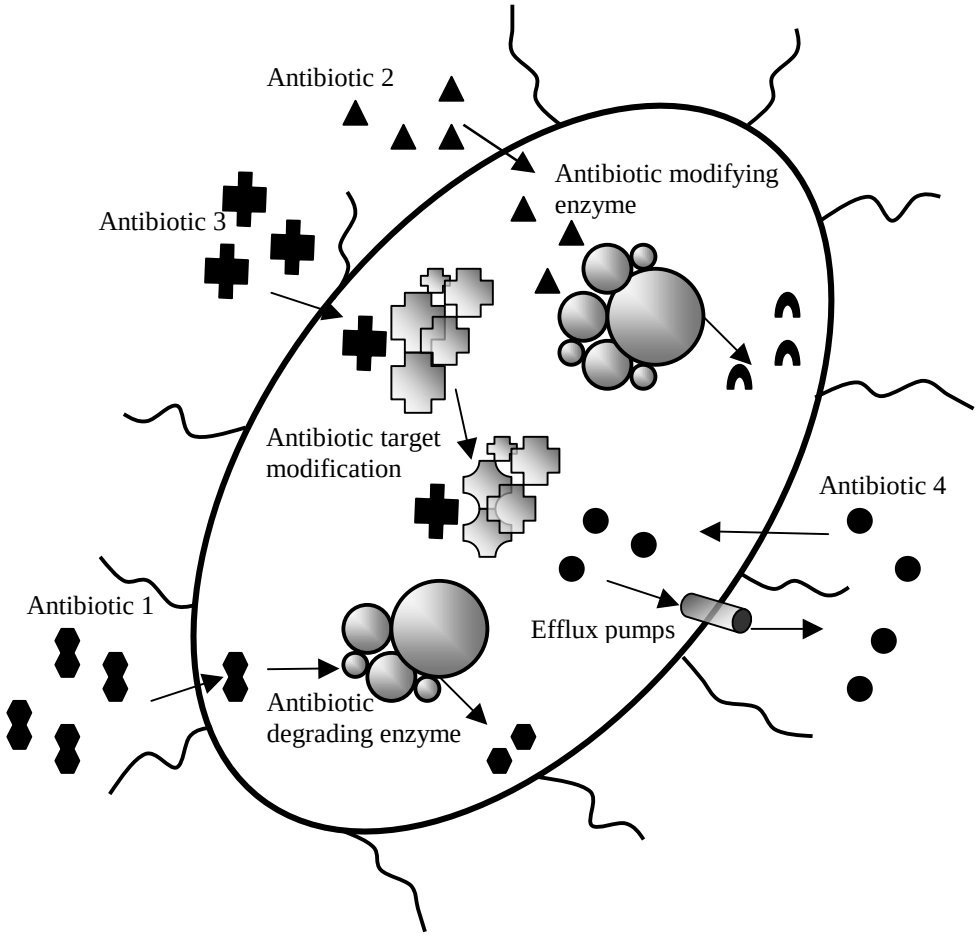


Figure 2.1. Schematic representation of the mechanisms of antimicrobial resistance (modified from http://www.chembio.uoguelph.ca/merrill/research/ enzyme_mechanisms. html).

There are several antimicrobial drug classes, however it is not the purpose to classify all antimicrobial groups in detail, but to classify the most common antimicrobials that *Salmonella* have been developed resistance in present thesis. They are namely β-lactams, quinolones/fluoroquinoles, aminoglycosides, phenicols, tetracyclines, sulfonamides and trimethoprim.

β-lactams are one of the critically important antibiotics in both human and veterinary medicine. Penicillins are also used for growth promoters of animals (Li *et al.*, 2007). β-lactams are bacteriocidal by blocking the transpeptidations peptidoglycan layer of bacterial cell wall where they bind to penicillin binding-proteins.

There are three major groups of β-lactams: penicillins, cephalosporins, and carbapenems. Possibly because of the widespread clinical use of penicillins, resistance to drugs such as ampicillin and methicillin has become common. In response to this problem, second class of β-lactams, the cephalosporins, was developed. Penicillins have a five-member thiazolidine ring fused to the β-lactam ring. Cephalosporins are the enzymatically converted form of penicillins by a ring expandase enzyme forming a six-member ring (a dihydrothiazine ring) fused to the β-lactam ring (Walsh, 2003; Alcaine *et al.*, 2007). These changes provide cephalosporins with a broader range of activity and greater stability in the presence of β-lactamases. Both penicillins and cephalosporins are fungal secondary metabolites produced by *Penicillium chrysogenum* (Walsh, 2003). There are four generations of cephalosporins, and each progressive generation is effective against a broader range of organisms. Although *Salmonella* isolates may appear susceptible to first- and second-generation of cephalosporins in vitro, the CLSI cautions that this antimicrobial drug class may not be clinically effective against *Salmonella* soon (Alcaine *et al.*, 2007).

The latest discovered group of β-lactams is carbapenems, which differs from penicillins and cephalosporins by lacking sulphur in five-membered ring fused to the four-member β-lactam ring. These β-lactams are sometimes paired with β-lactamase inhibitors. Carbapenems have a much broader range of activity against both Gram-negative and Gram-positive bacteria than do other β-lactams and are more stable against β-lactamases. Nevertheless, *Salmonella* isolates that possesses resistance to carbapenems such as imipenem already have been reported (Singh *et al.*, 2007).

β-lactam resistance mechanism in *Salmonella* is mostly mediated by the production of the enzymes β-lactamase. These enzymes work by hydrolysing the β-lactam ring structure, yielding beta-amino acids with no antimicrobial activity. The genes encoding for β-lactamases produced by *Salmonella* are typically carried on plasmids, although most of these genes are chromosomally encoded in other bacterial species.

β-lactamases are classified by two schemes called Ambler classes A-D (Ambler, 1980) and Bush classes 1-4 (Bush et al., 1995). Ambler's classification scheme is based on the primary structure and amino acid sequence identity of β-lactamases. According to Ambler's classification scheme there are class A, B, C, and D beta-lactamases (Alcaine *et al.*, 2007; Li *et al.*, 2007):

- In general, **class A β-lactamases** are the most commonly reported class of β-lactamases in *Salmonella*. They are plasmid encoded and provide a range of resistance against penicillins, early generation cephalosporins, and carbapenems. There are several different gene families encoding for enzymes in this class, and bla_{TEM-1} is the most prevalent among *Salmonella* isolates. Other Class A β-lactamase gene such as bla_{PSE-1} also has been found in a number of *Salmonella* isolates and chromosomally encoded (Li *et al.*, 2007). The emergence of cefotaximases (CTX-M), which are class A β-lactamases conferring resistance pirmarily to ceftiofur, is an important trend to watch. Variants of bla_{CTX-M} have been identified in isolates of *Salmonella* serotypes (Livermore *et al.*, 2007).
- **Class B β-lactamases** are metallo-β-lactamases which are not commonly found in *Salmonella*.
- **Class C β-lactamases** are typically encoded by chromosomal *ampC* genes and provide resistance against cephalosporins and ceftiofur. *Salmonella* has no chromosomal *ampC* gene; instead, these genes are harboured in plasmids. Currently researches are primarily focused on bla_{CMY-2}, which has been associated with resistance primarily to cephoxitin. The spread of bla_{CMY-2} is a public health concern because the presence of this gene appears to mediate resistance or at least reduced susceptibility to ceftriaxone, another extended spectrum cephalosporin that is the drug of choice for the treatment of *Salmonella* infections in children.
- **Class D β-lactamases** appear to be rare among *Salmonella* isolates. This class of enzymes provides resistance to β-lactams closely related to oxacillin and methicillin. The chromosomally encoded gene bla_{oxa-1} (=bla_{oxa-30}) was found in a *S.* Paratyphi, *S.* Muenchen and *S.* Typhimurium. This group is

resistant to inhibitors such as clavulanic acid (Hall and Collis, 1998; Alcaine *et al.*, 2007).

Quinolones and fluoroquinolones are synthetic bacteriocidal drugs. In 1962, nalidixic acid became the first quinolone approved for medical use. Several generations of quinolones have been developed, with each new generation having improved action against bacterial infections. The early generation quinolones target DNA gyrase, and the late generation quinolones both DNA gyrase and topoisomerase IV. The mode of action for quinolones is quite complex and not completely understood. Although quinolones target topoisomerases, they do not actually bind to the topoisomerase but to the double stranded DNA in the topoisomerase complex (Alcaine *et al.*, 2007).

There are documented cases of *Salmonella* isolates with resistance to nalidixic acid and low-level resistance to fluoroquinolones and high-level resistance to quinolones to be emerging. Quinolone resistance of *Salmonella* isolates has been linked to two mechanisms, target gene mutations and active efflux. The first mechanism is mediated by target mutations in the quinolone resistance determining region (QRDR) of *gyrA* and *gyrB*, the two genes that encode the subunits of DNA gyrase, and in the *parC* subunit of topoisomerase IV. The most frequently amino acid substitutions observed in *gyrA* are Ser-83 (to Phe, Tyr, or Ala) or Asp-87 (to Gly, Asn, or Tyr) and in *parC* is Thr-57 (to Ser) (Cloeckaert and Chaslus-Dancla, 2001). The second mechanism involves changes in the expression of the AcrAB-TolC efflux system, mostly due to mutations in the genes encoding regulators of this system (e.g., *marRAB*) that results in overexpression and consequently decreased quinolone sensitivity. No single mutation confers high-level resistance to fluoroquinolones but resistance results from the accumulation of multiple mutations. The facts that *Salmonella* isolates must acquire multiple unlinked mutations and that some of those mutations reduce fitness, particularly those involved in the regulation of the efflux pump, may explain why this kind of resistance is so infrequent (Alcaine *et al.*, 2007).

Quinolone resistance also has been linked to the expression of the plasmid-mediated *qnr* gene. This gene codes for a protein that appears to bind to DNA gyrase and protect it from quinolone inhibition. Research conducted on plasmids harbouring *qnr* revealed that this gene could be transferred from other bacterial species to *Salmonella* via conjugation. Although documented cases of plasmid-mediated quinolone resistance in *Salmonella* isolates are rare, a recent study indicated that the spread of such plasmids to *Salmonella* isolates has also occurred (Kehrenberg *et al.*, 2007). The appearance of plasmid-mediated quinolone resistance in *Salmonella* isolates is a very important emerging public health concern. Plasmids harbouring *qnr* also can harbour other resistance genes, suggesting that the treatment of infections with *Salmonella* strains containing this plasmid may be increasingly difficult. In a recent study, reduced susceptibility ciprofloxacin was conferred by a variant of the gene encoding aminoglycoside acetyltransferase AAC(6')-Ib. Even if the gene was detected among other *Enterobacteriaceae*, it has to the best of our knowledge that this gene has not been identified yet in *Salmonella* isolates (Kehrenberg *et al.*, 2007).

Aminoglycosides were first discovered in 1944 from *Streptomyces griseus* and since have been widely used. Other aminoglycosides are kanamycin, neomycin, amikacin, and gentamicin. They are hydrophilic sugars with multiple amino groups and target 16S rRNA on the 30S ribosome from the A site of aminoacyl-tRNA binding which leads to codon misreading and translation inhibition. Most aminoglycosides are bactericidal (destructive), with the exception of spectinomycin, which has a bacteriostatic (growth inhibiting) mode of action. (Walsh, 2003; Alcaine *et al.*, 2007).

Resistance to aminoglycosides in *Salmonella* is mainly associated with the modification of aminoglycoside molecules by enzymes (Sørum and L'Abée-Lund, 2002). These enzymes fall into three groups that are named according to the types of reactions they catalyse (Alcaine *et al.*, 2007):

- **Aminoglycoside acetyltransferases** are enzymes that primarily acetylate aminoglycoside-amino groups. Genes encoding these enzymes are typically designated *aac* and these genes have been found as part of *Salmonella*

genomic islands, integrons and plasmids. Aminoglycoside acetyltransferases provide resistance to gentamicin, tobramycin, and kanamycin.

- **Aminoglycoside phosphotransferases** are enzymes that catalyse ATP-dependent phosphorylation of specific aminoglycoside hydroxyl groups. Most genes encoding these enzymes are designated as *aph* provide resistance to kanamycin and neomycin. The genes *aph(3')-Ib* and *aph(6)-Id* are commonly referred in the literature as *strA* and *strB*, respectively and provide resistance to streptomycin.
- **Nucleotidyltransferases** also target the hydroxyl groups. Genes encoding nucleotidyltransferases are usually designated *aad* (for aminoglycoside adenyltransferases), although some are also designated as *ant* (for aminoglycoside nucleotidyltransferase). The *aadA* gene [or *ant(3')*] provides streptomycin resistance in *Salmonella* isolates. The *aadB* gene [or *ant(2')-Ia*] contributes resistance to gentamicin and tobramycin. Both *aadA* and *aadB* have been found as integron-borne gene cassettes.

Phenicols include chloramphenicol and florfenicol. Chloramphenicol was once the drug of choice for the treatment of typhoid fever. Production of chloramphenicols by *Streptomyces venezuelae* was discovered in 1947. Chloramphenicol works by binding to the peptidyltransferases centre of the 50S ribosomal unit, thus preventing formation of peptide bonds. Chloramphenicol's broad range activity against Gram-positive and Gram-negative bacteria and its ability to cross the blood-brain barrier make it a powerful choice for the treatment of systemic infections. Its toxicity, which can lead to bone marrow damage and aplastic anemia, and widespread resistance have generally limited chloramphenicol use to occasions where the risk of the infection, such as bacterial meningitis, is greater than the risk of adverse effects from the drug. Chloramphenicol is still widely used in developing countries because of its low cost (Walsh, 2003; Alcaine *et al.*, 2007).

Chloramphenicol resistance in *Salmonella* isolates is conferred through two mechanisms: (i) the enzymatic inactivation of the antibiotic via chloramphenicol *O*-acetyltransferase (CAT) and (ii) the removal of the antibiotic via an efflux pump.

The genes encoding for CAT are plasmid-borne and commonly found in *S.* Typhi isolates. CAT genes, such as *cat1* and *cat2*, have also been found in nontyphoidal *Salmonella* serotypes. Chloramphenicol efflux pumps in *Salmonella* isolates have been reported to be encoded by two closely related genes, *cmlA* and *floR*. The *floR* gene appears to be widespread in *Salmonella* isolates, whereas *cmlA* is less widely distributed. The highly mobile *floR* gene has been found in *Salmonella* genomic islands and in many different plasmids. It appears to be associated with multidrug resistance.

Tetracyclines were discovered in the 1940s. The first tetracycline, chlorotetracycline, was isolated from *Streptomyces aerofaciens*. Tetracyclines were popular because of their minimal adverse effects and broad-spectrum activity. They were effective against most bacteria, including chlamydias and mycoplasm, and even some protozoa. Tetracyclines act by preventing the binding of tRNA to the A site of the 30S ribosomal subunit, thus inhibiting protein synthesis. Unfortunately, the rise of resistant bacteria has severely limited the use of tetracyclines (Walsh, 2003; Alcaine *et al.*, 2007).

Tetracycline resistance of *Salmonella* isolates is attributed to production of an energy-dependent efflux pump, which removes this antimicrobial drug from the bacterial cell. Other mechanism of resistance, such as modification of the ribosomal target and enzymatic inactivation of tetracycline, have been attributed to other bacterial species but have yet to be reported in *Salmonella* isolates. Deletion or inactivation of *marRAB* operon also has been linked to the reduced susceptibility to tetracycline.

There are at least 32 different genes that confer resistance to tetracycline and oxytetracycline. Of these, *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(G), and *tet*(H) have been found in *Salmonella* isolates. The most commonly reported one of these genes is *tet*(A). It has been found in *Salmonella* genomic island 1, on integrons, and on transferable plasmids. The *tet*(A) gene has been detected in isolates of *Salmonella* serotypes. Like *tet*(A), *tet*(B) has also been located on transferable plasmids. These

genes appear to be easily transferred and widespread among *Salmonella* isolates. They also tend to be found in isolates that display multidrug resistance, making them an important marker in identifying potentially serious *Salmonella* infections. *tet*(G) is linked to *Salmonella* Genomic Island 1 (SGI1).

Sulfonamides and Trimethoprim prescribed separately and has been used in combination for the treatment of bacterial infection since the late 1960s. These compounds are bacteriostatic antimicrobial drugs that act by competitively inhibiting enzymes involved in the synthesis of tetrahydrofolic acid. Sulfonamides inhibit dihydropteroate synthetase (DHPS), and trimethoprim by inhibiting dihydrofolate reductase (DHFR). The combination of a sulfonamide and trimethoprim has been a popular form of treatment for decades, and although resistance among *Salmonella* isolates has emerged, this resistance does not appear to be common (Walsh, 2003; Alcaine *et al.*, 2007).

Sulfonamide resistance in *Salmonella* isolates has been attributed to the presence of an extra *sul* gene, which expressed an insensitive form of DHPS. Three main *sul* genes have been identified: *sul1, sul2, sul3*. The *sul1* gene has been found in a wide range of *Salmonella* serotypes. This gene is often associated with class I integrons that contain other resistance genes. These integron-borne gene cassettes have been found on transferable plasmids and as part of *Salmonella* genomic island variants. Although sometimes found in *Salmonella* isolates also harbouring *sul1, sul2* appears to be associated with plasmids, but not with class I integrons. Isolates of *Salmonella* serotypes Agona, Enteritidis, Typhimurium have been reported to carry *sul2*. The *sul3* gene has been identified only recently in *Salmonella*, and it has been associated with plasmids and class I integrons, suggesting that there may be further dissemination of this gene within *Salmonella* populations (Guerra *et al.*, 2004a). Deletion or inactivation of the *marRAB* also has been linked to reduced sulfonamide susceptibility.

Similar to sulfonamide resistance, trimethoprim resistance is attributed to the expression of DHFR that does not bind trimethoprim. There are minivariants of the

dhfr and *dfr* genes that encode this resistance, such as *dhfr1*, *dfrA1*, and *dhfr12*. These genes have been found as part of integron borne gene cassettes also associated with *sul1* and *sul3*, on transferable plasmids carrying other resistance genes, and *Salmonella* genomic islands.

2.6. Antimicrobial Patterns of *Salmonella* Isolates in Türkiye

Although there is limited information about the antimicrobial resistances of the *Salmonella* isolates in Türkiye, there are studies, which performed the antimicrobial susceptibility analysis. In a study from 13 *S.* Typhimurium and 22 *S.* Enteritidis clinical isolates, 10 different resistance patterns among *S.* Typhimurium and 4 different resistance patterns among *S.* Enteritidis were detected. ESBL production was also detected in 10 of *S.* Typhimurium *and* 3 of *S.* Enteritidis isolates (Anğ-Küçüker *et al.*, 2000). Resistance to multiple antimicrobials and reduced susceptibility to fluoroquinolones was also reported from the clinical *Salmonella* isolates (Erdem *et al.* 2005). Yazıcıoğlu *et al.* (2005) found that among 58 *Salmonella* isolates of avian origin, the most common resistances were against nalidixic acid in 26 strains (48,1%) and streptomycin in 10 strains (17,2%).

2.7. Horizontal Transfer of Antibiotic Resistance

The spreading of resistance genes between members of different bacterial species and genera under natural conditions requires a horizontal transmission by conjugation, mobilization, transduction or to a lesser extent by transformation. For efficient horizontal gene transfer, two key factors are of importance: (1) a sufficiently high bacterial density, which enables the close contact of the partners between which genes are exchanged, and (2) the location of the transferred genes on mobile genetic elements. Such elements include plasmids, transposons, integrons and gene cassettes, and chromosomal genomic islands (Schwarz *et al.*, 2006). Studies have shown that one or more of these elements may be present in antimicrobial resistant *Salmonella* strains.

2.7.1. Plasmids

Plasmids are double-stranded DNA elements of variable in size. Due to their replication system, they can replicate independently from the chromosomal DNA in the bacterial cell. Plasmids may be present in single or multiple copies per bacterial cell. They can harbour resistance genes, but also virulence genes or the genes for metabolic activities. Large plasmids may also carry the *tra* gene complex, which enables the transfer of plasmid horizontally by conjugation. Plasmids can act as vectors for transposons and integrons/gene cassettes (Guerra *et al.*, 2000b; Schwarz *et al.*, 2006). Plasmids are classified based on their replication origin into incompatibility (Inc) groups. Plasmids having same replication origin are "incompatible" whereas plasmids with different replication origin are "compatible" (Carattoli *et al.*, 2005).

2.7.2. Transposons

Transposons are also double-stranded DNA elements of variable in size, which – in contrast to plasmids – cannot replicate autonomously. Since they lack an own replication system, they have to integrate into a replication-proficient molecule in the bacterial cell, such as the chromosomal DNA or plasmids. Large transposons have also been identified to be conjugative whereas small transposons can only change their host cell as part of a plasmid into which they have integrated. Transposons can change their location within the bacterial cell by transposition (Schwarz *et al.*, 2006). In this regard, transposons integrate into very specific sites whereas others just integrate into host DNA without any known preference (Waturangi *et al.*, 2003).

2.7.3. Integrons

Integrons are known as natural cloning and expression vectors that mediate the integration or excision of gene cassettes (Recchia and Hall, 1995; Carattoli, 2001, Rowe-Magnus and Mazel, 2002; Mazel, 2006). Gene cassettes are small, double-stranded mobile genetic elements. A resistance gene cassette consists of a resistance gene and a recombination site, designated *attC*. This site can vary in size and contains the binding sites for the integrase which catalyses the integration and excision of the gene cassettes. After excision and before integration, gene cassettes

form an intermediate circular form (Fig. 2.2). The *attC* sites commonly have a central axis of symmetry and represent imperfectly inverted repeats. A gene cassette usually does not have its own promoter, but its cassette-borne gene is transcribed from a promoter located in the 5' conserved segment of the integron. Class 1 and 2 integrons of commonly consist of two conserved segments (CS), 5'-CS and 3'-CS, which bracket a variable region that can contain one or more gene cassettes (Schwarz *et al.*, 2006). The essential components of an integron are the integrase gene (*intI*), the attachment site (*attI*) and the promoter, which is essential for the expression of the integrated gene cassettes (Carattoli, 2001). Different classes of integrons have been defined based on the homology of the integrase proteins. Classes 1 and 2 are most commonly found in Gram-negative bacteria. The 5'-CS part contains the integrase gene, the attachment site and the promoter. The 3'-CS harbours the *qacEΔ1* gene, a semi-functional derivative of the quaternary ammonium compounds resistance gene *qacE*, the sulfonamide gene *sul1* and an open reading frame of unknown function named ORF5 in class 1 integrons whereas it contains transposition genes in class 2 integrons. The development of multiple resistance is based on the capacity of integrons to cluster the gene cassettes and to express antimicrobial resistance genes (Recchia and Hall, 1995; Carattoli, 2001; Schwarz *et al.*, 2006). Integrons are not mobile by themselves, but may be integrated into transposable elements, such as Tn*1696* (Partridge *et al.*, 2001), or located on plasmids, and then can spread with these elements. Moreover, integrons can also be located on chromosomal DNA or in chromosomal genomic islands, such as the *Salmonella* Genomic Island 1 (SGI1) (Boyd *et al.*, 2001).

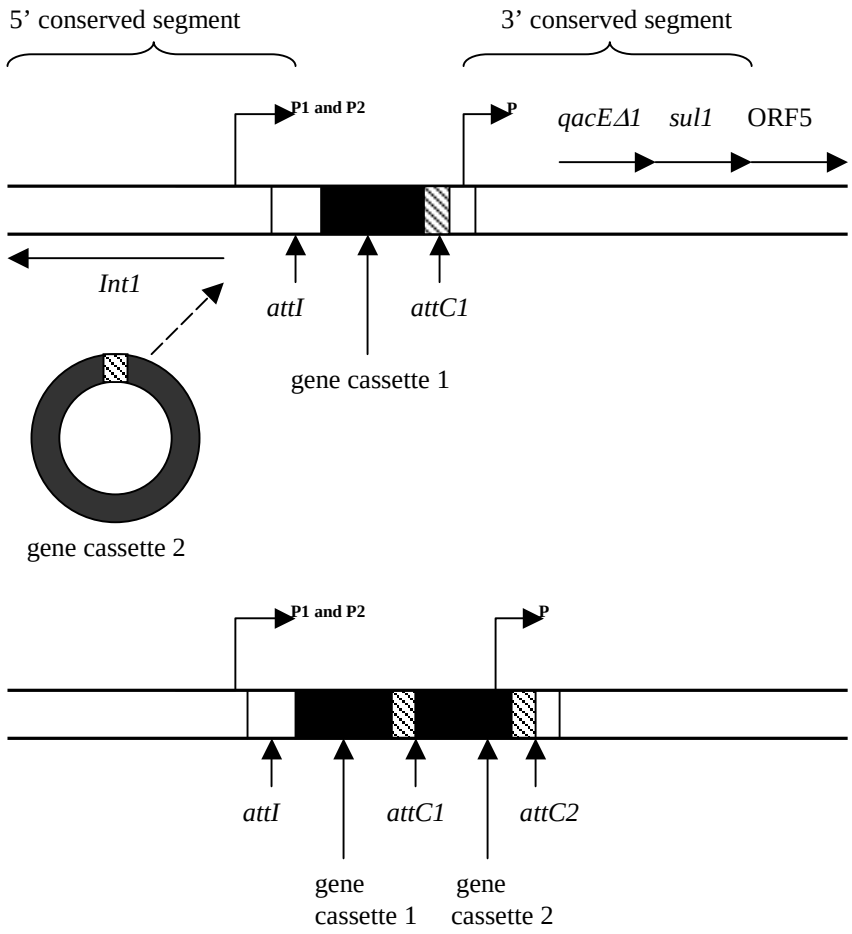


Figure 2.2. Typical structure of a class 1 integron (modified from Carattoli *et al.*,2001).

2.7.4. Genomic Islands

Genomic islands are large elements found in the chromosomal DNA of bacteria. The best studied genomic islands associated with antimicrobial resistance are the 43-kb *Salmonella* genomic island 1 (SGI1) in various *Salmonella* serotypes (Boyd *et al.*, 2001), the 100-kb SXT element of *Vibrio cholerae* (Hochhut *et al.*, 2001), and SCCmec elements of different sizes and structures in *Staphylococcus aureus* (Katayama *et al.*, 2000). These genomic islands integrate site-specifically into the *S.* Typhimurium DT104 (Boyd *et al.*, 2001). In the meantime SGI1 was also identified in other *Salmonella enterica* serovars, including Agona, Albany, Newport, Meleagridis, and Paratyphi B (Boyd *et al.*, 2001; Doublet *et al.*, 2003; Doublet *et al.*, 2004a; Doublet *et al.*, 2004b; Ebner *et al.*, 2004; Meunier *et al.*, 2002). SGI1 is

horizontally transferable and has been identified as an integrative mobilizable element (Doublet *et al.*, 2005). The 14 kb antibiotic resistance gene cluster within SGI1 (Briggs and Fratamico, 1999) consists of a complex integron related to the IN4 group of integrons. In most known cases, the pentaresistance phenotype associated with SGI1 includes ampicillin resistance via bla_{pse-1}, chloramphenicol/florfenicol resistance via *floR*, streptomycin/spectinomycin resistance via *aadA2*, sulfonamide resistance via *sul1*, and tetracycline resistance via *tet(R)-tet(G)*. During recent years, variant clusters have been identified containing additional or other resistance genes such as *dfrA1* and *dfrA10* conferring resistance to trimethoprim, *aadA7* conferring resistance to streptomycin, and *aac(3)-Id* conferring resistance to gentamicin (Boyd *et al.*, 2002; Doublet *et al.*, 2003; Doublet *et al.*, 2004a; Doublet *et al.*, 2004b).

Currently, research activities on antimicrobial resistance in *Salmonella* isolates from animal sources focus on quinolone/fluoroquinolone resistance – with particular emphasis on transferable *qnr*-based quinolone resistance – the detection of transferable genes coding for extended-spectrum β-lactamases, as well as the identification of the genetic basis of multi-resistance due to either SGI1 variants or multi-resistance integrons.

2.7. Aims of the Present Study

There is still considerable lack of information with regard to (i) the prevalent serotypes, (ii) the molecular characteristics (iii) the antimicrobial patterns, and (iv) the genetic basis of antimicrobial resistances of *Salmonella* strains isolated from foods in Türkiye. The objective of the present study was to contribute some information to close the gaps in these areas of research. Besides, it was also aimed to search for the genetic basis of horizontal gene transfer, based primarily on class 1 integrons, of antimicrobial resistances found in Turkish isolates. The second part of the study was to perform the same determinations with the German isolates of *Salmonella* to form a comparative base of the current situations in both countries.

CHAPTER 3

MATERIALS AND METHODS

This chapter explains the materials and the methods used throughout the thesis in detail. Collection of food samples, isolation of *Salmonella* strains of Türkiye origin, and biochemical confirmations were performed in Ankara University, Department of Biology, Prokaryotic Genetics Laboratories, whereas serotyping, phage typing antibiotyping and all the molecular biological analyses were done in Federal Institute of Risk Assessment, *Salmonella* Reference Laboratories in Germany. *Salmonella* strains, which constitute the base material of the thesis, were isolated from foods of animal origin. Their confirmation was done first by biochemical analyses. Furthermore, in the frame of the objectives of the thesis strains were characterized by selected methods covering both phenotypic and genotypic analysis namely serotyping, phage typing, and plasmid profiling and pulsed field gel electrophoresis. Subsequently, strains were examined for their antimicrobial susceptibilities by both disk diffusion and minimal inhibitory concentration analysis. When the resistance was observed, the genotypic properties of the phenotypes were investigated. In order to associate the resistance genotype with mobile genetic elements, thesis focused on class 1 integrons. To characterize the important plasmids obtained, plasmid incompatibility group detection was done. For genotypic analysis, in general, polymerase chain reaction, southern-blotting, DNA-hybridization and DNA-sequencing methods were used. All the materials other than biological materials were given in Appendix I.

3.1. Bacterial Strains and Bacteriophages

59 epidemiologically unrelated *Salmonella enterica* subsp. *enterica* serovars were isolated from foods of avian and bovine origin and 49 *Salmonella enterica* subsp. *enterica* serovars together with 17 control strains were obtained from Federal Institute of Risk Assessment, *Salmonella* Reference Laboratories Culture Collection. The bacteria were routinely grown in Luria Bertani (LB) broth at 37ºC with shaking at 200 rpm when necessary. The bacteria were stored at -80ºC in 60% glycerol containing LB broth. Bacteriophages (phages) for phage typing sets were obtained as stock cultures from Health Protection Agency, Specialist and Reference Microbiology Division, Centre for Infections, Laboratory of Enteric Pathogens (HPA, London, UK) and kept as concentrated high titre stocks at +4ºC. The stocks were diluted to appropriate concentrations when they were used.

3.2. Bacterial Isolations

To isolate *Salmonella* spp., food samples were collected from free-markets, supermarkets and one dairy market in Türkiye. The food samples were from animal origin namely poultry meat (85 samples), minced meat (44 samples), raw milk (53 samples) and mayonnaise containing salads (13 samples). They were kept at +4ºC until they were analysed. The isolation was done in four steps by following the ISO 6579 manual of detection of *Salmonella* spp. from food and animal feeding staff (Figure 3.1):

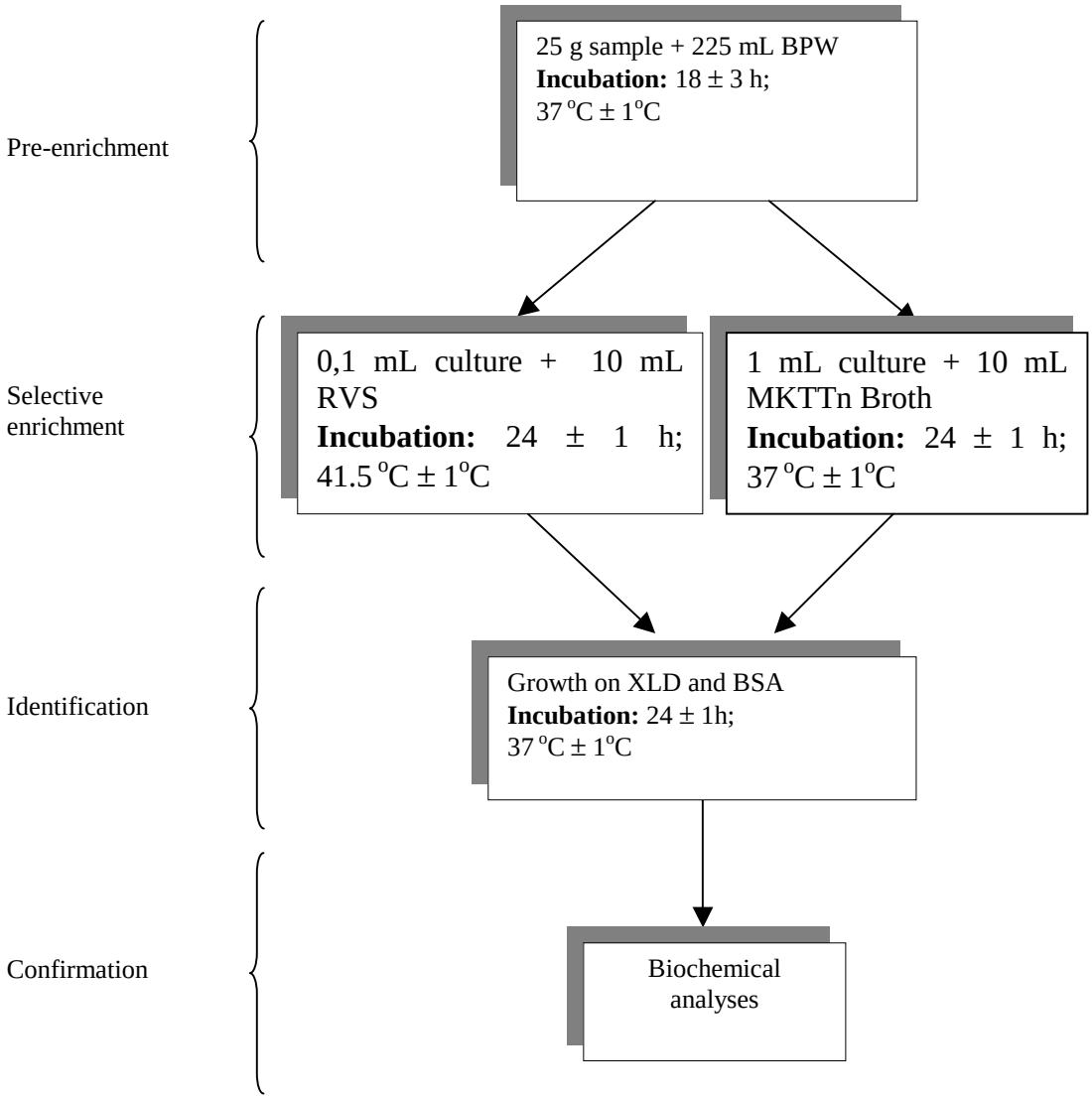


Figure 3. 1. Schematic representation of serotyping analyses of *Salmonella*.

1. Pre-enrichment in non-selective liquid media: 225 mL buffered peptone water (BPW) was inoculated at ambient temperature with the test portion of 25 g (1/10, w/v), then incubated at 37ºC ± 1 ºC for 18 ± 1h to permit the detection of low numbers of *Salmonella* or injured *Salmonella*.
2. Enrichment in selective liquid media: Rappaport-Vassiliadis (RVS) and Müller-Kauffmann-tetrathionate/novobiocin (MKTTn) broths were inoculated respectively with the culture obtained from pre-enrichment. 0,1 mL of the culture was transferred to 10 mL of the RVS broth and incubated at 41,5ºC ± 1 ºC for 24 ± 3h. In parallel to this, 1 mL of the culture

was inoculated into 10 mL MKTTn broth and incubated at 37ºC ± 1 ºC for 24 ± 3h.

3. Identification: From the cultures grown in the enrichment step, two selective media were inoculated: xylose lysine deoxycholate (XLD) agar and bismuth sulphite agar (BSA). Both solid media were streaked with the cultures obtained from enrichment broths making four solid media plates for one sample and incubated at 37ºC ± 1 ºC for 24 ± 3h. After incubation typical *Salmonella* colonies which were black centred and slightly transparent zone of reddish colour on XLD agar and black centred, light edges surrounded by a black precipitate with metallic sheen (so-called rabbit's or fish-eye) on BSA agar were selected.
4. Confirmation: Selected colonies of presumptive *Salmonella* were sub-cultured in LB broth containing 60% glycerol to possess pure cultures and stored at -20ºC until the confirmation which was done by means of Gram-staining, oxidase test and other biochemical tests. Gram staining was performed according to the conventional method. Gram-negative strains were analysed by oxidase test performed with 1% solution of N,N,N',N'-tetramethyl-p-phenylenediamine.2HCL. Both Gram-negative and oxidase-negative isolates were further tested. Biochemical tests other than oxidase test were done by using API 20E test kit (bioMérieux, Inc., France). The plastic strips holding twenty mini-test tubes were inoculated with the saline suspensions of the cultures according to manufacturer's directions. This process also rehydrated the desiccated medium in each tube. A few tubes were completely filled (CIT, VP and GEL), and some tubes were overlaid with mineral oil such that anaerobic reactions could be carried out (ADH, LDC, ODC, H2S, URE) (Figure 3.2).

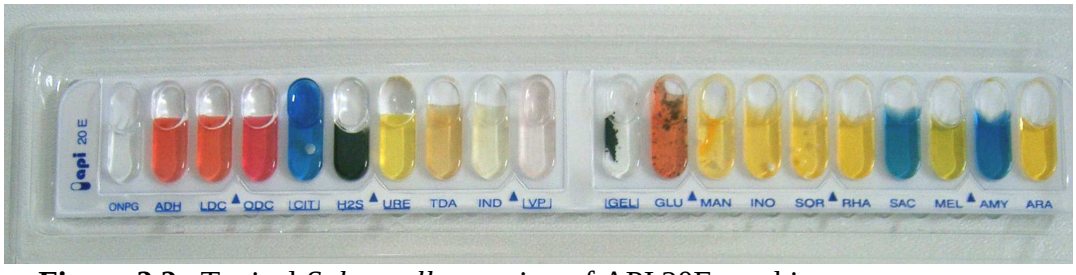


Figure 3.2. Typical *Salmonella* reaction of API 20E test kit.

After incubation in a humidity chamber for 18-24 hours at 37°C, the colour reactions were read (some with the aid of added reagents as supplied by the kit). The data were analysed by the manufacturer's software and positive results with ≥89% probabilities were confirmed as *Salmonella*. The list of the biochemical tests performed by API 20E test kit and typical reactions exhibited by *Salmonella* spp. are given in Table 3.1.

Table 3.1. Biochemical reactions involved in API 20E (bioMérieux, Inc., France) test kits and typical *Salmonella* reactions.

Tests	**Substrate**	**Reaction**	**(-) Results**	**(+) Results**	***Salmonella* spp.**
ONPG	ONPG	beta-galactosidase	colorless	yellow	-
ADH	arginine	arginine dihydrolase	yellow	red/orange	-
LDC	lysine	lysine decarboxylase	yellow	red/orange	+
ODC	ornithine	ornithine decarboxylase	yellow	red/orange	+
CIT	citrate	citrate utilization	pale to green/yellow	blue-green/ blue	-
H_2S	Na-thiosulfate	H_2S production	colorless/gray	black deposit	+
URE	urea	urea hydrolysis	yellow	red/orange	-
TDA	tryptophan	deaminase	yellow	brown-red	-
IND	tryptophan	indole production	yellow	red (in 2 min)	-
VP	Na-pyruvate	acetoin production	colorless	pink/red (in 10 min)	-
GEL	charcoal gelatin	gelatinase	no diffusion of black	black diffusion	-

Table 3.1. Biochemical reactions involved in API 20E (bioMérieux, Inc., France) test kits and typical *Salmonella* reactions (continued).

Tests	**Substrate**	**Reaction**	**(-) Results**	**(+) Results**	***Salmonella* spp.**
GLU	glucose	fermentation/oxidation	blue/ blue-green	yellow	+
MAN	mannitol	fermentation/oxidation	blue/ blue-green	yellow	+
INO	inositol	fermentation/oxidation	blue/ blue-green	yellow	-
SOR	sorbitol	fermentation/oxidation	blue/ blue-green	yellow	+
RHA	rhamnose	fermentation/oxidation	blue/ blue-green	yellow	+
SAC	sucrose	fermentation/oxidation	blue/ blue-green	yellow	-
MEL	melibiose	fermentation/oxidation	blue/ blue-green	yellow	+
AMY	amygdalin	fermentation/oxidation	blue/ blue-green	yellow	-
ARA	arabinose	fermentation/oxidation	blue/ blue-green	yellow	+

3.3. Serotyping

Serotype assessments were done in *Salmonella* Reference Laboratories (Federal Institute for Risk Assessment, Berlin Germany). The detection of the presence of *Salmonella* O- and H- antigens were tested by slide agglutination with the commercially available antisera. One loop of appropriate antisera was dropped onto a cleaned glass slide. One loop of overnight culture grown on agar was dispersed in the drop to obtain a homogeneous and turbid suspension. The slide was rocked gently for 30 s and clumping was monitored by a magnifying glass. The scheme to obtain the serotype was given in Figure 3.3. To detect the O-group antigens, cultures were grown on Gassner agar (Merck KGaA., Germany) for overnight at 37°C; while semi-solid agar was the media for the determination of H-antigens with the same incubation conditions. To find out phase-2 flagellar antigens (H2), detected phase-1 flagellar antigens (H1) was suppressed by adding the appropriate antiserum to the semi-solid agar and the culture grown on it for overnight at 37°C. Slide agglutination was performed again to determine the H2 antigens. Antisera set used for the analyses were as follows:

a) *Salmonella* polyvalent I (A-E) (Sifin, Germany; Statens, Denmark),
b) *Salmonella* somatic (O) group A, B, C, D, and E (Sifin, Germany; Statens, Denmark),
c) *Salmonella* flagellar (H) antisera set (Sifin, Germany; Statens, Denmark).

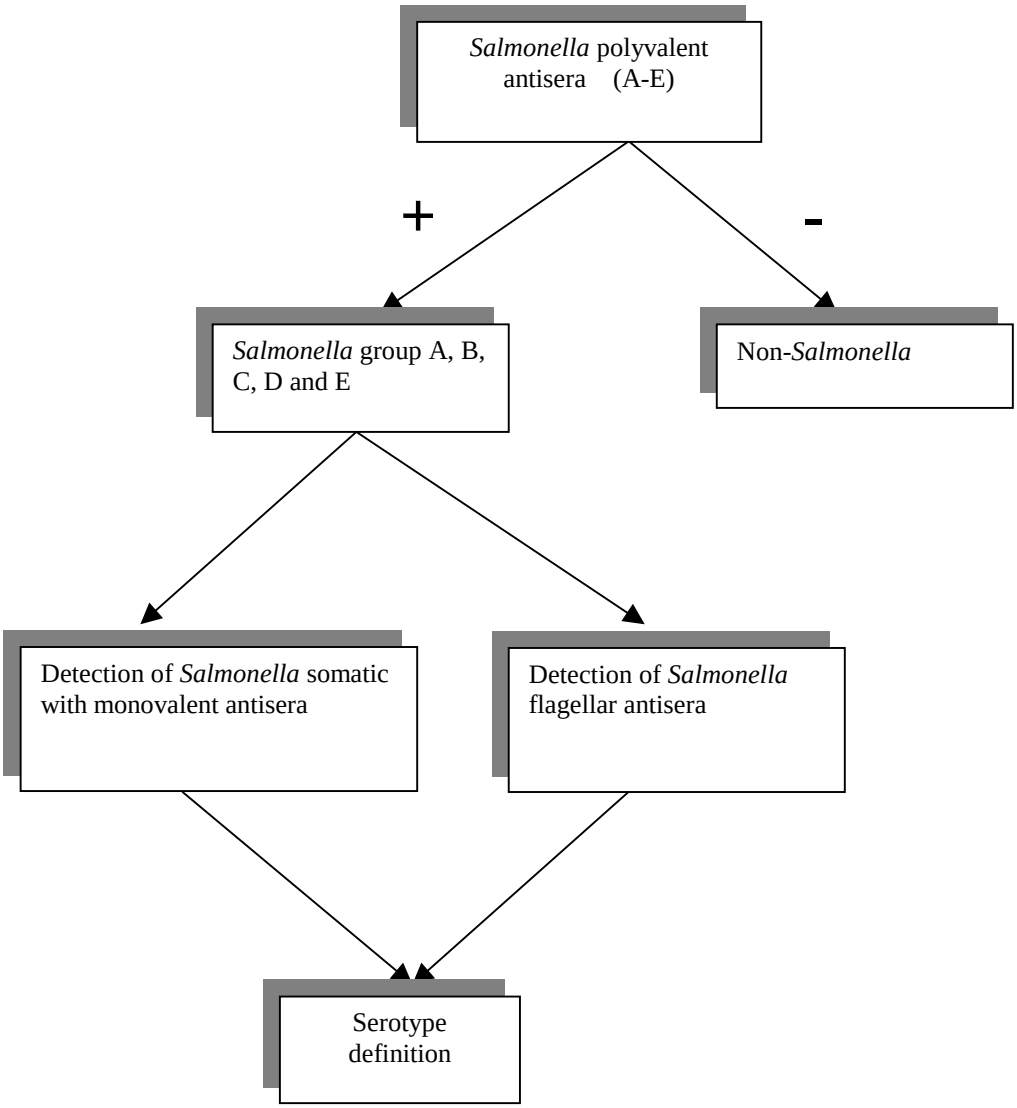


Figure 3.3. Serotyping analysis scheme for *Salmonella*.

3.4. Phage Typing

Phage typing analyses were performed in *Salmonella* Reference Laboratories (Federal Institute for Risk Assessment, Berlin Germany). Typing phage sets were obtained as stock cultures from Health Protection Agency, Specialist and Reference Microbiology Division, Centre for Infections, Laboratory of Enteric Pathogens (HPA, London, UK).

By means of a sterile inoculation loop, the test culture was inoculated into a test tube containing 4 mL double strength nutrient broth with a special care for heavy inoculum to give visible turbidity for *S.* Enteritidis and a very light inoculum for *S.* Typhimurium to give a barely visible turbidity. The culture was incubated by shaking at 200 rpm at 37°C for 1-1,5 h for *S.* Enteritidis and for *S.* Typhimurium 1,5 h without agitation to obtain a very light growth in early log phase. After incubation, it was flooded over the surface of double strength nutrient agar using a flooding pipette and the excess of culture was removed. As soon as the surface of agar dried, the appropriate typing phages at routine test dilutions were applied to the dried surface by a multipoint inoculation loop. When the phage spots dried, the agar plate was incubated at 37°C for 18 h. At the end of the incubation, the agar plate was read using a magnifying glass through the bottom of the plate (Anderson *et al.*, 1977; Ward *et al.*, 1987).

Phage susceptibilities were evaluated by means of the plaque number, size and transparency. The pattern was compared with known phage type patterns in the database and defined. If the culture did not react with any of the typing phages, it was defined as non-typable (NT); and if the culture reacted with the typing phages, but gave a different pattern other than those in the database, it was considered as reacting with the typing phages, but lytic pattern did not correspond to any recognized phage types, so called RDNC (= **R**eacting with the typing phage, but lytic pattern **D**id **N**ot **C**orrespond to any recognized phage types).

3.5. Plasmid Profiling

3.5.1. Plasmid Isolation

Plasmid analysis was performed with the modification of method described by Kado and Liu (Kado and Liu, 1981; Helmuth *et al.*, 1985). 2 mL LB-broth was inoculated with one loop of LB-agar culture and incubated at 37ºC for 18h. 1,5 mL of the culture transferred into a microcentrifuge tube and centrifuged at 14.000 rpm for 5 min at room temperature. The supernatant was discarded and the pellet was dried by aspiration. Bacterial pellet was resuspended in 20 µL of KADO-buffer by vigorous shaking. Freshly prepared 100 µL Lysis Mix solution was added to the suspension and mixed very gently by inverting the tubes until a homogenous mixture was obtained. Than the tubes were placed into a water-bath and incubated 27 min at 58ºC. Right after the incubation, freshly prepared 100 µL phenol:chloroform (1:1) solution was added and again mixed gently until a uniform white colour was formed. Thereafter, it was centrifuged at 14.000 rpm for 30 min at room temperature. 90 µL supernatant was mixed with 10 µL DNA-sample-buffer by flicking the tubes and kept in ice-bath for 10 min.

3.5.2. Agarose Gel Electrophoresis and Gel Documentation

The plasmid DNAs were run on 2 mm-thick vertical 0,8% agarose gel in 1XTBE-buffer solution under 50 V/15 min followed by 100 V/2 h electrophoresis conditions. To view the plasmid pattern, agarose gel was stained with ethidium bromide solution (0,5 µg/mL) for 20 min and after washing with sterile bidest water for 15 min, it was visualised by Stratagene EagleEye II Imaging System (Stratagene, USA) under UV light.

3.6. Pulsed Field Gel Electrophoresis (PFGE)

PFGE analyses were carried out following the method suggested by PulseNet (http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf).

3.6.1. Preparation of PFGE Plugs From Agar Cultures

The culture grown on LB-agar plates at 37ºC for overnight was transferred to 4 mL cell suspension buffer solution by means of a sterile plastic loop. The optic density of the suspension was adjusted to 1,3-1,4 absorbance at 610 nm (Ultraspec 2000 Spectrophotometer; Amersham Pharmacia Biotech. Inc., Sweden). The suspensions were kept in ice-bath until the preparation of agarose plugs. 300 µL cell suspension, 15 µL proteinase K (end concentration of 0,5 mg/mL), and 300 µL Seakem Agarose (1%)-SDS was mixed in a pre-warmed (50ºC) 1,5 mL microcentrifuge tubes. As quick as possible, it was transferred to the PFGE-molds with a special care to avoid bubble formation. During this step, in order not to solidify the agarose, the mixture was prepared in the waterbath (50ºC). The PFGE-molds were kept at +4 ºC for 30 min and the plugs were solidified.

3.6.2. Lysis of Cells in Agarose Plugs

The agarose plugs were removed from the mold by means of a sterile thin spatula and transferred to 5 mL cell lysis buffer solution in tubes. The lysis was performed at 54ºC for 2 h in shaking waterbath (175-200rpm).

3.6.3. Washing of Agarose Plugs

The washing process was carried out at 50ºC in shaking incubator (175-200 rpm). It was carried out with pre-warmed (50ºC) sterile bidest water twice (~10-15 mL and 15 min intervals) and pre-warmed (50ºC) TE-buffer solution four times (~10-15 mL and 15 min intervals). After washing process, the plugs were cut by means of a scalpel to 2,5 mm slices and kept in a fresh TE-buffer solution at +4ºC in a microcentrifuge tube.

3.6.4. Restriction Enzyme Digestion with *Xba*I

One of the 2,5 mm slices was kept in 120 µL SureCut H-buffer solution (Roche GmbH., Germany) for 15 min to maintain the solution balance and replaced by 100 µL *Xba*I (50U/cell) enzyme solution(Roche GmbH., Germany). Restriction reaction was carried out at 37ºC in waterbath for 4 h. To stop the enzyme activity, enzyme solution was discarded and 500 µL 0,5XTBE solution was added.

3.6.5. Casting Pulsed Field Agarose Gel

2,5 mm agarose slices was placed onto the teeth of horizontally positioned electrophoresis comb by means of a sterile spatula and the excess of buffer solution was removed by the help of a pipette. The slices were kept for 5 min horizontally on the teeth to dry and the comb was placed into the gel mold tray with 90ºA. The Seakem Agarose (1%)-TBE at 50ºC was poured into the tray slowly with a special care not to replace the agarose slices from the teeth. It was kept at room temperature for 30 min to solidify.

3.6.6. Electrophoresis, Staining and Documentation of Pulsed Field Agarose Gel

Pulsed Field chamber (CHEF-DR II, Variable Angle System, BioRad, USA) was filled with 2,5 L of 0,5XTBE solution (with 0,016 g thiourea) and cooled down to 14ºC. When the Pulsed Field agarose gel solidified, it was placed into the chamber and electrophoresis was performed under 6V/cm (200V), 2-64 s and 120ºA conditions for 20 h. Later, the gel was stained with ethidium bromide solution (0,5 µg/mL) and destained with sterile bidest water for 20 min each. PFGE pattern was documented under UV light by EagleEye II (Strartagene, USA).

3.6.7 Cluster Analysis

PFGE results were analysed by using BioNumerics Software (Applied-Maths, Kortrijk, Belgium). The banding patterns were compared using Dices coefficient with a 1,5% band position tolerence and unweighted pair group method with arithmetic averages (UPGMA) (Zhao *et al.*, 2007)

3.7. Antimicrobial Susceptibility Testing

3.7.1. Disk Diffusion Method

The cultures were inoculated into 4 mL LB-broth by the aid of a sterile plastic loop and incubated at 37ºC for 18 h. After incubation, they were diluted in the range of 1:100 with sterile bidest water and 100 µL were spread on Müller-Hinton agar (Oxoid, UK) plates. 6 mm paper disks containing antimicrobial agents (Oxoid, UK) were placed onto the agar surface and incubated at 37ºC for 18 h. For disk diffusion, 16 different antimicrobials were used and 11 additional β-lactams were tested when β-lactam resistance was obtained. The table showing used antimicrobial disks (Table A.I.1) is given in Appendix I. When the incubation was complete, the zone diameters around the disks were measured and compared with the break points of Clinical Laboratory Standards Institute (CLSI) and decided as susceptible (S), intermediate (I), and resistant (R).

3.7.2. Minimal Inhibitory Concentration (MIC) Method

Müller-Hinton agar plate was streaked with the culture to be tested and incubated at 37ºC for 18 h. The culture was taken by the aid of a sterile plastic loop and suspended in 5 mL sterile saline solution (0,85% NaCl). Then the turbidity of suspensions were measured to achieve 0,5 McFarland turbidity in Sensititer System (Autoinoculator INO2, Trek Diagnostic Systems Ltd., UK). From the suspension 15 µL was transferred into 11 mL Müller-Hinton-broth (Oxoid, UK) and mixed. 100 µL from the inoculated Müller-Hinton Broth was dispensed in microtiter plates (NLMV1A, Trek Diagnostic Systmes Ltd., UK) having different lyophilized antimicrobial agent with different concentrations in its each well. 17 antimicrobials were the same set of those used for disk diffusion with the addition of Colistin. Table showing antimicrobial concentrations of the microtiter plate used (Table A.I.2) is given in Appendix I. Thereafter, the plates were covered with a plastic sticker and incubated at 37ºC for 18 h. After incubation the plate was read under semi-automatic Sensitouch System (Accumed International Ltd., UK) and the antimicrobial concentration corresponding the first well where there was any growth determined as the MIC value. The concentrations (µg/mL) obtained for the culture was compared with the CLSI breakpoints and concluded as S, I, and R.

3.8. Genotypic Characterization of Antimicrobial Resistance

3.8.1. Raw-DNA Isolation

Raw-DNA of the culture was isolated using DNA-boiling method suggested by Sambrook *et al.* (1989). 1 mL fresh culture (grown at 37ºC for 18 h) was transferred to a microcentifuge tube and centrifuged for 6 min at 14.000 rpm (Model 5415D, Eppendorf GmbH., Germany). The supernatants were discarded and the pellets were dried by aspiration. Dried pellets were resuspended in 300 µL TE-buffer solution. Then the suspension was boiled in waterbath (100 ºC) for 10 min and immediately transferred into ice-bath. When it cooled down, it was centrifuged at 14.000 rpm for 2 min and the supernatant (or raw-DNA) was transferred into a new sterile 1,5 mL microcentrifuge tube. The raw-DNA was dispensed in aliquots and stored at -20ºC and used in all PCR amplifications.

3.8.2. PCR Amplifications

GeneAmp9700 Thermocycler (Applied Biosystems, USA) was used for PCR amplifications. The specific primers used for the antimicrobial resistance gene amplifications are given in Table 3.2. The reaction mixture and conditions for the amplifications are as follows:

PCR reaction mixture (for single reaction):

- 12 µL sterile dH_2O (Merck KGaA., Germany)
- 2,5 µL 10X PCR buffer solution (Finnzyme Oy, Finland)
- 2,5 µL dNTP mix, 200µM end concentration (dNTP Set 1, Roth GmbH., Germany)
- 2,5 µL Forward primer, 1ρmol end concentration (Table 3.2)
- 2,5 µL Reverse primer, 1 ρmol end concentration (Table 3.2)
- 0,5 µL DyNazyme II DNA-polymerase (2U/µl) (Finnzyme Oy, Finland)
- 2,5 µL Raw-DNA

PCR amplification conditions:

- 94^oC, 5 min
- 30 cycle; 94^oC/30 s; T_a/30 s; $72^oC/t_e$
- 72^oC, 5 min
- 4^oC, ∞

T_a (annealing temperature, ^oC) was calculated from the formula $T_a = [2 X (A + T)] + [4 X (G + T)]$ whereas t_e (elongation time, s) was determined according to the expected PCR product size in terms of base pairs (bp) i.e. for an expected PCR product of ≤500 bp, t_e was adjusted to 30s, 500-700 bp to 40 s, and ≥1000 bp to 60 s or more.

3.8.3. Agarose Gel Electrophoresis and Gel Documentation

When the PCR amplification completed, 8 µL PCR product and 2 µL DNA-sample-buffer were mixed and load on 1,5% agarose gel together with the DNA-size ladder (Hyperladder II, Bioline, Germany). The buffer used for the gel preparation and electrophoresis was 1XTBE solution and the running condition was 50 V/15 min followed by 80 V/45 min (power supply: PowerPac 300, BioRad, USA). After electrophoresis, the gel was stained with ethidium bromide solution (0,5 µg/mL) and washed with sterile bidest water for 15 min each. Gel documentation was done with EagleEye II (Stratagene, USA) under UV light.

3.8.4. DNA Sequence Analysis

DNA Sequence analysis was done to detect the mutation type resistance. In order to sequence DNA fragments, PCR amplification of the fragment was performed with 100 µL of total reaction volume. Thereafter, it was purified by GFX PCR DNA/Gel Band Purification Kit (GE, Healthcare, UK) to 50 µL in TE-buffer solution. Sensitivity of purification was controlled by running 10 µL purified DNA on 1,5% agarose gels as described above. To sequence the purified DNA, 10 µL volume (20 ng DNA/µL) was mixed with 4 µL forward primer (5 pmol/µL) and sent to AGOWA GmbH., Germany. When the results were received, they were analysed by SeqMan Pro software program (DNAStar Inc., USA) and by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

Table 3.2. Primer pairs and PCR conditions.

Resistance	Gene/Region	Control Strain	Primer pair (Forward/Reverse)	T_a/t_e	PCR Product	Reference
Class I Integron	*5'-3'CS*	SUO1	GGCATCCAAGCAGCAAGC/ AAGCAGACTTGACCTGAT	58°C / 02:30 min	variable	Guerra *et al.*, 2004b
Sulfonamides	*sul 1*	SUO1	CTTCGATGAGAGCCGGCGGC/ GCAAGGCGGAAACCCCGCC	65°C / 00:30 min	436 bp	Guerra *et al.*, 2004b
	sul 2	SUO5	TCAACATAACCTCGGACAGT/ GATGAAGTCAGCTCCACCT	60°C / 00:40 min	707 bp	Guerra *et al.*, 2004b
	sul 3	S65	GAGCAAGATTTTTGGAATCG/ CATCTGCAGCTAACCTAGGGC TTTGGA	51°C / 00:40 min	~ 750 bp	Perreten, 2003
β-lactams	bla_{tem-1}	SUO8	TTGGGTGCACGAGTGGGT/ TAATTGTTGCCGGGAAGC	55°C / 00:30 min	503 bp	Guerra *et al.*, 2004b
	bla_{oxa-1}	SUO5	AGCAGCGCCAGTGCATCA/ ATTCGACCCCAAGTTTCC	60°C / 00:30 min	708 bp	Guerra *et al.*, 2004b
	bla_{pse-1}	SUO1	CGCTTCCCGTTAACAAGTAC/ CTGGTTCATTTCAGATAGCG	60°C / 00:30 min	419 bp	Guerra *et al.*, 2004b

Table 3.2. Primer pairs and PCR conditions (continued).

Resistance	Gene/Region	Control Strain	Primer pair (Forward/Reverse)	T_a/t_e	PCR Product	Reference
Streptomycin/ Spectinomycin	*bla*$_{CMY}$	β4	GACAGCCTCTTTCTCCACA/ TGGAACGAAGGCTACGTA	55^{o}C / 01:00 min	1000 bp	Zhao *et al.*, 2003
	bla$_{CTX-M}$	RL102/05	CGATGTGCAGTACCAGTAA/ TTAGTGACCAGAATCAGCGG	60^{o}C / 00:30 min	585 bp	Guerra *et al.*, 2004b
	aadA1-like	SUO5	GTGGATGGCGGCCTGAAGCC/ ATTGCCCAGTCGGCAGCG	70^{o}C / 00:30 min	526 bp	Guerra *et al.*, 2004b
	aadA2	SUO1	TGTTGGTTACTGTGGCCGTA/ GATCTCGCCTTTCACAAAGC	60^{o}C / 01:00 min	600 bp	Walker *et al.*, 2001
	strA	S65	CCTGGTGATAACGGCAATTC/ CCAATCGCAGATAGAAGG	60^{o}C / 00:40 min	~ 500 bp	Guerra *et al.*, 2004b
	strB	S65	ATCGTCAAGGGATTGAAACC/ GGATCGTAGAACATATTGGC	60^{o}C / 00:40 min	~ 500 bp	Guerra *et al.*, 2004b
Kanamysin/ Neomysin	*aphAl*	SUO7	AAACGTCTTGCTCGAGGC/ CAAACCGTTATTCATTCGTGA	60^{o}C / 00:40 min	~ 500 bp	Guerra *et al.*, 2004b
Trimethoprim/ Sulfamethoksazole	*dfrA1-like*	P304	GTGAAACTATCACTAATGG/ CCCTTTTGCCAGATTTGG	55^{o}C / 00:30 min	473 bp	Guerra *et al.*, 2004b

Table 3.2. Primer pairs and PCR conditions (continued).

Resistance	Gene/Region	Control Strain	Primer pair (Forward/Reverse)	T_a/t_e	PCR Product	Reference
Tetracycline	*dfrA12*	SUO8	ACTCGGAATCAGTACGCA/ GTGTACGGAATTACAGCT	55°C / 00:30 min	462 bp	Guerra *et al.*, 2004b
	dfrA5-14	SUO6	GATTGGTTGCGGTCCA/ CTCAAAAACAACTTCGAAGG	55°C / 00:30 min	379 bp	Guerra *et al.*, 2004b
	dfrA7-17	NRL1	CAGAAAATGGCGTAATCG/ TCACCTTCAACCTCAACG	55°C / 00:30 min	345 bp	Guerra *et al.*, 2004b
	tet(A)	SUO8	GCTACATCCTGCTTGCCT/ CATAGATCGCCGTGAAGA	60°C / 00:30 min	210 bp	Guerra *et al.*, 2004b
	tet(B)	SUO5	TTGGTTAGGGGCAAGTTTTG/ GTAATGGGCCAATAACACCG	60°C / 00:30 min	600 bp	Guerra *et al.*, 2004b
	tet(G)	SUO1	GCTCGGTGGTATCTCTGC/ AGCAACAGAATCGGGAAC	60°C / 00:30 min	500 bp	Guerra *et al.*, 2004b
Phenicols	*cat*	SUO5	CCTGCCACTCATCGCAGT/ CCACCGTTGATATATCCC	60°C / 00:30 min	623 bp	Guerra *et al.*, 2004b
	cmlA	SUO8	TGTCATTTACGGCATACTCG/ ATCAGGCATCCCATTCCCAT	55°C / 00:30 min	435 bp	Guerra *et al.*, 2004b

Table 3.2. Primer pairs and PCR conditions (continued).

Resistance	Gene/Region	Control Strain	Primer pair (Forward/Reverse)	T_a/t_e	PCR Product	Reference
Gentamicin	*floR*	SUO1	CACGTTGAGCCTCTATAT/ ATGCAGAAGTAGAACGCG	55°C / 00:40 min	868 bp	Guerra *et al.*, 2004b
	aac(3)-IV	SUO8	GTTACACCGGACCTTGGA/ AACGGCATTGAGCGTCAG	60°C / 00:40 min	674 bp	Guerra *et al.*, 2004b
	aac(3)-II	99-4068	ATTCGAAAACTCGGAGTC/ CGGAGTGGCTCCGAAGTG	60°C / 00:40 min	800 bp	Guerra *et al.*, 2004b
Quinolones	*gyrA*	*S.* Typhimurium LT2	TGTCCGAGATGGCCTGAAGC/ TACCGTCATAGTTATCCACG	60°C / 00:30 min	346 bp	Guerra *et al.*, 2003
	parC	*S.* Typhimurium LT2	TCGTGCGTTGCCGTTTATTG/ CATGGTGCCGTCGAAGTTTG	60°C / 00:30 min	369 bp	Guerra *et al.*, 2003
	qnrA	*E. coli* pMG252	TCAGCAAGAAGGATTTCTCA/ GGCAGCACTATTACTCCCAA	60°C / 00:30 min	627 bp	Wang *et al.*, 2003
	qnrB	-	ATGACGCCATTACTGTATAA/ GATCGCAATGTGTGAAGTTT	55°C / 00:30 min	562 bp	Jacoby *et al.*, 2006
	qnrS	-	ACGACATTCGTCAACTGCAA/ TAAATTGGCACCCTGTAGGC	60°C / 00:30 min	416 bp	Gay *et al.*, 2006

Table 3.2. Primer pairs and PCR conditions (continued).

Resistance	Gene/Region	Control Strain	Primer pair (Forward/Reverse)	T_a/t_e	PCR Product	Reference
Sequencing pRQ2006	*pre-qnrS1 / qnrS-B1*	-	CTGATAACACTTCAACCATC/ TGGAAACTTGCATCACGAAG	60°C / 00:30 min	621	Poirel *et al.*, 2006; present study
Sequencing pRQ2006	*qnrS-F / pre-qnrS2*	-	ACGACATTCGTCAACTGCAA/ TCGTTTTATAAATTTGAGCG	60°C / 00:30 min	579	Gay *et al.*, 2006; Poirel *et al.*, 2006
Sequencing pRQ2006	*post-qnrF1-F/B*	-	GAAGCACTGGGTATTGTTGT/ GCTTGTCTTCGGTATCTTTG	55°C / 00:30 min	639	present study
Sequencing pRQ2006	*post-qnrF2-F/B*	-	GGCAGAATATCTTTCAGCAG/ AGATAGTCATCCACCACAGC	60°C / 00:30 min	482	present study
Sequencing pRQ2006	*tnpIS26-F/B*	-	CAGGGGATCACCATAATAAA/ AACCTACGTGAAGGTCAATG	58°C / 00:30 min	420	present study
pIV2 vector primer	*M13-1224S*	-	CGCCAGGGTTTTCCCAGTCAC GAC	-	-	New England Biolabs
pIV2 vector primer	*M13-1233S*	-	AGCGGATAACAATTTCACACA GGA	-	-	New England Biolabs

3.9. Detection of Class I Integrons

The universal class I integron primers designed for the conserved regions (5CS'/3CS'; Table 3.2) was used to detect class 1 integrons for the antimicrobial resistant strains. DNA amplification, agarose gel electrophoresis and gel documentation was done as described in Section 3.8. Detected integrons were subjected to PCR-RFLP analysis. For this aim, *Taq*I endonuclease (Roche GmbH., Germany) enzyme was used. After the DNA amplification of integron with the primers, 100 µL end volume of the PCR product was purified by PCR DNA/Gel Band Purification Kit (GE, Healthcare, UK) to 50 µL in 1XTE buffer solution. 8 µL purified PCR product mixed with 1,5 µL buffer-B (Roche GmbH., Germany), 0,5 µL *Taq*I endonuclease (5U/mL; Roche GmbH., Germany), and 5 µL PCR grade water to obtain 15 µL mix. The mixture was incubated at 65^{o}C for 1 h in the waterbath. Immediately after incubation, it was transferred to ice-bath in order to stop the enzyme reaction. Agarose gel electrophoresis and gel documentation was performed as given in Section 3.8.3. When the difference was detected, DNA sequence analyses were performed in order to reveal the gene cassette in variable region of the class I integron. DNA sequence analyses were done by the method explained in Section 3.8.4.

3.10. Conjugation

E. coli J53 (Rif^{R}) strain was used as the recipient of conjugation analyses. From fresh cultures of donor and recipient strains, 10 mL LB-broth was inoculated in 1/20 (vol/vol) range and incubated in a shaking incubator at 37^{o}C until 95-110 Klett Unit (~1,2X10^{8}) was achieved. The strains were mixed 1:2 (donor:recipient) in two parallels and filtered through 0,45 µm pore-sized membrane filters. Then the filters were placed onto the surface of LB-agar plates and one was incubated at room temperature whereas the other at in parallel for 6 h to conjugate. At the end of incubation, the filter was transferred into 2 mL LB-broth and washed by vigorous agitation. Eosin Methylene Blue (EMB) agar plates containing ampicillin (100 µg/mL) and rifampicin (50 µg/mL) was inoculated with serial dilutions of the culture for 24-48 h at 37^{o}C and typical *E. coli* colonies (if any) were selected as conjugants. The conjugants were characterized by means of plasmid analysis and

PCR amplification of the gene expected to be transferred. After these confirmations, conjugants were stored at -20^{o}C in LB-broth containing 60% glycerol.

3.11. Southern-Blotting and DNA-Hybridization Analysis

3.11.1. Southern-Blotting

Southern-Blotting was performed following the method suggested by Sambrook *et al.* (1989). The plasmid was isolated, run and documented as described in Section 3.5. Later, the plasmid gel was transferred to a tank containing sterile bidest water and washed twice to remove the ethidium bromide from agarose gel. After washing, 250 mL HCl solution (0,25 N) was applied 15 min on a shaker in order to perform depurination. To stop depurination the gel was washed with sterile bidest water for 5 min and denatured with 250 mL NaOH solution (0,5 N) for 30 min on a shaker. To discard NaOH completely, the gel was washed 5 min more and left in 10XSSC for 2 min to maintain the solution balance. Thereafter, the gel was placed on to the vacuum blotter (Model 785, BioRad, USA) in such away that a filter paper (Whatmann No. 1) at the bottom, a positively charged nylon membrane (Roche GbmH., Germany) in the middle, and the gel to be blotted on the top. The blotting was performed in 10XSSC solution (~500 ml) at 5 Hg-vacuum for 2 h. Right after blotting, the membrane was dried by aspiration at room temperature and cross-linked under UV-light for 2 min. The membrane was kept at in a plastic bag +4^{o}C until DNA hybridization assay.

3.11.2. DNA Probe Preparation

DNA fragment was amplified by PCR as described in Section 3.8.2 with the exception by using digoxigenin-labelled dNTPs (PCR-DIG Labelling Mix, Roche GmbH., Germany). The PCR product was run on agarose gel as explained before and the band of concern was cut under UV-light by means of a scalpel. The DNA fragment was purified from the agarose by GFX PCR DNA/Gel Band Purification Kit (GE, Healthcare, UK). The purified DNA probe was than kept at - 20^{o}C.

3.11.3. DNA Hybridization

Hybridization solution prepared with hybridization solution (Roche GmbH., Germany) was prewarmed to 42^{o}C in waterbath (GFL 1002, GFL GmbH., Germany). The plastic bag containing the membrane was filled with this solution (~15-30 mL) and incubated at 42^{o}C for 30-60 min in a shaking waterbath (GFL 1083, GFL GmbH., Germany). Just before to the end of the incubation, 3 µL DNA-probe mixed with 80 µL hybridization solution and denatured at 100ºC for 5 min in the Thermocycler (GenAmp9700, Thermocycler, Applied Biosystems, USA). When the denaturation of DNA-probe was completed, it was cooled in ice-bath immediately and applied to the membrane in plastic bag. Hybridization was carried out for overnight at 42ºC in shaking incubator.

3.11.4. Post-Hybridization Process

Following the incubation the membrane was performed to a series of washing steps by shaking in each step of post-hybridization. Initially the membrane was washed with post-hybridization-wash solution-I (~100 mL) twice for 5 min each. In the meanwhile, post-hybridization-wash solution-II was pre-warmed to 68ºC in waterbath. Thereafter, the membrane was transferred, to a new plastic bag together with ~15-30 mL post-hybridization washing solution-II and washed twice at 68ºC for 15 min each in waterbath.

After washing the membrane ~100 mL 1Xblocking reagent (Roche GmbH., Germany) was applied at room temperature for 30 min. DIG-AP antibody solution (Roche GmbH., Germany) was diluted with 1Xblocking reagent in the range of 1:5000, the membrane was transferred into this solution (~100 mL) and kept at room temperature for 30 min. In order to remove antibody solution, the membrane was washed with detection-wash solution (~100 mL) following maleic acid buffer solution (~100 mL) for 15 min each at room temperature. The membrane was kept in the detection-buffer solution (~100 mL) for 2 min to maintain the solution balance and transferred to a new plastic bag. ~15-30 mL NBT/BCIP-mix (NBT/BCIP Stock Solution, Roche GmbH., Germany) was prepared by adding 200 µL NBT/BCIP stock solution to each 10 mL detection-buffer solution. The membrane was kept in dark, at room temperature with this solution without shaking. When the hybridization

was developed, the reaction was stopped by washing the membrane with sterile bidest water. After documentation, the membrane was kept in a new plastic bag with 1XTE solution at +4ºC.

3.12. Cloning

3.12.1. Restriction Analyses

The plasmid to be cloned was first subjected to restriction cutting with 13 different restriction enzymes not only to define the restriction profile appropriate for the aim of cloning but also to characterize the plasmid. The culture having the plasmid was inoculated (1%, vol/vol) into 200 mL LB-broth containing ampicillin (end concentration of 100 μg/mL) and incubated at 37ºC for 18 h. Plasmid isolation was done by using commercially available Qiagen MidiPrep kit (Qiagen, Germany). The set of enzymes used for restriction digestion analysis were *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*I, *Sal*I, *Hin*cII, *Xba*I, *Sma*I, *Eco*RV, *Sac*I, *Bsp* 120-I, and *Xho*I (Fermentas, Germany). The restriction mixture was prepared for each enzyme contained 2 μL plasmid DNA, 6 μL sterile bidest water, 1 μL buffer solution of the enzyme used, and 1 μL restriction enzyme. The mix was incubated at 37ºC for 2 h for each of the restriction reaction. Following the reaction, 8 μL restriction product with 2 μL loading buffer solution was loaded on 0,8% horizontal agarose gel in TBE. Electrophoresis and gel documentation was performed as described in Section 3.5.

3.12.2. Ethanol Precipitation

When the restriction enzyme was selected for cloning, the restriction digestion was carried out once more with a mixture having 50 μL end volume. 47 μL restriction product, 4.7 μL sodium acetate (3M) and 120 μL ice-cold pure ethanol was mixed and DNA was precipitated at -20ºC for 1 h. Thereafter, the mixture was centrifuged at 14.000 rpm for 20 min. The pellet was resuspended in 800 μL ice-cold ethanol (80%) and centrifuged again at 14.000 rpm for 15 min to get rid of the salt contamination. The supernatant was discarded, pellet was dried by aspiration and then dissolved in 20 μL sterile bidest water. Besides the plasmid to be cloned, the vector plasmid (pIV2, Kan^R) was also subjected to the same treatments.

3.12.3. Ligation

The vector plasmid and the plasmid to be cloned were treated with T4 DNA-ligase (Fermentas, Germany). 8 μL plasmid of concern, 8 μL vector plasmid, 2 μL T4 DNA-ligase buffer solution, and 2 μL T4 DNA-ligase enzyme were mixed and incubated at room temperature for 18 h to perform the ligation.

3.12.4. Tranformation

For transformation, competent *E. coli* cell preparation (NEB 5α, Biolabs Inc. USA) was used. 5 μL ligation mix, 50 μL competent cell preparation, and 50 μL SOC medium was mixed and incubated 30 min in ice-bath. To maintain the heat shock, immediately after incubation, the mixture was incubated in a waterbath at 42ºC for 30 s and transferred to the ice-bath. 900 μL SOC medium was added and incubated at 37ºC for 1 h in a shaking incubator (200 rpm). Thereafter, the culture was centrifuged at 5000 rpm for 5 min and the supernatant was removed by leaving 100 μL to resuspend the pellet. The suspension was spread on the surface of LB-agar plates containing ampicillin (100 μg/mL). After incubation at 37ºC for 18 h, transformant colonies were selected, grown in 5 mL LB-broth containing ampicillin (100 μg/mL) at 37ºC for 18 h, and plasmid analysis was performed with the commercial plasmid isolation kit (Qiagen MiniPrep Kit, Qiagen, Germany). The cloned DNA fragment was detected with restriction digestion with the restriction enzyme used in cloning.

3.12.5. DNA Sequencing by Primer Walking

DNA sequence of the cloned DNA fragment was analysed by primer walking method. First the DNA fragment was sequenced with the primers designed for vector plasmid's multiple cloning site (MCS) from both 5' and 3' sites by forward and reverse primers (Table 3.2). When the sequence obtained, the end of the sequenced fragment was used as the primer for the next part of the fragment sequence. This was repeated until the both sequences from left- and right-hand sides were overlapped. The complete sequence was analysed by SeqMan Pro software program (DNAStar Inc., USA) and by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

3.13. Plasmid Incompatibility Group Detection

Plasmid incompatibility group detection was performed following the method suggested by Carattoli *et al.* (2005). The boiled DNA's (Section 3.8.1) were used as the template DNA, the known plasmids having the corresponding incompatibility group as (+) control and sterile PCR grade water as (-) control (Table 3.3). The list of the primers used in plasmid incompatibility studies is given in Table 3.3. The control plasmids were obtained from Federal Institute of Risk Assessment, *Salmonella* Reference Laboratories with the permission of Dr. Alessandra Carattoli (Department of Infectious, Parasitic, Immune-mediated Diseases, Istituto Superiore di Sanità Roma, Italy).

To detect the incompatibility group, PCR amplification was done with the primers specially designed corresponding to 18 different replication origins of each incompatibility group as given in the method and performed with GeneAmp9700 Thermocycler (Applied BioSystems, USA). Reaction mixture and amplification conditions are given below. By following the method, 5 multiplex- and 3 simplex-PCR analyses were carried out (Table 3.3). While simplex-PCR reaction mix was used as described in Section 3.8.2, multiplex-PCR was performed with the mixture as described below. Except F_{repB} incompatibility group, PCR conditions were the same for both multiplex- and simplex-PCR analyses. The F_{repB}-simplex-PCR was performed with the same amplification program but at an annealing temperature of 52 °C. If any positive result was obtained from the multiplex-PCR, in order to confirm the PCR product, simplex-PCR was performed with the corresponding primers.

Multiplex-PCR reaction mixture (for single reaction):

- 2µL sterile dH_2O (Merck KGaA., Germany)
- 2,5µL 10X PCR buffer solution (Finnzyme Oy, Finland)
- 2,5µL dNTP mix, 200µM end concentration (dNTP Set 1, Roth GmbH., Germany)
- 2,5µL Forward primerI, 1ρmol end concentration
- 2,5µL Reverse primerI, 1ρmol end concentration
- 2,5µL Forward primerII, 1ρmol end concentration
- 2,5µL Reverse primerII, 1ρmol end concentration
- 2,5µL Forward primerIII, 1ρmol end concentration
- 2,5µL Reverse primerIII, 1ρmol end concentration
- 0,5µL DyNazyme II DNA-polymerase (2U/µL) (Finnzyme Oy, Finland)
- 2,5µL Raw-DNA

Multiplex- ve Simplex-PCR amplification conditions:

- 94^oC, 5 min
- 30 cycle: 94^oC/1 min; $60\ ^oC$/30 s; 72^oC/1 min
- 72^oC, 5 min
- 4^oC, ∞

Table 3.3. Primer pairs used for the replicon typing analyses (Carattoli *et al.*, 2005).

Multiplex PCR Group	Incompatibility Group	Primer Pair (Forward/Reverse)	PCR Product
1	HI1	GGAGCGATGGATTACTTCAGTAC/TGCCGTTTCACCTCGTGAGTA	471 bp
	HI2	TTTCTCCTGAGTCACCTGTTAACAC/GGCTCACTACCGTTGTCATCCT	644 bp
	I1	CGAAAGCCGGACGGCAGAA/TCGTCGTTCCGCCAAGTTCGT	139 bp
2	X	AACCTTAGAGGCTATTTAAGTTGCTGAT/GAGAGTCAATTTTTATCTCATGTTTTAGC	376 bp
	L/M	GGATGAAAACTATCAGCATCTGAAG/CTGCAGGGGCGATTCTTTAGG	785 bp
	N	GTCTAACGAGCTTACCGAAG/GTTTCAACTCTGCCAAGTTC	559 bp
3	FIA	CCATGCTGGTTCTAGAGAAGGTG/GTATATCCTTACTGGCTTCCGCAG	462 bp
	FIB	GGAGTTCTGACACACGATTTTCTG/CTCCCGTCGCTTCAGGGCATT	702 bp
	W	CCTAAGAACAACAAAGCCCCCG/GGTGCGCGGCATAGAACCGT	242 bp
4	Y	AATTCAAACAACACTGTGCAGCCTG/GCGAGAATGGACGATTACAAAACTTT	765 bp
	P	CTATGGCCCTGCAAACGCGCCAGAAA/TCACGCGCCAGGGCGCAGCC	534 bp
	FIC	GTGAACTGGCAGATGAGGAAGG/TTCTCCTCGTCGCCAAACTAGAT	262 bp

Table 3.3. Primer pairs used for the replicon typing analyses (continued) (Carattoli *et al.*, 2005).

Multiplex PCR Group	Incompatibility Group	Primer Pair (Forward/Reverse)	PCR Product
5	A/C	GAGAACCAAAGACAAAGACCTGGA/ACGACAAACCTGAATTGCCTCCTT	465 bp
	T	TTGGCCTGTTTGTGCCTAAACCAT/CGTTGATTACACTTAGCTTTGGAC	750 bp
	FII_S	CTGTCGTAAGCTGATGGC/CTCTGCCACAAACTTCAGC	270 bp
Simplex-1	F_{repB}	TGATCGTTTAAGGAATTTTG/GAAGATCAGTCACACCATCC	270 bp
Simplex-2	K/B	GCGGTCCGGAAAGCCAGAAAAC/TCTTTCACGAGCCCGCCAAA	160 bp
Simplex-3	B/O	GCGGTCCGGAAAGCCAGAAAAC/TCTGCGTTCCGCCAAGTTCGA	159 bp

CHAPTER 4

RESULTS

The results of the study are given through this chapter. Briefly there were 203 food samples obtained from Turkish markets analysed and from those 59 epidemiologically unrelated strains were obtained. In addition to these strains, 49 strains originated from Germany were added to the analyses. The antimicrobial resistances of the isolates were found to be variable. Among Turkish strains the highest antimicrobial resistance was observed against NAL, whereas it was against SUL for strains from German origin. Molecular typing of all strains exhibited different plasmid profiles and PFGE patterns. There were 1-4 plasmids/profile for Turkish strains and 1-7 plasmids/profile for German strains. The PFGE patterns revealed 42 different groups, having two major clusters with 44,3% arbitrary homology. The genetic determinants of the antimicrobial resistance were shown similar results to those reported in the literature by the antimicrobial resistance genes, integron analyses, DNA sequencing and lateral gene transfer studies.

4.1. Isolation and Typing of the Strains

4.1.1. Isolation and Biochemical Characterization of Strains

Out of 203 food samples collected 108 *Salmonella* isolates were obtained (Table A.I.3). The rate of isolation was 71% for poultry, 60% for meat and 33% for raw milk. There was only 2 isolates from 18 mayonnaise-based salad samples. Biochemical test results exhibited 100 of the isolates as *Salmonella* spp. strains with 89,4-99,9% probability (Table 4.1). These strains have shown differences in their biochemical pattern in arginine dihydrolysis, lysine decarboxylation, ornithin decarboxylation, gelatinase activity, inositol-oxidation, and mellibiose-fermentation. In comparison with the control strain *S.* Typhimurium ATCC 14028, 14 strains were arginine dihydrolysis, 2 strains were lysine decarboxylation, 2 strains were ornithine decarboxylation, 50 strains were inositol-oxidation, and 1 strain was mellibiose-fermentation deficient. On the other hand, 2 strains were gelatinase positive. These strains revealed the atypic biochemical characteristics of *Salmonella* spp.

4.1.2. Serotyping and Phage Typing

Serological analysis revealed that the most prevalent serotype in the food samples analysed were *S.* Enteritidis (16 isolates) and followed by *S.* Virchow (14 isolates), *S.* Kentucky (11 isolates), and *S.* Infantis (12 isolates). The rest of the isolates exhibited 13 different serotypes corresponding 47% of total isolates (Table 4.2). The DMC52 and DMC85 were found to be mixed culture and two different serotypes were obtained for each. Hence these isolates were renamed as DMC52a (*S.* Kentucky), DMC52b (*S.* subsp. I Roughform), DMC85a (*S.* Kentucky) and DMC85b (*S.* subsp. I Roughform). Phage typing results are given in Table 4.2. Among *S.* Enteritidis serotypes PT21 was the most prevalent but PT3 and PT6 phage types were also detected. The two *S.* Typhimurium isolates were found not to correspond any phage type in the database so called RDNC phage type.

According to the results, out of 100 isolates, 59 were considered as epidemiologically unrelated strains by comparing isolation date, isolation source, serotype and phage type. The rest of the study was performed with these 59 epidemiologically unrelated strains.

Table 4.1. Biochemical analyses results of the strains isolated.

Strain	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Computer Analysis
Conrtol*	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC1	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC2	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC3	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC4	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC5	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC6	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC7	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC8	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC9	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC10	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC11	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC12	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC13	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC14	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC15	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC16	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC17	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC18	-	+	-	-	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	+	*Salmonella* spp.(93.8%)
DMC19	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC20	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC21	-	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC22	-	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC23	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC24	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC25	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC26	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC27	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC28	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC29	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC30	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC31	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC32	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC33	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC34	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)

Table 4.1. Biochemical analyses results of the strains isolated (continued).

Strain	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Computer Analysis
DMC35	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC36	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC37	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC38	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC39	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC40	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC41	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC42	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC43	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC44	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC45	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC46	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC47	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC48	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC49	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC50	-	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.8%)
DMC51	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.8%)
DMC52	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC53	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC54	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC55	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC56	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC57	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC58	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC59	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC60	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC61	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC62	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC63	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC64	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC65	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC66	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC67	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC68	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC69	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)

Table 4.1. Biochemical analyses results of the strains isolated (continued).

Strain	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Computer Analysis
DMC70	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC71	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC72	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC73	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC74	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC75	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC76	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC77	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC78	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC79	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC80	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC81	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC82	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC83	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC84	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC85	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC86	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC87	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC88	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC89	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC90	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC91	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC92	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC93	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	*Salmonella* spp.(99.9%)
DMC94	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC95	-	-	+	-	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(98.2%)
DMC96	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC97	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC98	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(95.1%)
DMC99	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)
DMC100	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	*Salmonella* spp.(89.4%)

ONPG: β -galactosidase; ADH: arginine dihydrolase; LDC: lysine decarboxylase; ODC: ornithine decarboxylase; CIT: citrate utilization; H_2S: H_2S production; URE: urea hydrolysis; TDA: deaminase; IND: indole production; VP: acetoin production; GEL: gelatinase; GLU: glucose-; MAN: mannitol-; INO: inositol-; SOR: sorbitol-; RHA: rhamnose-; SAC: sucrose-; MEL: melibiose-; AMY: amygdalin-; ARA: arabinose-fermentation/oxidation. *Control Strain: *S.* Typhimurium ATTC 14028.

Table 4.2. Serotyping and phage typing results of the strains isolated.

Strain	Serotype	O Antigen*	H1 Antigen*	H2 Antigen*	Phage type**
DMC 1	*S.* Group C1	6,7	k	-	
DMC 2	*S.* Group C1	6,7	k	-	
DMC 3	*S.* Enteritidis	9,12	g,m	-	PT1
DMC 4	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
DMC 5	*S.* Virchow	6,7	r	1,2	
DMC 6	*S.* Virchow	6,7	r	1,2	
DMC 7	*S.* Infantis	6,7	r	1,5	
DMC 8	*S.* Enteritidis	9,12	g,m	-	PT6
DMC 9	*S.* Virchow	6,7	r	1,2	
DMC 10	*S.* Virchow	6,7	r	1,2	
DMC 11	*S.* Virchow	6,7	r	1,2	
DMC 12	*S.* Infantis	6,7	r	1,5	
DMC 13	*S.* subsp. I Roughform	-	-	-	
DMC 14	*S.* Enteritidis	9,12	g,m	-	PT6
DMC 15	*S.* Nchanga	3,10	l,v	1,2	
DMC 16	*S.* Virchow	6,7	r	1,2	
DMC 17	*S.* Virchow	6,7	r	1,2	
DMC 18	*S.* Virchow	6,7	r	1,2	
DMC 19	*S.* Virchow	6,7	r	1,2	
DMC 20	*S.* Infantis	6,7	r	1,5	
DMC 22	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 23	*S.* Infantis	6,7	r	1,5	
DMC 24	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 25	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 26	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 27	*S.* Group C1	6,7	k	-	
DMC 28	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 29	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 30	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 31	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 32	*S.* Virchow	6,7	r	1,2	
DMC 33	*S.* Virchow	6,7	r	1,2	
DMC 34	*S.* Kentucky	8,20	i	z6	
DMC 35	*S.* Kentucky	8,20	i	z6	
DMC 36	*S.* Corvallis	8,20	z4,z23	-	
DMC 37	*S.* Group C1	6,7	k	-	
DMC 38	*S.* Group C1	6,7	k	-	
DMC 39	*S.* Thompson	6,7	k	1,5	

Table 4.2. Serotyping and phage typing results of the strains isolated (continued).

Strain	Serotype	O Antigen*	H1 Antigen*	H2 Antigen*	Phage type**
DMC 40	*S.* Infantis	6,7	r	1,5	
DMC 41	*S.* Infantis	6,7	r	1,5	
DMC 42	*S.* Virchow	6,7	r	1,2	
DMC 43	*S.* Enteritidis	9,12	g,m	-	PT3
DMC 44	*S.* Thompson	6,7	k	1,5	
DMC 45	*S.* Thompson	6,7	k	1,5	
DMC 46	*S.* Group C1	6,7	k	-	
DMC 47	*S.* Thompson	6,7	k	1,5	
DMC 48	*S.* Thompson	6,7	k	1,5	
DMC 49	*S.* Group C1	6,7	k	-	
DMC 50	*S.* Group C1	6,7	k	-	
DMC 51	*S.* Thompson	6,7	k	1,5	
DMC 52a	*S.* Kentucky	8,20	i	z6	
DMC 52b	*S.* subsp. I Roughform	-	-	-	
DMC 53	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 54	*S.* subsp. I Roughform	-	-	-	
DMC 55	*S.* Senftenberg	1,3,19	g,[s],t	-	
DMC 56	*S.* Kentucky	8,20	i	z6	
DMC 57	*S.* Infantis	6,7	r	1,5	
DMC 58	*S.* Infantis	6,7	r	1,5	
DMC 59	*S.* Agona	4,12	f,g,s	-	
DMC 60	*S.* Agona	4,12	f,g,s	-	
DMC 61	*S.* Agona	4,12	f,g,s	-	
DMC 62	*S.* Agona	4,12	f,g,s	-	
DMC 63	*S.* subsp. I Roughform	-	-	-	
DMC 64	*S.* Agona	4,12	f,g,s	-	
DMC 65	*S.* Kentucky	8,20	i	z6	
DMC 66	*S.* Telaviv	28	y	e,n,z15	
DMC 67	*S.* Telaviv	28	y	e,n,z15	
DMC 68	*S.* Virchow	6,7	r	1,2	
DMC 69	*S.* Virchow	6,7	r	1,2	
DMC 70	*S.* Infantis	6,7	r	1,5	
DMC 71	*S.* Infantis	6,7	r	1,5	
DMC 72	*S.* Kentucky	8,20	i	z6	
DMC 73	*S.* Kentucky	8,20	i	z6	
DMC 74	*S.* Kentucky	8,20	i	z6	
DMC 75	*S.* Infantis	6,7	r	1,5	
DMC 76	*S.* Infantis	6,7	r	1,5	
DMC 77	*S.* Infantis	6,7	r	1,5	
DMC 78	*S.* Bispebjerg	4,[5[,12	a	e,n,x	

Table 4.2. Serotyping and phage typing results of the strains isolated (continued).

Strain	Serotype	O Antigen*	H1 Antigen*	H2 Antigen*	Phage type**
DMC 79	*S.* Kentucky	8,20	i	z6	
DMC 80	*S.* Kentucky	8,20	i	z6	
DMC 81	*S.* Montevideo	6,7	g,m,[p],s	[1,2,7]	
DMC 82	*S.* Montevideo	6,7	g,m,[p],s	[1,2,7]	
DMC 83	*S.* Virchow	6,7	r	1,2	
DMC 84	*S.* Virchow	6,7	r	1,2	
DMC 85a	*S.* Kentucky	8,20	i	z6	
DMC 85b	*S.* subsp. I Roughform	-	-	-	
DMC 86	*S.* Corvallis	8,20	z4,z23	z6	
DMC 87	*S.* Corvallis	8,20	z4,z23	z6	
DMC 88	*S.* Montevideo	6,7	g,m,[p],s	[1,2,7]	
DMC 89	*S.* Montevideo	6,7	g,m,[p],s	[1,2,7]	
DMC 90	*S.* Anatum	3,10	e,h	1,6	
DMC 91	*S.* Anatum	3,10	e,h	1,6	
DMC 92	*S.* Anatum	3,10	e,h	1,6	
DMC 93	*S.* Salford	16	l,v	e,n,x	
DMC 94	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 95	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
DMC 96	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 97	*S.* subsp. I Roughform	-	-	-	
DMC 98	*S.* Enteritidis	9,12	g,m	-	PT21
DMC 99	*S.* Nchanga	3,10	l,v	1,2	
DMC 100	*S.* Corvallis	8,20	z4,z23	-	

*****Antigens are numbered by following Kauffmann-White scheme.
******PT: Phage Type; RDNC: **R**eact but **D**oes **N**ot **C**onform.

4.1.3. Selected Strains from Germany

In addition to those isolated, 49 antibiotic resistant strains were added from Germany (Table 4.3). These strains were selected from the culture collection of *Salmonella* Reference Laboratories by considering the period of isolation (2005-2006), the isolation material (food of avian origin) and the serotypes of the strains originated from Türkiye. Serotyping and phage typing of the selected strains had been done previously when they were added to the culture collection. The serotype distribution was *S.* Agona, *S.* Enteritidis, *S.* Infantis (15 strains), *S.* Kentucky (2 strains), *S.* subsp. I Roughform (2 strains), *S.* Thompson (2 strains), *S.* Typhimurium (25 strains), and *S.* Virchow (2 strains). The most common phage type for *S.* Typhimurium strains were DT104, whereas the only *S.* Enteritidis strain was PT4 phage type.

Table 4.3. Serotyping and phage typing results strains obtained from Germany.

Strain	Serotype	O Antigen*	H1 Antigen*	H2 Antigen*	Phage type**
BFR1	*S.* Agona	4,12	f,g,s	-	
BFR2	*S.* Enteritidis	9,12	g,m	-	PT4
BFR3	*S.* Kentucky	8,20	i	z6	
BFR4	*S.* Kentucky	8,20	i	z6	
BFR5	*S.* subsp. I Roughform	-	-	-	
BFR6	*S.* subsp. I Roughform	-	-	-	
BFR7	*S.* Thompson	6,7	k	1,5	
BFR8	*S.* Virchow	6,7	r	1,2	
BFR9	*S.* Virchow	6,7	r	1,2	
BFR10	*S.* Infantis	6,7	r	1,5	
BFR11	*S.* Infantis	6,7	r	1,5	
BFR12	*S.* Infantis	6,7	r	1,5	
BFR13	*S.* Infantis	6,7	r	1,5	
BFR14	*S.* Infantis	6,7	r	1,5	
BFR15	*S.* Infantis	6,7	r	1,5	
BFR16	*S.* Infantis	6,7	r	1,5	
BFR17	*S.* Infantis	6,7	r	1,5	
BFR18	*S.* Infantis	6,7	r	1,5	
BFR19	*S.* Infantis	6,7	r	1,5	
BFR20	*S.* Infantis	6,7	r	1,5	
BFR21	*S.* Infantis	6,7	r	1,5	
BFR22	*S.* Infantis	6,7	r	1,5	
BFR23	*S.* Infantis	6,7	r	1,5	
BFR24	*S.* Infantis	6,7	r	1,5	
BFR25	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR26	*S.* Typhimurium	1,4,[5],12	i	1,2	NT
BFR27	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR28	*S.* Typhimurium	1,4,[5],12	i	1,2	U302
BFR29	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR30	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104B low
BFR31	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR32	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR33	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104B low
BFR34	*S.* Typhimurium	1,4,[5],12	i	1,2	DT120
BFR35	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
BFR36	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
BFR37	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
BFR38	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
BFR39	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC

Table 4.3. Serotyping and phage typing results strains obtained from Germany (continued).

Strain	Serotype	O Antigen*	H1 Antigen*	H2 Antigen*	Phage type**
BFR40	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104B low
BFR41	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR42	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR43	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR44	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR45	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR46	*S.* Typhimurium	1,4,[5],12	i	1,2	RDNC
BFR47	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR48	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L
BFR49	*S.* Typhimurium	1,4,[5],12	i	1,2	DT104L

*****Antigens are numbered by following Kauffmann-White scheme.
******PT: Phage Type; DT : Definitive Type; RDNC: **R**eact but **D**oes **N**ot **C**onform.

4.2. Antibiotic Susceptibilities of the Strains

Antibiotic susceptibility results of the strains are given in Table 4.4 for disk diffusion and in Table 4.5 for MIC values. The Turkish origin strains gave 39% resistance (23 out of 59 strains) for the tested antibiotics. The most prevalent resistance profile was KAN-NEO-NAL-STR-SPE-SUL-TET-TMP-SXT (8 strains), which was obtained only from *S.* Infantis strains. Other resistance profiles were AMP-EFT-NAL-STR-SUL-TMP-SXT (DMC 19), NAL-STR-SPE-SUL-TET-TMP-SXT (DMC40), AMP-SUL-TMP-SXT (DMC15), STR-SPE-SUL (DMC34), AMP- NAL (3 strain), NAL (6 strain).

Among 19 strains resistant to NAL, 7 had reduced susceptibility to CIP (MIC value of 0.5-1 μg/mL). Besides, 7 strains had intermediate STR resistance (16 μg/mL). The antimicrobials CHL (≤ 2-8 μg/mL), FFC (≤ 2-8 μg/mL) and COL (≤ 4 μg/mL) were found to be effective against *Salmonella* strains from Türkiye. On the other hand, the highest resistance was obtained against NAL with MIC value of >128 μg/mL. This corresponded to 82% of resistance strains and 32% of epidemiologically unrelated strains.

Antibiotic resistance profiles among German strains were variable (Table 4.4). Out of 49 strains, 12 exhibited NAL-TET-STR-SPE-SUL which were all *S.* Infantis serotype. In addition, 6 strains had AMP-CHL-FFC-TET-STR-SPE-SUL; 5 strains had AMP-TET-STR-SUL; 5 strains had AMP-CHL-FFC-NAL-TET-STR-SPE-SUL; 4 strains had AMP-SUL-TMP-SXT; 3 strains had AMP-SUL and 3 strains had only NAL resistance phenotype. One strain exhibited resistance to extended spectrum β-lactams (BFR3).

As shown in Table 4.5, reduced susceptibilty to CIP (0.5-1 μg/mL) was also found in 12 German strains. Intermediate resistance to AMC (16/8 μg/mL) in 19 strains and CHL (16 μg/mL) in 5 strains were detected. The most effective antibiotic was COL (≤ 4 μg/mL), where there was no resistance observed among German strains. Subsequently, the least effective antibiotic was found as SUL (>512 μg/mL) corresponding 91% of the German origin strains.

Table 4.4. Antibiotic susceptibilities of the strains by disk diffusion method.

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT
DMC1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC5	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S
DMC7	S	S	S	S	S	R	R	S	R	I	R	R	R	R	R	R
DMC8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC9	R	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S
DMC12	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R
DMC13	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC15	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R
DMC16	R	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S
DMC18	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S
DMC19	R	I	R	S	S	S	S	S	R	S	S	R	S	R	R	R
DMC20	S	S	S	S	S	R	R	S	R	I	R	R	R	R	R	R
DMC22	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC23	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R
DMC24	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
DMC25	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC27	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC28	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC31	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC32	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S
DMC34	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S
DMC36	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC37	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC39	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC40	S	S	S	S	S	S	S	S	R	I	R	R	R	R	R	R

Table 4.4. Antibiotic susceptibilities of the strains by disk diffusion method (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT
DMC42	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S
DMC43	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC44	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC46	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC49	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC52a	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC52b	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC53	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC55	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC56	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC57	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R
DMC58	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R
DMC59	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC63	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC65	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC66	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC68	R	S	S	S	S	S	S	S	R	I	S	I	S	S	S	S
DMC70	S	S	S	S	S	R	R	S	R	I	R	R	R	R	R	R
DMC72	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC75	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R
DMC78	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC79	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC81	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC83	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
DMC85a	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC85b	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC86	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DMC88	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 4.4. Antibiotic susceptibilities of the strains by disk diffusion method (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT
DMC90	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S
DMC91	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S
DMC93	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
BFR1	S	S	S	S	S	S	S	S	S	I	R	S	S	R	R	R
BFR2	R	S	S	S	S	S	S	S	R	S	R	R	R	R	R	R
BFR3	R	R	R	S	S	S	S	S	S	S	S	R	S	R	S	S
BFR4	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R
BFR5	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S
BFR6	R	I	S	I	S	I	S	I	R	I	R	R	R	R	S	S
BFR7	S	S	S	S	S	R	R	S	R	I	R	S	S	S	S	S
BFR8	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
BFR9	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
BFR10	S	S	S	S	S	S	S	S	R	S	R	R	R	R	S	S
BFR11	S	S	S	S	S	S	S	S	R	I	R	R	R	R	S	S
BFR12	S	S	S	S	S	S	S	S	R	I	R	R	R	R	S	S
BFR13	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R
BFR14	S	S	S	I	S	S	S	S	R	I	R	R	R	R	S	S
BFR15	S	S	S	I	I	S	S	S	R	I	R	R	R	R	S	S
BFR16	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R
BFR17	S	S	S	S	S	S	S	S	R	S	R	R	R	R	S	S
BFR18	S	S	S	S	S	S	S	S	R	I	R	R	R	R	S	S
BFR19	S	S	S	S	S	S	S	S	R	I	R	R	R	R	S	S
BFR20	S	S	S	I	S	S	S	S	R	I	R	R	R	R	S	S
BFR21	S	S	S	S	S	S	S	S	R	S	R	R	R	R	S	S
BFR22	S	S	S	S	S	S	S	S	R	I	R	R	R	R	S	S
BFR23	R	S	S	S	S	S	S	S	S	S	S	I	S	R	R	R
BFR24	S	S	S	I	S	S	S	S	R	I	R	R	R	R	S	S
BFR25	R	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S

Table 4.4. Antibiotic susceptibilities of the strains by disk diffusion method (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT
BFR26	R	I	S	R	R	R	R	S	R	S	R	R	R	R	S	S
BFR27	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S
BFR28	R	I	S	R	R	S	S	S	R	S	R	R	R	R	S	S
BFR29	R	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
BFR30	R	I	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR31	R	I	S	R	R	S	S	S	R	S	R	R	R	R	S	S
BFR32	R	I	S	R	R	S	S	S	R	S	R	R	R	R	S	S
BFR33	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S
BFR34	R	S	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR35	R	S	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR36	R	S	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR37	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R
BFR38	R	S	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR39	R	S	S	S	S	S	S	S	S	S	R	R	R	R	S	S
BFR40	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S
BFR41	R	I	S	R	R	R	R	S	S	S	R	R	R	R	S	S
BFR42	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S
BFR43	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S
BFR44	R	I	S	S	S	S	S	S	S	S	R	R	S	R	S	S
BFR45	R	I	S	R	R	S	S	S	R	S	R	R	R	R	S	S
BFR46	R	S	S	R	R	S	S	S	S	S	R	R	S	R	S	S
BFR47	R	I	S	R	R	S	S	S	R	S	R	R	R	R	S	S
BFR48	R	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
BFR49	R	I	S	R	R	S	S	S	S	S	R	R	R	R	S	S

AMP: Ampicillin; AMC: Amoxicillin/clavulonic acid; CIP: Ciprofloxacin; CHL: Chloramphenicol; EFT: Ceftiofur; FFC: Florfenicol; GEN: Gentamicin; KAN: Kanamycin; NEO: Neomycin; NAL: Nalidixic acid; STR Streptomycin; SPE: Spectinomycin; SUL: Sulfonamide compounds; TET: Tetracycline; TMP: Trimethoprim; SXT: Sulfamethoxazole/Trimethoprim; S: Susceptible; I: Intermediate; R: Resistant.

Table 4.5. Antibiotic susceptibilities of the strains by MIC values (μg/mL).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT	COL
DMC5	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S	S
	(2)	(≤2/1)	(≤0.5)	(8)	(8)	(≤4)	(≤2)	(≤1)	(>128)	(0.12)	(≤2)	(16)	(32)	(64)	(≤4)	(≤1/19)	(≤4)
DMC7	S	S	S	S	S	R	R	S	R	I	R	R	R	R	R	R	S
	(≤1)	(≤2/1)	(≤0.5)	(4)	(4)	(>64)	(32)	(≤1)	(>128)	(0.5)	(>32)	(32)	(>128)	(>512)	(>32)	(>8/152)	(≤4)
DMC9	R	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S	S
	(>32)	(8/4)	(≤0.5)	(8)	(4)	(≤4)	(≤2)	(≤1)	(>128)	(1)	(≤2)	(8)	(32)	(≤32)	(≤4)	(≤1/19)	(≤4)
DMC12	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R	S
	(≤1)	(≤2/1)	(1)	(4)	(4)	(>64)	(>32)	(≤1)	(>128)	(0.25)	(>32)	(32)	(>128)	(>512)	(>32)	(>8/152)	(≤4)
DMC15	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S
	(>32)	(8/4)	(≤0.5)	(4)	(4)	(≤4)	(≤2)	(≤1)	(≤4)	(≤0.03)	(≤2)	(8)	(32)	(>512)	(>32)	(>8/152)	(≤4)
DMC16	R	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S	S
	(>32)	(8/4)	(1)	(8)	(4)	(≤4)	(≤2)	(≤1)	(128)	(1)	(≤2)	(8)	(32)	(≤32)	(≤4)	(≤1/19)	(≤4)
DMC18	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S	S
	(2)	(≤2/1)	(≤0.5)	(8)	(8)	(≤4)	(≤2)	(≤1)	(128)	(0.25)	(≤2)	(16)	(32)	(64)	(≤4)	(≤1/19)	(≤4)
DMC19	R	I	R	S	S	S	S	S	R	S	S	R	S	R	R	R	S
	(>32)	(16/8)	(>8)	(8)	(8)	(≤4)	(≤2)	(≤1)	(128)	(0.25)	(≤2)	(>64)	(32)	(>512)	(>32)	(>8/152)	(≤4)
DMC20	S	S	S	S	S	R	R	S	R	I	R	R	R	R	R	R	S
	(2)	(≤2/1)	(1)	(8)	(8)	(>64)	(32)	(≤1)	(>128)	(1)	(>32)	(32)	(>128)	(>512)	(>32)	(>8/152)	(≤4)
DMC23	S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R	S
	(≤1)	(≤2/1)	(≤0.5)	(8)	(8)	(>64)	(>32)	(≤1)	(>128)	(0.25)	(>32)	(32)	(>128)	(>512)	(>32)	(>8/152)	(≤4)
DMC24	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
	(≤1)	(≤2/1)	(1)	(8)	(4)	(≤4)	(≤2)	(≤1)	(>128)	(0.25)	(≤2)	(≤4)	(16)	(64)	(≤4)	(≤1/19)	(≤4)
DMC32	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S	S
	(≤1)	(≤2/1)	(≤0.5)	(8)	(4)	(≤4)	(≤2)	(≤1)	(>128)	(0.25)	(≤2)	(16)	(32)	(64)	(≤4)	(≤1/19)	(≤4)
DMC34	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S
	(≤1)	(≤2/1)	(1)	(4)	(4)	(≤4)	(≤2)	(≤1)	(≤4)	(0.12)	(≤2)	(32)	(>128)	(>512)	(≤4)	(≤1/19)	(≤4)
DMC40	S	S	S	S	S	S	S	S	R	I	R	R	R	R	R	R	S
	(2)	(≤2/1)	(1)	(8)	(8)	(≤4)	(≤2)	(≤1)	(>128)	(1)	(>32)	(32)	(>128)	(>512)	(>32)	(>8/152)	(≤4)
DMC42	S	S	S	S	S	S	S	S	R	S	S	I	S	S	S	S	S
	(≤1)	(≤2/1)	(≤0.5)	(8)	(4)	(≤4)	(≤2)	(≤1)	(>128)	(0.25)	(≤2)	(16)	(32)	(64)	(≤4)	(≤1/19)	(≤4)

Table 4.5. Antibiotic susceptibilities of the strains by MIC values (μg/mL) (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT	COL
DMC57	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	R (>64)	R (>32)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (64)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
DMC58	S (≤1)	S (≤2/1)	S (≤0.5)	S (≤2)	S (≤2)	R (>64)	R (>32)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (32)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
DMC68	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (1)	S (≤2)	I (16)	S (64)	S (64)	S (≤4)	S (≤1/19)	S (≤4)
DMC70	S (2)	S (≤2/1)	S (1)	S (8)	S (4)	R (>64)	R (>32)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (32)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
DMC75	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	R (>64)	R (>32)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (64)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
DMC83	S (≤1)	S (≤2/1)	S (1)	S (8)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	S (≤2)	S (8)	S (32)	S (≤32)	S (≤4)	S (≤1/19)	S (≤4)
DMC90	S (≤1)	S (≤2/1)	S (≤0.5)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	I (16)	S (32)	S (≤32)	S (≤4)	S (≤1/19)	S (≤4)
DMC91	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	I (16)	S (64)	S (≤32)	S (≤4)	S (≤1/19)	S (≤4)
BFR1	S (≤1)	S (≤2/1)	S (1)	S (8)	S (8)	S (≤4)	S (≤2)	S (≤1)	S (16)	I (0.5)	R (>32)	S (8)	S (32)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR2	R (>32)	S (8/4)	S (1)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (>64)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR3	R (>32)	R (32/16)	R (>8)	S (≤2)	S (≤2)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	R (64)	S (32)	R (128)	S (≤4)	S (≤1/19)	S (≤4)
BFR4	R (>32)	S (8/4)	S (≤0.5)	S (4)	S (≤2)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (64)	R (>128)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR5	S (≤1)	S (≤2/1)	S (≤0.5)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	S (≤2)	S (≤4)	S (16)	R (64)	S (≤4)	S (≤1/19)	S (≤4)
BFR6	R (>32)	I (16/8)	S (2)	I (16)	S (8)	I (32)	S (≤2)	I (8)	R (>128)	I (1)	R (>32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR7	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	R (>64)	R (>32)	S (≤1)	R (>128)	I (0.5)	R (>32)	S (8)	S (64)	S (64)	S (≤4)	S (≤1/19)	S (≤4)

Table 4.5. Antibiotic susceptibilities of the strains by MIC values (μg/mL) (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT	COL
BFR8	S (≤1)	S (≤2/1)	S (≤0.5)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	S (≤2)	S (≤4)	S (32)	S (64)	S (≤4)	S (≤1/19)	S (≤4)
BFR9	S (≤1)	S (≤2/1)	S (1)	S (8)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	S (≤2)	S (8)	S (32)	S (64)	S (≤4)	S (≤1/19)	S (≤4)
BFR10	S (≤1)	S (≤2/1)	S (≤0.5)	S (≤2)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR11	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR12	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR13	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S(≤2)	S (8)	S (64)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR14	S (2)	S (≤2/1)	S (2)	I (16)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (1)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR15	S (2)	S (≤2/1)	S (1)	I (16)	I (16)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (1)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR16	R (>32)	S (4/2)	S (≤0.5)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (8)	S (32)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR17	S (≤1)	S (≤2/1)	S (1)	S (8)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR18	S (≤1)	S (≤2/1)	S (1)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR19	S (≤1)	S (≤2/1)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR20	S (2)	S (≤2/1)	S (1)	I (16)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (1)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR21	S (≤1)	S (≤2/1)	S (1)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR22	S (≤1)	S (≤2/1)	S (1)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (>64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)

Table 4.5. Antibiotic susceptibilities of the strains by MIC values (μg/mL) (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT	COL
BFR23	R (>32)	S (8/4)	S (1)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	I (16)	S (32)	R (>512)	R (>32)	R (>8/152)	S (≤4)
BFR24	S (≤1)	S (≤2/1)	S (2)	I (16)	S (8)	S (≤4)	S (≤2)	S (≤1)	R (>128)	I (0.5)	R (>32)	R (32)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR25	R (>32)	I (16/8)	S (1)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (8)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR26	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (>32)	R (>64)	R (>32)	S (≤1)	R (>128)	S (0.25)	R (32)	R (>64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR27	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (32)	R (>64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR28	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR29	R (>32)	I (16/8)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (8)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR30	R (>32)	I (16/8)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR31	R (>32)	I (16/8)	S (1)	R (>64)	R (>64)	S (≤4)	S (4)	S (≤1)	R (>128)	S (0.25)	R (>32)	R (>64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR32	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR33	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR34	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR35	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (8)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR36	R (>32)	S (4/2)	S (1)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR37	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (8)	S (32)	R (>512)	R (>32)	R (>8/152)	S (≤4)

Table 4.5. Antibiotic susceptibilities of the strains by MIC values (μg/mL) (continued).

Strain	AMP	AMC	EFT	CHL	FFC	KAN	NEO	GEN	NAL	CIP	TET	STR	SPE	SUL	TMP	SXT	COL
BFR38	R (>32)	S (8/4)	S (1)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR39	R (>32)	S (8/4)	S (≤0.5)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR40	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR41	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	R (>64)	R (>32)	S (≤1)	S (≤4)	S (≤0.03)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR42	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR43	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR44	R (>32)	I (16/8)	S (1)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (32)	R (64)	S (≤4)	S (≤1/19)	S (≤4)
BFR45	R (>32)	I (16/8)	S (≤0.5)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR46	R (>32)	S (8/4)	S (1)	R (>64)	R (>64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (>32)	R (>64)	S (64)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR47	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	R (>128)	S (0.25)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR48	R (>32)	I (16/8)	S (1)	S (8)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (8)	S (64)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
BFR49	R (>32)	I (16/8)	S (1)	R (>64)	R (64)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	R (32)	R (64)	R (>128)	R (>512)	S (≤4)	S (≤1/19)	S (≤4)
ATTC 200406*	S (2)	S (≤2/1)	S (≤0.5)	S (4)	S (4)	S (≤4)	S (≤2)	S (≤1)	S (≤4)	S (≤0.03)	S (≤2)	S (≤4)	S (16)	S (≤32)	S (≤4)	S (≤1/19)	S (≤4)

* ATTC 200406: Control *E. coli* strain.

AMP: Ampicillin; AMC: Amoxicillin/clavulonic acid; CIP: Ciprofloxacin; CHL: Chloramphenicol; EFT: Ceftiofur; FFC: Florfenicol; GEN: Gentamicin; KAN: Kanamycin; NEO: Neomycin; NAL: Nalidixic acid; STR Streptomycin; SPE: Spectinomycin; SUL: Sulfonamide compounds; TET: Tetracycline; TMP: Trimethoprim; SXT: Sulfamethoxazole/Trimethoprim; S: Susceptible; I: Intermediate; R: Resistant.

4.3. Molecular Typing of the Strains

4.3.1. Plasmid Profiles

The plasmid profiles for the strains are given in Figure 4.1-4.7. The molecular typing of the strains by plasmid profiling revealed that 36 Turkish origin and 45 German origin strains had plasmid content out of 108 strains studied. There were 1-4 plasmids/profile ranging in size <5-206 kb for Turkish strains and 1-7 plasmids/profile with <5-238 kb size for German strains. Plasmid contents were variable not only between serotypes but also some strains of the same serotypes. The distribution of different plasmid profiles of the serotypes was *S.* Montevideo (2), *S.* subsp. I Roughform (3), *S.* Virchow (4), and *S.* Enteritidis (5) for Turkish origin strains; whereas *S.* Kentucky (2), *S.* Infantis (2), and *S.* Typhimurium (13) for German origin strains. On the other hand, the strains DMC 1, DMC27, DMC37, DMC46, DMC49 DMC88, DMC39, DMC44, DMC85a, DMC85b, DMC18, DMC83, DMC36, DMC86, DMC34, DMC52, DMC56, DMC65, DMC72, DMC79, DMC90, DMC92, DMC66, BFR8, BFR9, BFR29, and BFR 46 did not possess any plasmid.

S. Infantis strains of Turkish origin did not differ in plasmid content having the ~206 kb size plasmid. *S.* Virchow strains had the ~45 kb plasmid in common. The *S.* Infantis strains of German origin were divided into two groups one having ~238 kb plasmid and the other having ~108,6 kb plasmid. Besides, almost all the *S.* Typhimurium strains had the big plasmid varying in size between ~85-112 kb which is suspected to be previously described virulence plasmid psLT90.

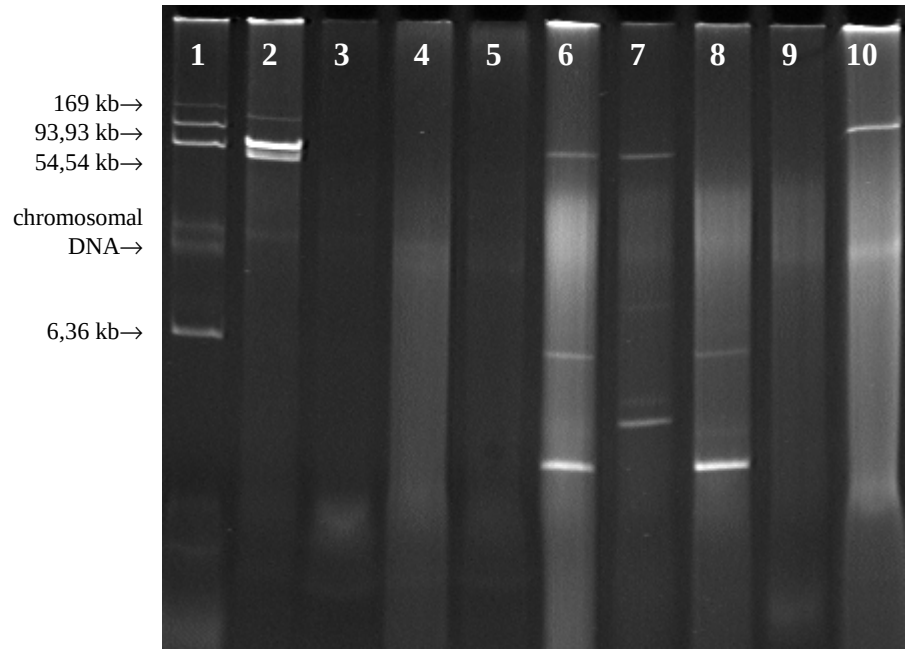


Figure 4.1. Plasmid profiles of Turkish origin *S.* Montevideo, *S.* Thompson and *S.* subsp. I Roughform strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	Marker	-	169; 93,93; 54,54; 6,36
2	DMC81	*S.* Montevideo	116,5; 54,03; 50,95
3	DMC88	*S.* Montevideo	-
4	DMC39	*S.* Thompson	-
5	DMC44	*S.* Thompson	-
6	DMC52b	*S.* subsp. I Roughform	51,98; <5
7	DMC13	*S.* subsp. I Roughform	51,98; <5
8	DMC63	*S.* subsp. I Roughform	<5
9	DMC85b	*S.* subsp. I Roughform	-
10	(+) control	*S.* Typhimurium	86

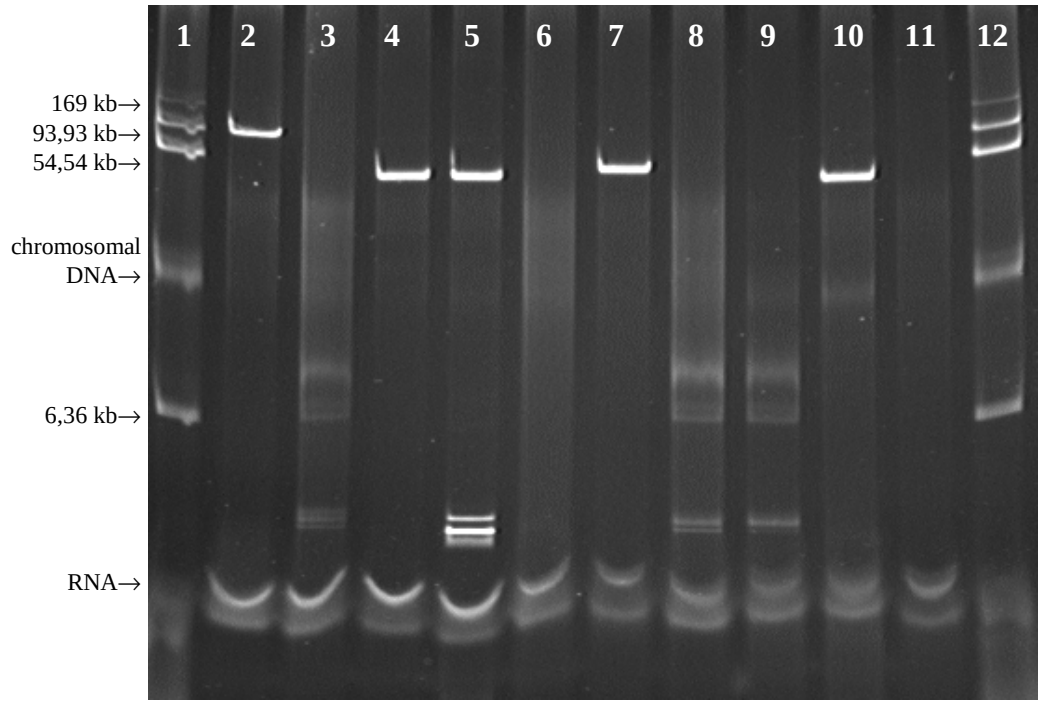


Figure 4.2. Plasmid profiles of Turkish origin *S.* Virchow strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	Marker	-	169; 93,93; 54,54; 6,36
2	(+)control	*S.* Typhimurium	86
3	DMC5	*S.* Virchow	5,5; <5 (3 small ones)
4	DMC9	*S.* Virchow	50,3
5	DMC16	*S.* Virchow	50,3; <5 (3 small ones)
6	DMC18	*S.* Virchow	-
7	DMC19	*S.* Virchow	50,3
8	DMC32	*S.* Virchow	5,5; <5 (2 small ones)
9	DMC42	*S.* Virchow	5,5; <5
10	DMC68	*S.* Virchow	50,3
11	DMC83	*S.* Virchow	-
12	Marker	-	169; 93,93; 54,54; 6,36

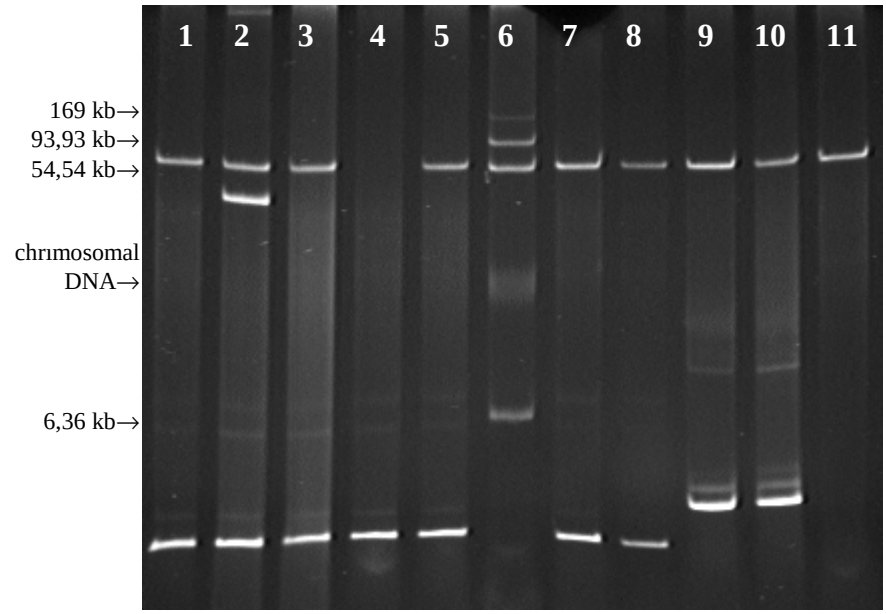


Figure 4.3. Plasmid profiles of Turkish origin *S.* Enteritidis strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	DMC3	*S.* Enteritidis	57,57; <5
2	DMC8	*S.* Enteritidis	57,57; 48,66; <5
3	DMC14	*S.* Enteritidis	57,57; <5
4	DMC21	*S.* Enteritidis	<5
5	DMC22	*S.* Enteritidis	57,57; <5
6	Marker	-	169; 93,93; 54,54; 6,36
7	DMC24	*S.* Enteritidis	57,57; <5
8	DMC28	*S.* Enteritidis	57,57; <5
9	DMC30	*S.* Enteritidis	57,57; <5 (2 small ones)
10	DMC43	*S.* Enteritidis	57,57; <5 (2 small ones)
11	DMC53	*S.* Enteritidis	57,57

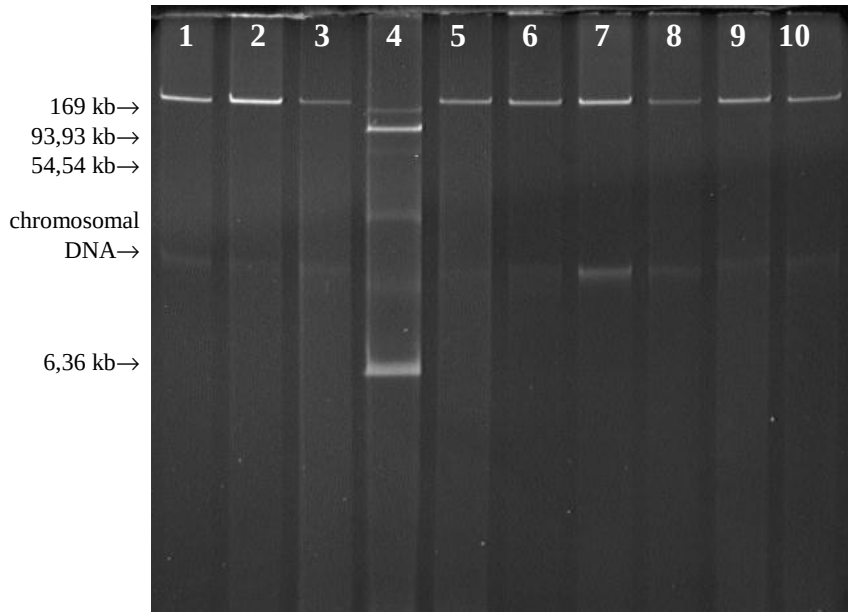


Figure 4.4. Plasmid profiles of Turkish origin *S.* Infantis strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	DMC7	*S.* Infantis	206,5
2	DMC12	*S.* Infantis	206,5
3	DMC20	*S.* Infantis	206,5
4	Marker	-	169; 93,93; 54,54; 6,36
5	DMC23	*S.* Infantis	206,5
6	DMC40	*S.* Infantis	206,5
7	DMC57	*S.* Infantis	206,5
8	DMC58	*S.* Infantis	206,5
9	DMC70	*S.* Infantis	206,5
10	DMC75	*S.* Infantis	206,5

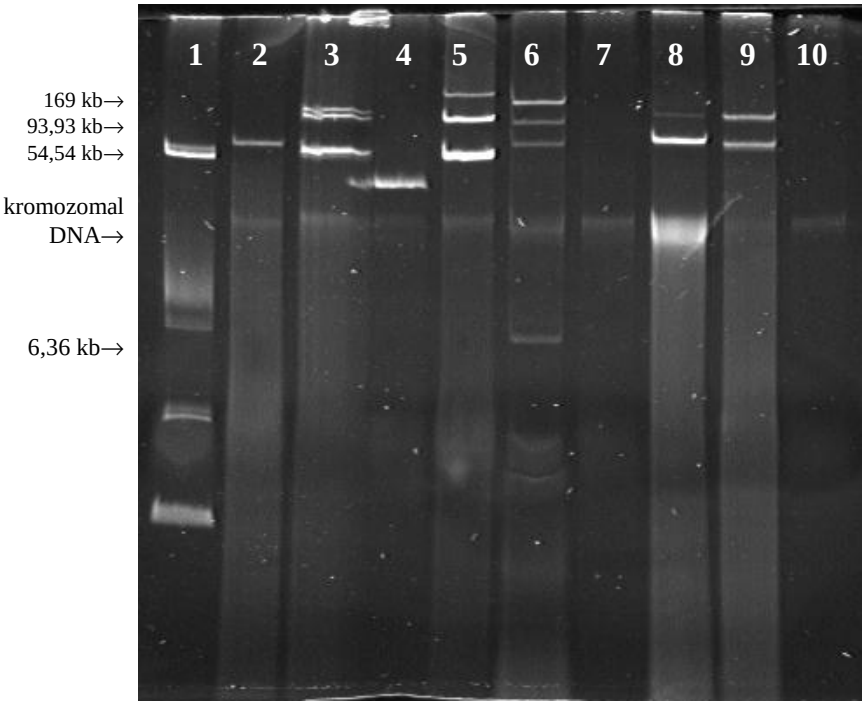


Figure 4.5. Plasmid profiles of German origin *S.* Agona, *S.* subsp. I Rough form, *S.* Kentucky, *S.* Thompson, *S.*Virchow and *S.* Enteritidis strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	BFR1	*S.* Agona	46,66; 7,41; 2,70; 0,95
2	BFR5	*S.* subsp. I Roughform	56,87
3	BFR4	*S.* Kentucky	143,99; 114,24; 51,52
4	BFR7	*S.* Thompson	35,22
5	BFR3	*S.* Kentucky	220,72; 104,52; 49,59
6	Marker	-	169; 93,93; 54,54; 6,36
7	BFR8	*S.* Virchow	-
8	BFR6	*S.* subsp. I Roughform	116,30; 59,30
9	BFR2	*S.* Enteritidis	110; 54,54
10	BFR9	*S.* Virchow	-

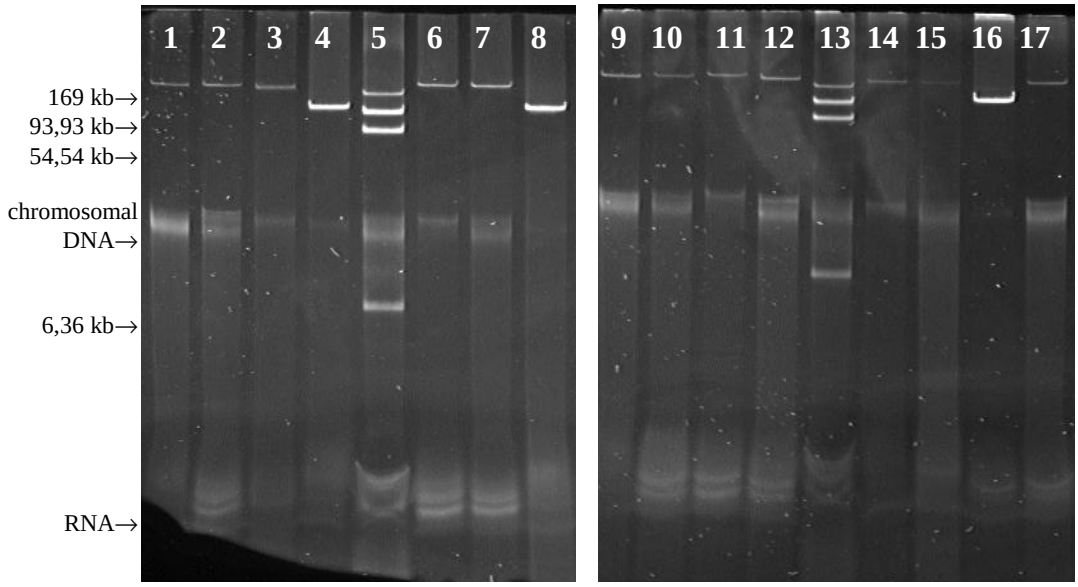


Figure 4.6. Plasmid profiles of German origin *S.* Infantis strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	BFR10	*S.* Infantis	238,06
2	BFR11	*S.* Infantis	238,06
3	BFR12	*S.* Infantis	238,06
4	BFR13	*S.* Infantis	108,79
5	Marker	-	169; 93,93; 54,54; 6,36
6	BFR14	*S.* Infantis	238,06
7	BFR15	*S.* Infantis	238,06
8	BFR16	*S.* Infantis	108,59
9	BFR17	*S.* Infantis	238,06
10	BFR18	*S.* Infantis	238,06
11	BFR19	*S.* Infantis	238,06
12	BFR20	*S.* Infantis	238,06
13	Marker	-	169; 93,93; 54,54; 6,36
14	BFR21	*S.* Infantis	238,06
15	BFR22	*S.* Infantis	238,06
16	BFR23	*S.* Infantis	108,05
17	BFR24	*S.* Infantis	238,06

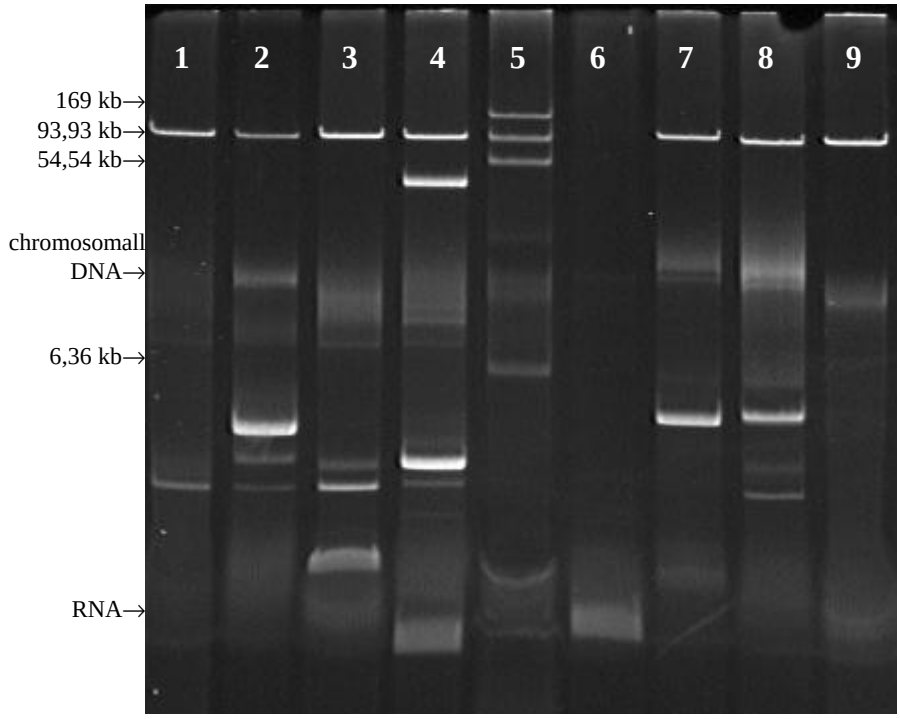


Figure 4.7. Plasmid profiles of German origin *S.* Typhimurium strains.

Lane	Strain	Serotype	Plasmid(s) in kb
1	BFR25	*S.* Typhimurium	99,08; 1,94
2	BFR26	*S.* Typhimurium	99,08; 3,44; 2,64; 1,94
3	BFR27	*S.* Typhimurium	99,08; 2,64; 1,94
4	BFR28	*S.* Typhimurium	99,08; 43,55; 2,64; 1,94
5	Marker	-	169; 93,93; 54,54; 6,36
6	BFR29	*S.* Typhimurium	-
7	BFR30	*S.* Typhimurium	99,08; 3,44
8	BFR31	*S.* Typhimurium	99,08; 3,44; 1,94
9	BFR32	*S.* Typhimurium	99,08

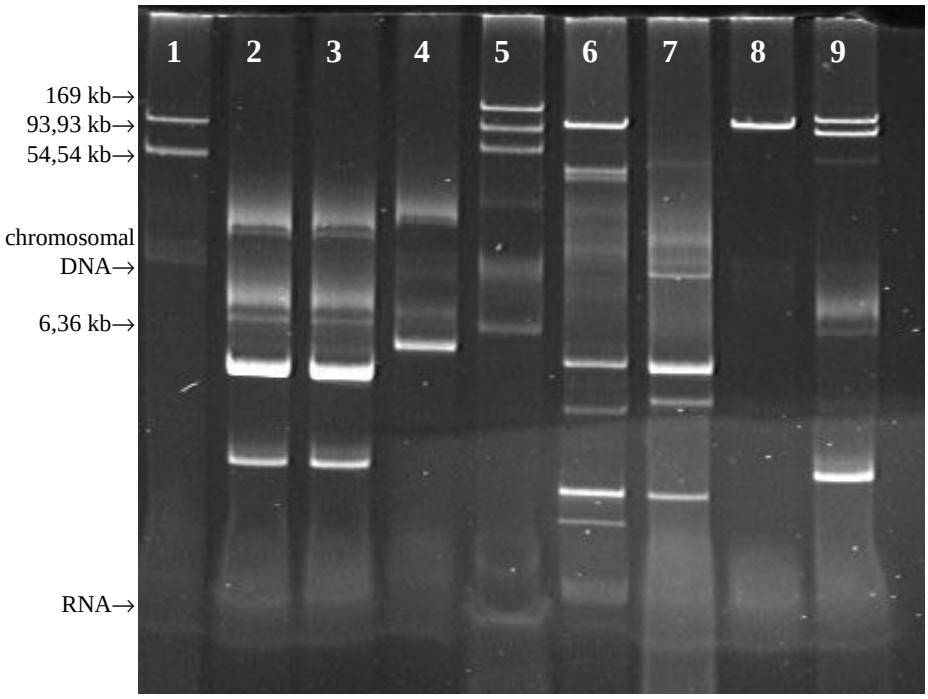


Figure 4.7. Plasmid profiles of German origin *S.* Typhimurium strains (continued).

Lane	Strain	Serotype	Plasmid(s) in kb
1	BFR33	*S.* Typhimurium	118,81; 53,24
2	BFR34	*S.* Typhimurium	3,92; 1,26
3	BFR35	*S.* Typhimurium	3,92; 1.26
4	BFR36	*S.* Typhimurium	5,12
5	Marker	-	169; 93,93; 54,54; 6,36
6	BFR37	*S.* Typhimurium	99,08; 42,84; 38,90; 4,12; 2,36; 0,88; 0,61
7	BFR38	*S.* Typhimurium	11,92; 3,83; 2,60; 0,84
8	BFR39	*S.* Typhimurium	99,08
9	BFR40	*S.* Typhimurium	112,03; 80,99; 6,06; 1,07

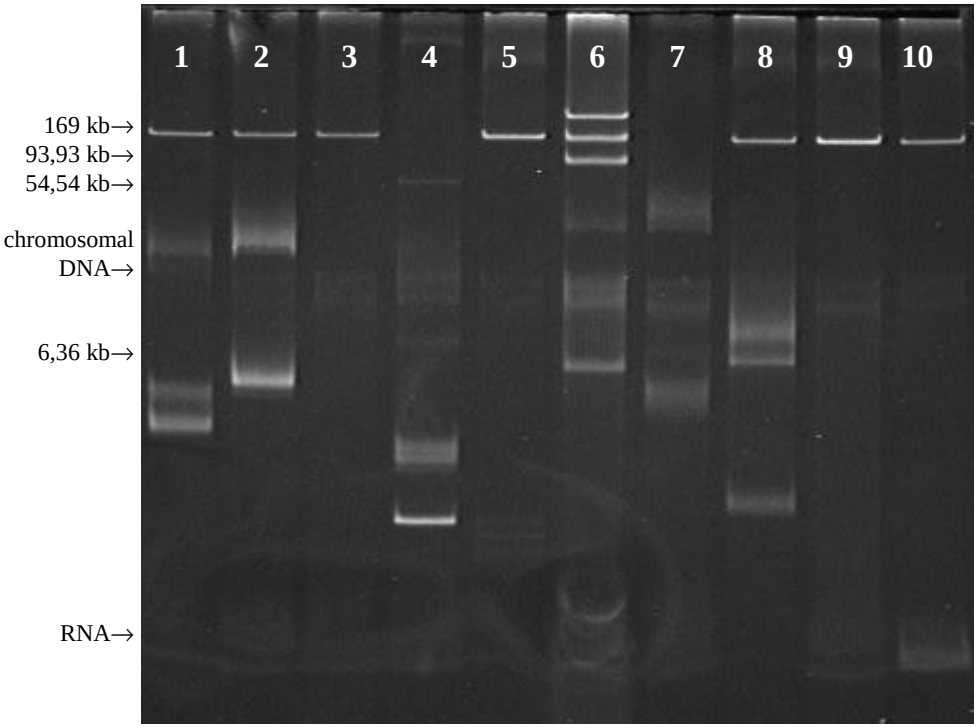


Figure 4.7. Plasmid profiles of German origin *S.* Typhimurium strains (continued).

Lane	Strain	Serotype	Plasmid(s) in kb
1	BFR41	*S.* Typhimurium	105,64; 3,38
2	BFR42	*S.* Typhimurium	99,61; 5,37
3	BFR43	*S.* Typhimurium	99,61
4	BFR44	*S.* Typhimurium	2,41; 1,26
5	BFR45	*S.* Typhimurium	93,93
6	Marker	-	169; 93,93; 54,54; 6,36
7	BFR46	*S.* Typhimurium	-
8	BFR47	*S.* Typhimurium	85,79; 6,63; 1,49
9	BFR48	*S.* Typhimurium	85,79
10	BFR49	*S.* Typhimurium	85,79

4.3.2. PFGE Profiles

108 strains were analysed for their PFGE profiles. The profiles obtained were compared and they were grouped according to the difference between the profiles where any band difference in the pattern was considered as one profile (Table 4.6). There were 42 groups obtained and each group had given a profile name. According to this groups, the serotypes having different profiles were *S.* Montevideo (2); *S.* Virchow (4); *S.* Infantis (4); *S.* Enteritidis (3); *S.* Kentucky (5l); *S.* Thompson (2); *S.* Typhimurium (11). The highest profile variabilities obtained among the serotypes were 55% for *S.* Kentucky and 42% for *S.* Typhimurium. Within these groups, the most common profiles among serotypes were V-X1 and V-X2, I-X3, K-X1, E-X1, Ty-X1, and Th-X1.

The strains DMC13, DMC52 and DMC63 were considered as rough strains of *S.* subsp. I, and exhibited 100% same PFGE profiles with *S.* Enteritidis serotype. Thus, these strains were confirmed by phage typing analyses and shown susceptibility pattern with *S.* Enteritidis typing phages revealing PT35 phage type. *S.* Group C1 serotyped strains DMC1, DMC27, DMC37, and DMC46 were exhibited one of the characteristic PFGE pattern (TH-X1) obtained from *S.* Thompson strains. DMC85b, which was serotyped as *S.* subsp. I Roughform also exhibited the same pattern as one of the *S.* Kentucky PFGE profile (K-X1). All these strains were serotyped once more to rule out the experimental error.

Table 4.6. PFGE profile groups of the strains.

Serotype	Profile Name	Strain
S. Virchow	V-X1	DMC9, DMC16, DMC18, DMC68
	V-X2	DMC19, DMC32, DMC42, DMC83
	V-X3	DMC5
	V-X4	BFR8, BFR9
S. Infantis	I-X1	DMC20, DMC23, DMC40, DMC57, DMC58, DMC70
	I-X2	DMC7, DMC12, DMC75
	I-X3	BFR10, BFR11, BFR12, BFR14, BFR15, BFR16, BFR17, BFR18, BFR19, BFR20, BFR21, BFR22, BFR24
	I-X4	BFR13, BFR23
S. Kentucky	K-X1	DMC52a, DMC56, DMC65, DMC72, DMC85a
	K-X2	DMC79
	K-X3	DMC34
	K-X4	BFR3
	K-X5	BFR4
S. Enteritidis	E-X1	DMC3, DMC22, DMC24, DMC25, DMC31, DMC43, DMC 53
	E-X2	DMC8, DMC14, BFR2
	E-X3	DMC 28
S. Thompson	Th-X1	DMC39, DMC44
	Th-X2	BFR7
S. Typhimurium	Ty-X1	BFR25, BFR27, BFR31, BFR32, BFR40, BFR41, BFR42, BFR43, BFR45, BFR47, BFR48, BFR49
	Ty-X2	BFR26
	Ty-X3	BFR46
	Ty-X4	BFR34, BFR35, BFR36, BFR38, BFR39
	Ty-X5	BFR44
	Ty-X6	BFR28
	Ty-X7	BFR29
	Ty-X8	BFR33
	Ty-X9	BFR30
	Ty-X10	BFR37
	Ty-X11	DMC4
S. Group C1	Th-X1	DMC1, DMC27, DMC37, DMC46
	GC-X1	DMC49

Table 4.6. PFGE profile groups of the strains (continued).

Serotype	Profile Name	Strain
S. subsp. I Roughform	E-X1	DMC13
	E-X2	DMC52B, BFR5
	E-X3	DMC63
	K-X1	DMC85b
	R-X1	BFR6
S. Agona	Ag-X1	DMC59
	Ag-X2	BfR1
S. Anatum	An-X1	DMC90, DMC91
S. Bispebjerg	B-X1	DMC78
S. Corvallis	C-X1	DMC36, DMC86
S. Montevideo	M-X1	DMC81
	M-X2	DMC88
S. Nchanga	N-X1	DMC15
S. Salford	Sa-X1	DMC93
S. Senftenberg	Se-X1	DMC55
S. Telaviv	Te-X1	DMC66

The dendogram showing the cluster analysis of the PFGE profile groups is given in Figure 4.8. Cluster analysis of the PFGE profiles showed the genetic variability within 42 different groups. The similarity level ranged from 44,3-100%. The major clusters were obtained by considering an arbitrary homology of about 60% and below, but the clusters were dispersed and minor cluster were obtained above this homology. The similarities among serotypes were 80,1% for *S.* Infantis, 91,8% for Turkish *S.* Virchow strains, which represented 67,7% similarity with German *S.* Virchow strain, and 68,5% for *S.* Typhimurium strains. Any of the subtypes were found to be in 100% similarity.

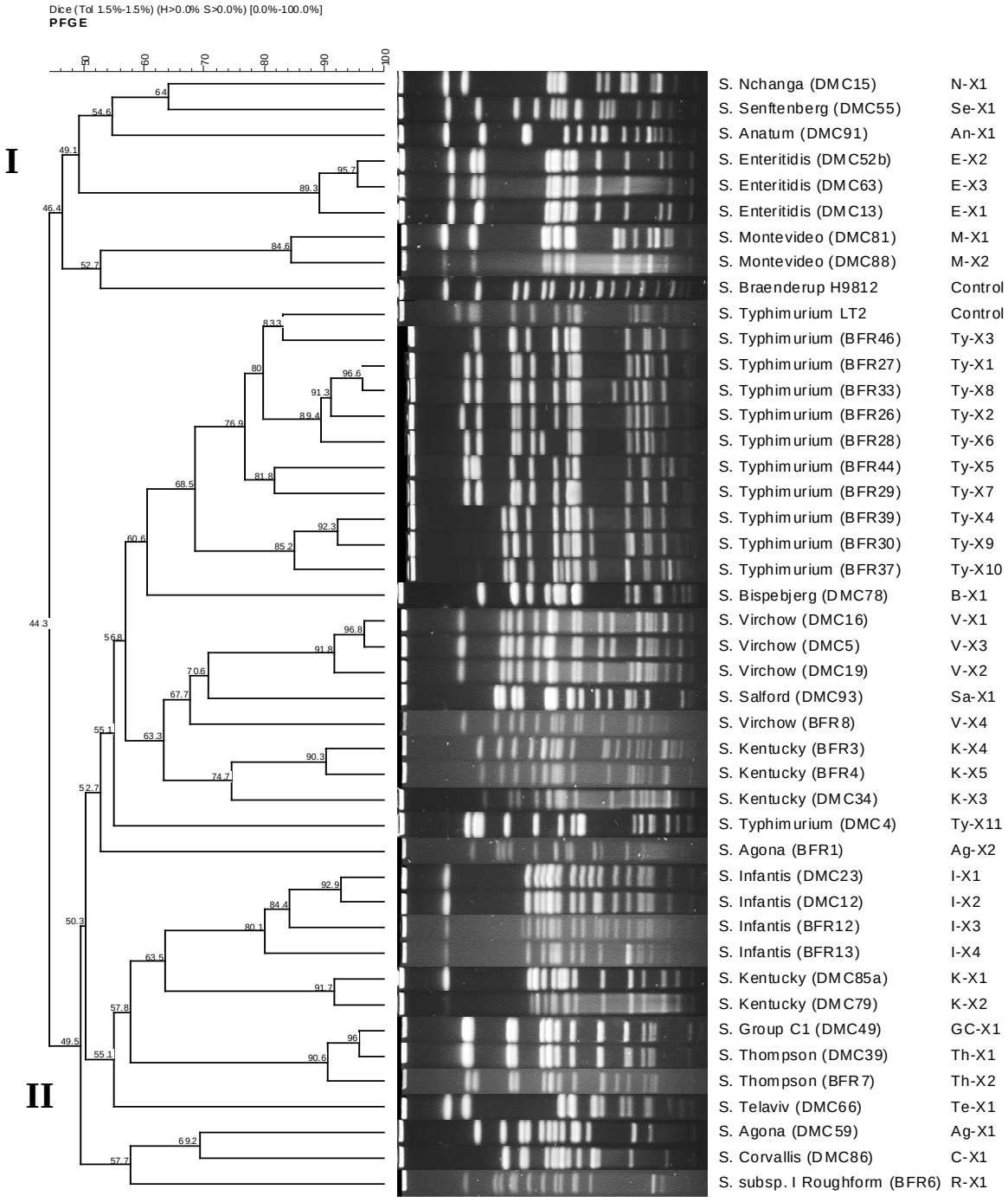


Figure 4.8. Dendogram showing the similarities between the strains having different PFGE patterns.

4.4. Genetic Determinants of Antimicrobial Resistance

The antimicrobial resistance genes observed from *Salmonella* strains are given in Table 4.7. The strains having intermediate resistance to GEN and STR revealed no antibiotic resistance genes tested. Besides, in the strain BFR1, the TMP-SXT resistance phenotype did not correspond to any of the analysed genes.

Among 72 resistant strains, the most prevalent resistance genotypes were observed as bla_{tem-1} (56%, AMP resistance); *floR* (100%, CHL and FFC resistance); *aphA1* (100%, KAN and NEO resistance); *tet*(A) (53%, TET resistance); *aadA1* (82%, SPE and STR resistance); *sulI* (78%, SUL resistance). Besides, one strain (DMC19) had bla_{CTX-M3} type and one strain (BFR3) had bla_{CMY-2} type ESBL (Extended Spectrum Beta-Lactamase) gene and 3 strains (DMC9, DMC16 and DMC68) had *qnrS1* (plasmid encoded quinolone resistance) gene. All the NAL resistant strains had mutation in QRDR (Quinolone Resistance Determining Region). The most prevalent mutation was Ser83 amino acid substitution in *gyrA* region (55% among NAL resistant strains). Besides this mutation type, Asp87 amino acid substitution was also observed. Only BFR7 had a double mutation in *gyrA* region with both Ser83 and Asp87 amino acid substitutions. All the mutants of *parC* region (51%) had Thr57 amino acid substitution. There were 22 strains obtained carrying mutations in both *gyrA* and *parC* regions.

Table 4.7. Antimicrobial resistance genes obtained from the antimicrobial resistant strains.

Strain	Gene(s) and/or Mutation(s)*
DMC5	*gyrA* (Asp87→Tyr87)
DMC7	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC9	bla_{tem-1}; *gyrA* (Asp87→Tyr87); *qnrS1*
DMC12	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC15	bla_{tem-1}; *sul2*; *dfrA1-like*
DMC16	bla_{tem-1}; *gyrA* (Asp87→Tyr87); *qnrS1*
DMC18	*gyrA* (Asp87→Tyr87)
DMC19	bla_{tem-1}; bla_{CTX-M9}; *strA*; *strB; sul2*; *dfr5-14*; *gyrA* (Asp87→Tyr87)
DMC20	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC23	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC24	*gyrA* (Asp87→Asn87)
DMC32	*gyrA* (Asp87→Tyr87)
DMC34	*aadA1-like*; *sul1*
DMC40	*aadA1-like*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC42	*gyrA* (Asp87→Tyr87)
DMC57	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC58	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC68	bla_{tem-1}; *gyrA* (Asp87→Tyr87); *qnrS1*
DMC70	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC75	*aadA1-like*; *aphA1*; *sul1*; *tet*(A); *dfr5-14*; *gyrA* (Ser83→Tyr83); *parC* (Thr57→Ser57)
DMC83	*gyrA* (Asp87→Tyr87)
BFR1	*sul1*; *tet*(A); *dfr25*
BFR2	bla_{tem-1}; *aadA1-like*; *strA*; *strB*; *sul1*; *sul2*; *tet*(A); *dfrA1-like*; *gyrA* (Asp87→Tyr87)
BFR3	bla_{CMY2}; *strA*; *strB*
BFR4	bla_{tem-1}; *aadA1-like*; *strA*; *strB*; *sul1*; *tet*(A); *tet*(B); *dfrA1-like*
BFR5	*gyrA* (Asp87→Phe87)
BFR6	bla_{tem-1}; *aadA1-like*; *aadA2*; *aadB*; *sul1*; *tet*(B); *gyrA* (Asp87→Phe87)
BFR7	*aphA1*; *tet*(A); *gyrA* (Ser83→Phe83; Asp87→Tyr87); *parC* (Thr57→Ser57)
BFR8	*gyrA* (Ser83→Phe83)
BFR9	*gyrA* (Ser83→Phe83)
BFR10	*aadA1-like*; *sul1*; *tet*(A); *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→Ser57)
BFR11	*aadA1-like*; *sul1*; *tet*(A); *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→Ser57)
BFR12	*aadA1-like*; *sul1*; *tet*(A); *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→Ser57)
BFR13	bla_{tem-1}; *sul2*; *dfrA1-like*

Table 4.7. Antimicrobial resistance genes obtained from the antimicrobial resistant strains (continued).

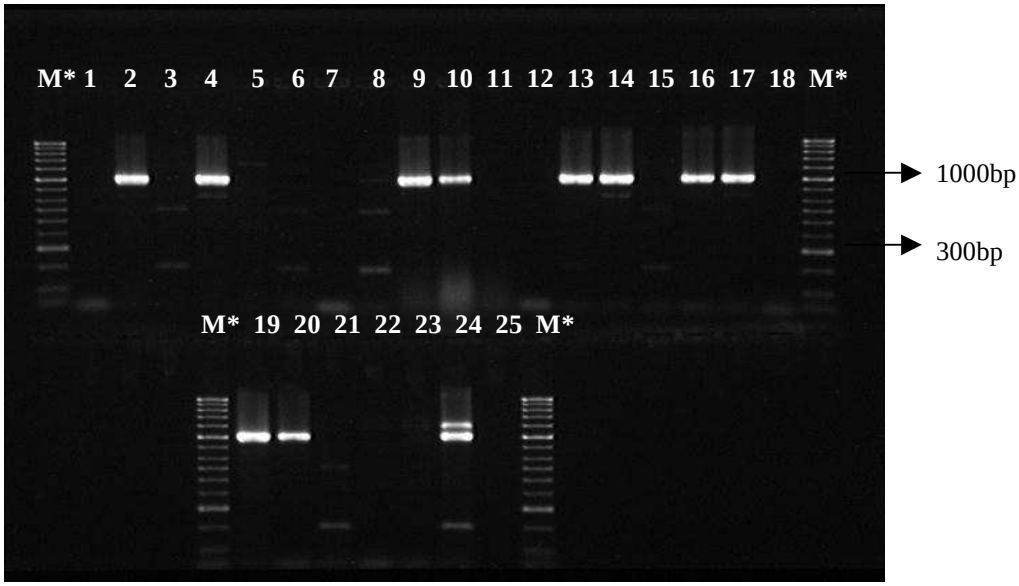
Strain	Gene(s) and/or Mutation(s)*
BFR14	*aadA1-like*; *sul1*; *tet*(A); *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR15	*aadA1-like*; *sul1*; *tet*(A); *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR16	bla_{tem-1}; *sul2*; *dfrA1-like*
BFR17	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR18	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR19	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR20	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR21	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR22	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR23	bla_{tem-1}; *sul2*; *dfrA1-like*
BFR24	*aadA1-like*; *sul1*; *tet*(A) ; *gyrA* (Ser83→ Tyr 83); *parC* (Thr57→ Ser57)
BFR25	bla_{pse-1}; *sul1*
BFR26	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *aphA1*; *sul1*; *tet*(G); *gyrA* (Asp87→ Tyr87)
BFR27	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)
BFR28	bla_{tem-1}; bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G); *gyrA* (Asp87→ Asn87)
BFR29	bla_{pse-1}; *sul1*
BFR30	bla_{tem-1}; *aadA1-like*; *strA*; *strB*; *sul2*; *tet*(B)
BFR31	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G); *gyrA* (Asp87→ Tyr87)
BFR32	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G); *gyrA* (Asp87→ Asn87)
BFR33	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)
BFR34	bla_{tem-1}; *strA*; *strB*; *sul2*; *tet*(B)
BFR35	bla_{tem-1}; *strA*; *strB*; *sul2*; *tet*(B)
BFR36	bla_{tem-1}; *strA*; *strB*; *sul2*; *tet*(B)
BFR37	bla_{tem-1}; *sul2*; *dfrA1-like*
BFR38	bla_{tem-1}; *aadA2*; *strA*; *strB*; *sul2*; *tet*(B)
BFR39	bla_{tem-1}; *aadA1-like*; *strA*; *strB*; *sul1*; *sul2*; *tet*(B)
BFR40	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)
BFR41	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *aphA1*; *sul1*; *tet*(G)
BFR42	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)
BFR43	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)
BFR44	bla_{tem-1}; *strA*; *strB*; *tet*(B)
BFR45	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G); *gyrA* (Asp87→ Asn87)
BFR46	bla_{tem-1}; *floR; strA*; *strB*; *sul2*; *tetB*
BFR47	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G); *gyrA* (Asp87→ Asn87)
BFR48	bla_{pse-1}; *sul1*
BFR49	bla_{pse-1}; *floR; aadA1-like*; *aadA2*; *sul1*; *tet*(G)

*****Mutations are indicated in parenthesis as amino acid substitutions.

The integron variable region analyses exhibited 700 bp (1 strain), 1000 bp (37 strain), 1200 bp (16 strain) and 1600 bp (3 strain) integrons (Figure 4.9 and 4.10). DNA sequence analyses of these integrons for their gene cassette(s) indicated that they were variable.

The Turkish origin strains which had 1000 bp variable region integron were all *S.* Infantis (9 strains) and one *S.* Kentucky (DMC33) serotype. In order to deduce the difference between these integrons, PCR-RFLP analysis was carried out. The results indicated that the variable region of these integrons were similar and produced the same fingerprint with four different sized fragments. Thus, only DMC33 and DMC58 were sequenced for their integron variable regions. Results exhibited that both serotypes had the same gene (*aadA1*) determining STR-SPE resistance.

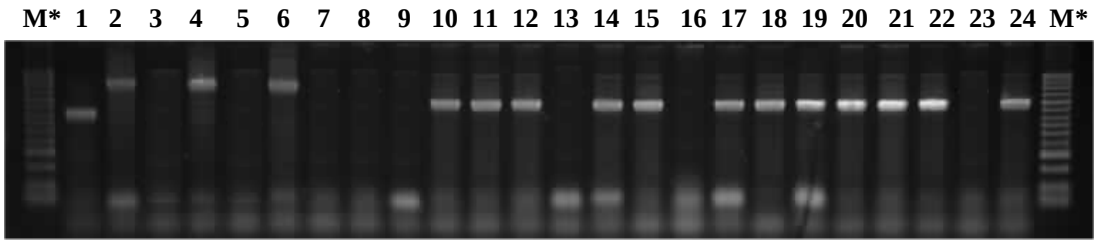
The strain BFR1 had the 700 bp integron and the gene cassette observed was *dfr25*. This result explained the TMP-SXT resistance of the strain and determined the genotype that was not confirmed by the genes screened. The other 1000 bp integron cassettes were belonging German origin strains BFR14 and BFR39 having *aadA1* gene cassette. BFR25 was one of the strain having 1200 bp integron cassette which showed bla_{PSE-1} gene cassette confering AMP resistance. The last integron variable region was the 1600 bp in BFR6 having two gene cassettes, namely *aadA1* and *aadB* (confering GEN resistance). The *aadB* gene explains the intermediate resistance in this strain.



*****M: Marker (50-2000 bp; Hyperladder II, Bioline, UK).

Figure 4.9. Results of the integron analysis of antimicrobial resistant strains from Turkish origin.

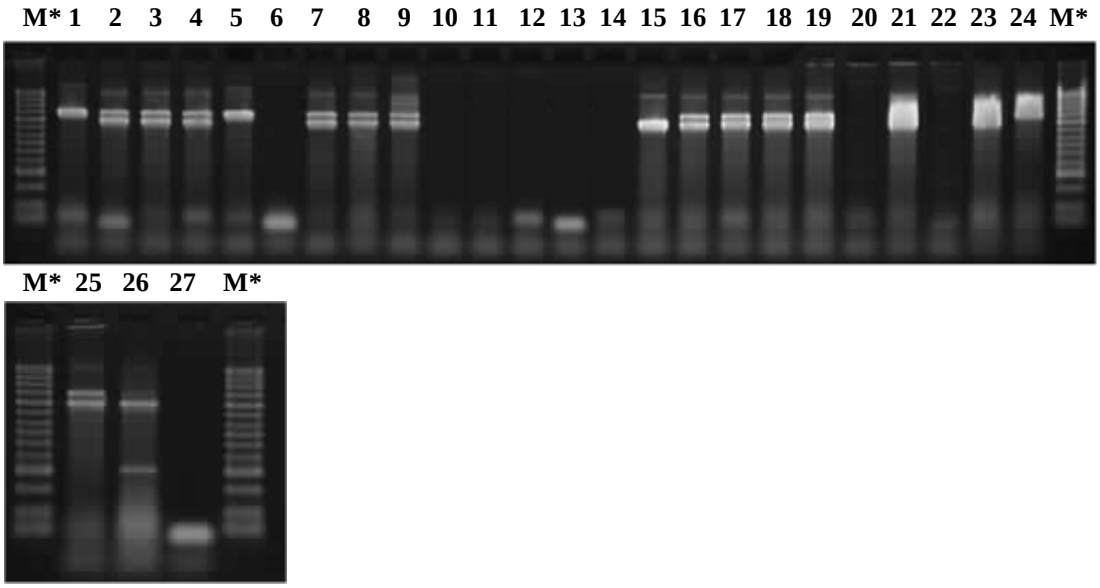
Lane	Strain	PCR product (bp)	Lane	Strain	PCR product (bp)
1:	DMC5	-	**14:**	DMC40	1000
2:	DMC7	1000	**15:**	DMC42	-
3:	DMC9	-	**16:**	DMC57	1000
4:	DMC12	1000	**17:**	DMC58	1000
5:	DMC15	-	**18:**	DMC68	-
6:	DMC16	-	**19:**	DMC70	1000
7:	DMC18	-	**20:**	DMC75	1000
8:	DMC19	-	**21:**	DMC83	-
9:	DMC20	1000	**22:**	DMC90	-
10:	DMC23	1000	**23:**	DMC91	-
11:	DMC24	-	**24:**	(+)control	1200; 1000
12:	DMC31	-	**25:**	(-)control	-
13:	DMC34	1000			



*****M: Marker (50-2000 bp; Hyperladder II, Bioline, UK).

Figure 4.10. Results of the integron analysis of antimicrobial resistant strains from German origin.

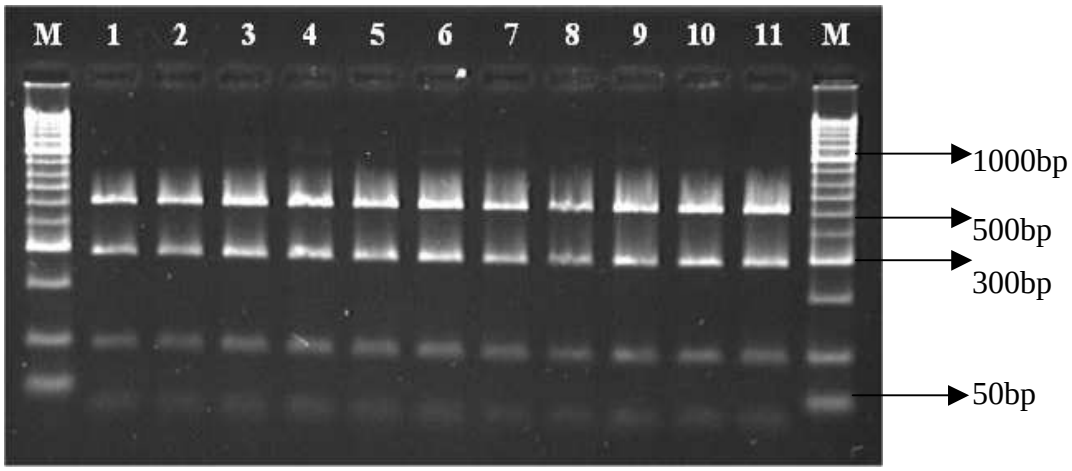
Lane	Strain	PCR product (bp)	Lane	Strain	PCR product (bp)
1:	BFR1	700	**13:**	BFR13	-
2:	BFR2	1600	**14:**	BFR14	1000
3:	BFR3	-	**15:**	BFR15	1000
4:	BFR4	1600	**16:**	BFR16	-
5:	BFR5	-	**17:**	BFR17	1000
6:	BFR6	1600	**18:**	BFR18	1000
7:	BFR7	-	**19:**	BFR19	1000
8:	BFR8	-	**20:**	BFR20	1000
9:	BFR9	-	**21:**	BFR21	1000
10:	BFR10	1000	**22:**	BFR22	1000
11:	BFR11	1000	**23:**	BFR23	-
12:	BFR12	1000	**24:**	BFR24	1000



*****M: Marker (50-2000 bp; Hyperladder II, Bioline, UK).

Figure 4.10. Results of the integron analysis of antimicrobial resistant strains from German origin (continued).

Lane	Strain	PCR product (bp)	Lane	Strain	PCR product (bp)
1:	BFR25	1200	**15:**	BFR39	1000
2:	BFR26	1200; 1000	**16:**	BFR40	1200; 1000
3:	BFR27	1200; 1000	**17:**	BFR41	1200; 1000
4:	BFR28	1200; 1000	**18:**	BFR42	1200; 1000
5:	BFR29	1200	**19:**	BFR43	1200; 1000
6:	BFR30	-	**20:**	BFR44	-
7:	BFR31	1200; 1000	**21:**	BFR45	1200; 1000
8:	BFR32	1200; 1000	**22:**	BFR46	-
9:	BFR33	1200; 1000	**23:**	BFR47	1200; 1000
10:	BFR34	-	**24:**	BFR48	1200
11:	BFR35	-	**25:**	BFR49	1200; 1000
12:	BFR36	-	**26:**	(+)control	1000
13:	BFR37	-	**27:**	(-)control	-
14:	BFR38	-			



M: Marker (50-2000 bp; Hyperladder II, Bioline, UK).

Figure 4.11. PCR-RFLP analysis of Turkish origin integron containing strains.

Lane	Strain	PCR-RFLP product (bp)
1:	DMC7	>500; 300; 100; <50
2:	DMC12	>500; 300; 100; <50
3:	DMC20	>500; 300; 100; <50
4:	DMC23	>500; 300; 100; <50
5:	DMC34	>500; 300; 100; <50
6:	DMC40	>500; 300; 100; <50
7:	DMC57	>500; 300; 100; <50
8:	DMC58	>500; 300; 100; <50
9:	DMC70	>500; 300; 100; <50
10:	DMC75	>500; 300; 100; <50
11:	(+)control	>500; 300; 100; <50

qnrS1 (DMC9) and *bla*$_{CTX-M3}$ (DMC19) genes were selected to be further characterized for their location since they were important antimicrobial resistance gene cassettes. To find out the location of the genes, Southern-blot and DNA-hybridization experiments were conducted (Figure 4.12 and 4.15). It was observed that both genes were plasmid encoded in the strains they belonged to. The plasmids were ~45 kb for both the *qnrS1* carrying one (pRQ2006) and the *bla*$_{CTX-M3}$ carrying one (pBD2006). To reveal the lateral gene transfer of these genes, conjugation analyses were carried out in two different incubation temperatures (room temperature and 37°C, respectively). The pRQ2006 was found to be conjugative in both temperatures, whereas any conjugants were not obtained for pBD2006.

The plasmid incompatibility groups were also studied for these two plasmids. pBD2006 was found to have IncN type replication origin, whereas pRQ2006 did not have any convenience with any of the 18 replicon studied.

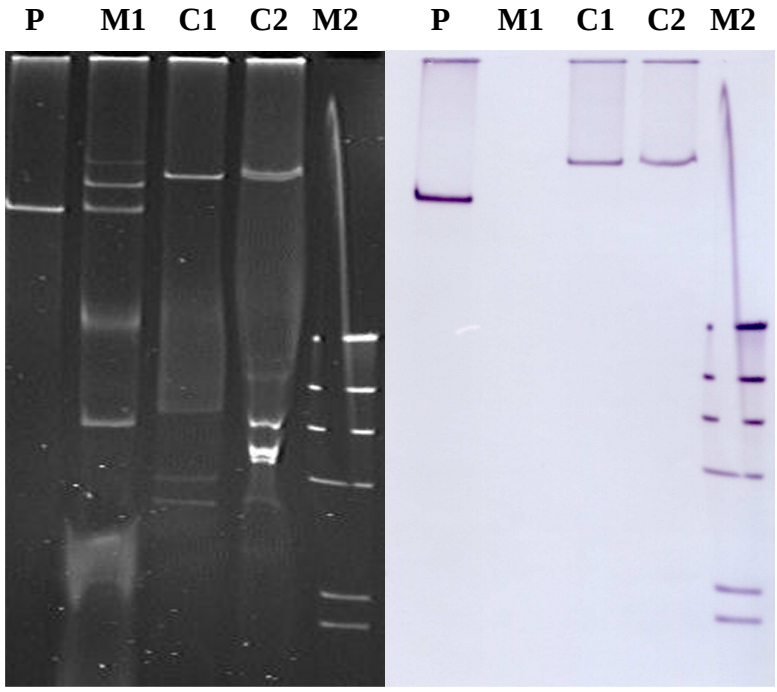


Figure 4.12. *bla*$_{CTX-M3}$ hybridization of the plasmid pBD2006. P: plasmid pBD2006; M1: marker (169 kb; 93,93 kb; 54,54 kb; 6,36 kb); C1: positive control 1; C2: positive control 2; M2: marker (Roche GmbH., Germany).

Thus, the pRQ2006 plasmid was further studied by its restriction pattern, cloning and DNA sequencing analysis. $bla_{TEM\text{-}1}$ gene was also found to be encoded by this plasmid (Figure 4.15). Restriction analysis of pRQ2006 was performed using several endonucleases, which is given in Figure 4.13. The restriction enzymes *Bam*HI, *Eco*RI, *Sal*I, *Xba*I, *Sma*I, *Bsp*120-I, *Xho*I and *Sac*I yielded fragments bigger than 14 kb. On the other hand, *Pst*I and *Pvu*I enzymes did not give the fragments proper for cloning experiments. For these reasons, *Hin*dIII, *Hin*cII ve *Eco*RV enzymes were selected for cloning experiments. The transformation efficiency was very low, since only 6 transformants were obtained (Figure 4.14). From those transformants, 5 were the result of *Eco*RV and 1 was *Hin*dIII restriction cutting. There was not any colony obtained from *Hin*cII restriciton cutting.

The presence of a Tn*3*-like transposon (accession no. AB187515.1), which contained the $bla_{TEM\text{-}1}$ gene was confirmed by partial sequencing of a *Hin*dIII (8 kb fragment) and *Eco*RV (2,7 kb fragment) restriction fragments that had been inserted into the vector pIV2 and introduced into the *E. coli* strain NEB5 (Fig 4.14).

With the transformants, a region of about 3,430 bp of pRQ2006 was sequenced and found to carry the $bla_{TEM\text{-}1}$ gene, the *tnpR* gene (encoding the resolvase) and part of the *tnpA* gene (encoding the transposase) of the Tn*3*-like transposon. This region included a TAAAA direct repeat at the boundaries of the Tn*3* element. Then, a pRQ2600 region of about 1,677 bp containing *qnrS1* was sequenced and compared with the database. No homology was observed with the known *qnrS1* carrying plasmids.

RFLP-hybridisation with *qnrS1* and $bla_{TEM\text{-}1}$-probes had been carried out (Figure 4.15). *qnrS1* is located on a 2,6 kb *Hin*dIII-fragment of pRQ2006 and the $bla_{TEM\text{-}1}$ gene is located on an 8.4 kb fragment.

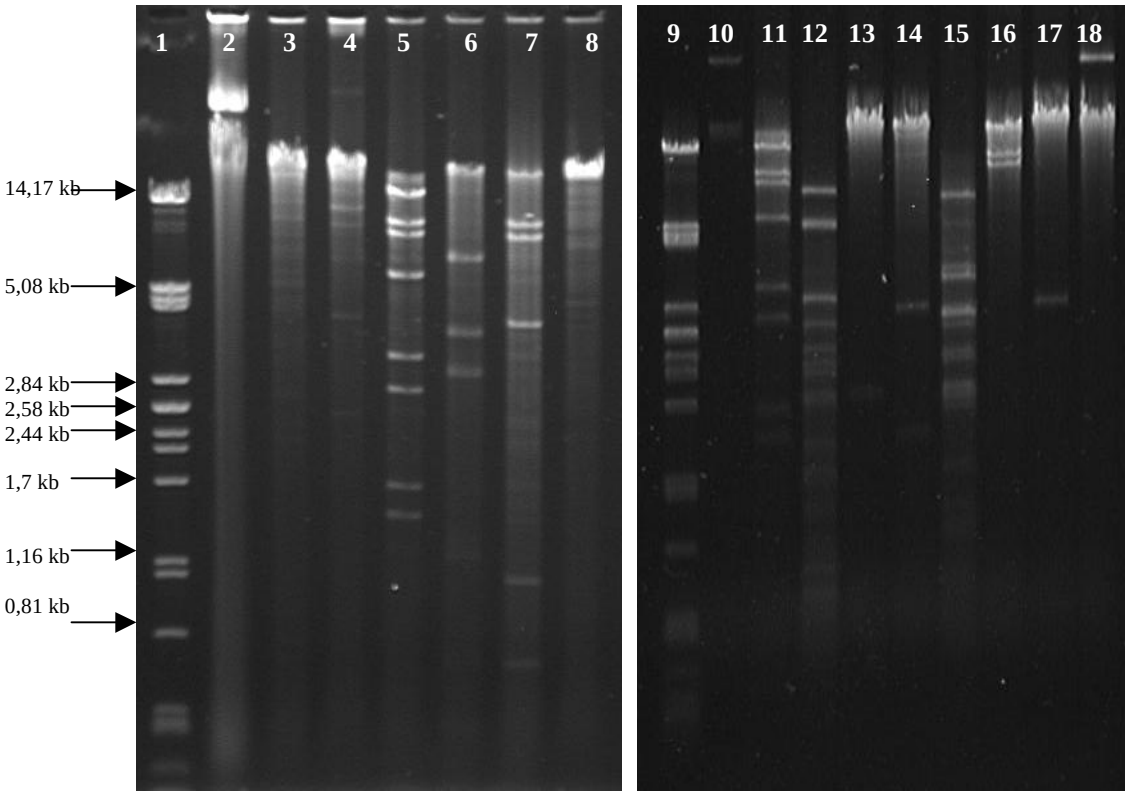


Figure 4.13. Restriction digestion pRQ2006; 1: Marker (λ DNA-*Pst*I), 2: DMC9 plazmid DNA, 3: *Bam*HI, 4: *Eco*RI, 5: *Hin*dIII, 6: *Pst*I, 7: *Pvu*I, 8: *Sal*I, 9: Marker, 10: DMC9 plasmid DNA, 11: *Hin*dIII, 12: *Hin*cII, 13: *Xba*I, 14: *Sma*I, 15: *Eco*RV, 16: *Sac*I, 17: *Bsp*120-I, 18: *Xho*I.

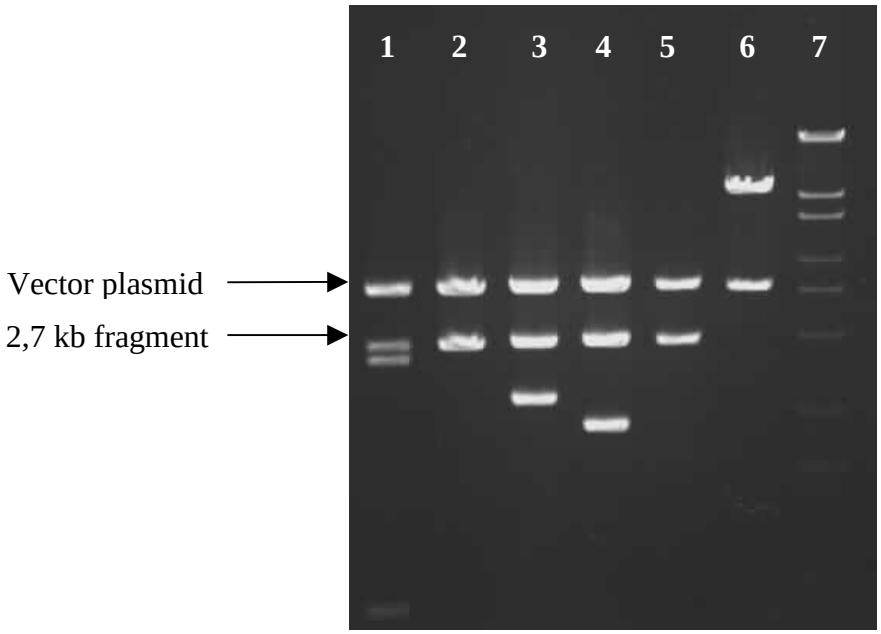


Figure 4.14. Restriction digestion of the vector plasmid from the transformant strains having 1-5: *Eco*RV restriction digest, 6: *Hin*dIII restriction digest, 7: Marker (Lambda DNA *Eco*RI-digest).

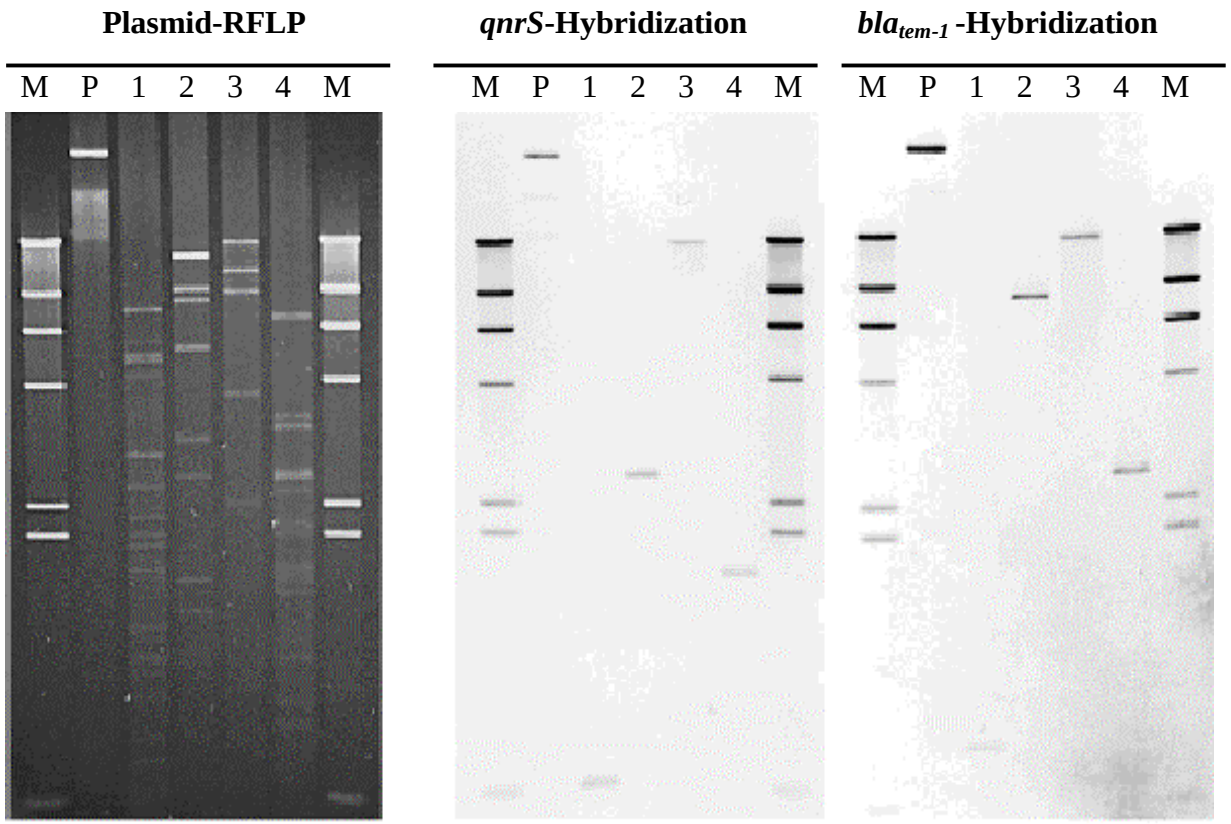


Figure 4.15. Restriction and hybridization analysis of the plasmid pRQ2006. 1: *Hin*cII; 2: *Hin*dIII; 3: *Eco*RI; 4: *Eco*RV; M: Molecular weight marker used phage lambda DNA digested with *Hin*dIII; and P: undigested pRQ2006.

CHAPTER 5

DISCUSSION

In this chapter, the results of the thesis are discussed in relation to the current literature. The isolation rate of *Salmonella* from Turkish food markets was found to be high, especially in poultry. Although biochemical reaction composition among isolated strains was not highly variable, a few atypical strains were also obtained. The serotype distribution of *Salmonella* in Türkiye was consistent with the literature by *S.* Enteritidis being the most common serotype. However, *S.* Typhimurium is a prevalent serotype worldwide, this observation was not reflected in our results. The epidemiology of the German strains was not discussed, since they were not randomly selected. Except *S.* Infantis strains no plasmid profile was predominated. On the other hand, there were unique plasmids, which were seen in all isolates of the serotypes they belonged. PFGE patterns revealed 42 different subgroups. The cluster analysis of subtypes exhibited two main clusters with 44,3% homology, where the majority of the strains belonged to the second cluster. Mostly, the subtypes of the same serotypes clustered together, but some exceptions were observed. The antimicrobial resistance exhibited by Turkish strains was remarkable. The high NAL resistance and reduced susceptibility to CIP is very worrisome, since quinolones are the antibiotic of choice for the treatment of such infectious diseases. The high SUL resistance of German strains showed that the isolates of both country origins differ in their antibiotic regimes. The genotypic analyses of antimicrobial resistance phenotype exhibited similar results with those explained in the literature. There were two important observations that are currently emerging worldwide, the presence of *qnrS1* and $bla_{CTX\text{-}M3}$. Class I integrons were obtained from both country origin strains and found to be consistent to those that reported in the literature.

The isolation data exhibited that *Salmonella* contaminations were high in poultry products followed by meat products in Türkiye. There were also considerable amount of isolation from raw milk, whereas mayonnaise-based salads did not exhibit an important result. This findings are due to the fact that of all the food samples other than salads were in raw form and did not have any sanitation process. However, ready-to-eat salads contained food additives that suppress the microbial flora. The contamination rates were quite high considering that the microbial load of the foods must be zero especially in terms of *Entereobacteriaceae*. The results found indicates the poor sanitation conditions in the Turkish food market.

The isolation rate (Anon. BS EN ISO 6579, 2002) was quite high since out of 108 isolates 100 were confirmed as *Salmonella* spp. Serotypes in *Salmonella enterica* subsp. I, do not differ in biochemical reactions. They exhibit variability in arginine dihydrolysis and inositol-oxidation. In our results this variability is also observed (Table 4.2). On the other hand, there were a few strains showing difference in other biochemical reactions, namely lysine decarboxylation, ornithine decarboxylation, mellibiose-fermentation and gelatinase activity. These isolates are considered as atypic strains of *Salmonella enterica* subsp. I.

Although there are not much studies, serotype prevalence of *Salmonella* isolates in Türkiye was reported by a few authors (Erol, 1999; Erdem *et al.*, 2005; Goncagül *et al.*, 2005; Yazcıoğlu *et al.*, 2005). Our results indicated that the most prevalent serotype was *S.* Enteritidis followed by *S.* Virchow, *S.* Infantis and *S.* Kentucky among foods tested. These results are consistent with the literature where *S.* Enteritidis was reported as the most prevalent strain in chicken meat (Goncagül *et al.*, 2005). In addition, it was also found in higher rates (47,7%) from human isolates (Erdem *et al.*, 2005). The same study was also reported the prevalence of *S.* Typhimurium isolates as 34,7%. *S.* Enteritidis and *S.* Typhimurium share the first two places in prevalence of *Salmonella* serotypes in Europe (http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm). Since the rate of isolation of *S.* Typhimurium serotype was very low a partial consideration was given for this strain. This low rate isolation may be due to the cross contaminations of other serotypes in the food chain. Serdaroğlu *et al.* (1996) reported the evaluation of

salmonellosis cases between 1989-1993 and obtained *S.* Typhimurium as the causative agent in 104 out of 110 isolates. The findings, that *S.* Virchow, *S.* Kentucky and *S.* Infantis were also in high prevalence, were in parallel with the Enter-Net results (http://www.hpa.org.uk/hpa/inter/enter-net/07q1summ.pdf). As in the results of this study, other serotypes were also reported to be isolated in Türkiye such as *S.* Anatum (Küplülü, 1999), *S.* Telaviv (Küplülü, 1999), and *S.* Group C1 (Erdem *et al.*, 2005).

Phage typing data on 16 *S.* Enteritidis strains in our studies have indicated the prevalence of PT21 phage type among Turkish isolates. But, the only *S.* Enteritidis strain in German isolates exhibited the PT4 phage type. PT4 phage type is common in Germany as reported in the literature (Schroeter *et al.*, 1994). Some other phage types were also detected from Turkish isolates (PT1, PT3 and PT6), however no observation of PT4 phage type indicates the lack of correlation between Turkish and German *S.* Enteritidis strains. There are reports indicating that the PT4 phage type is a common phage type in human isolates of *S.* Enteritidis from Türkiye (Anğ-Küçüker *et al.*, 2000), which was not observed by the strains used in our study for food isolates. Studies with human isolates also showed the other phage types such as PT1, PT6, PT7, PT8, PT18 and in all studies PT4 was the most prevalent phage type Anğ-Küçüker *et al.*, 2000). Our study is the first study that reveals the PT21 phage type prevalence in *S.* Enteritidis.

Because of there was only two *S.* Typhimurium isolates in Turkish origin strains, it was not logical to discuss the prevalence of the phage type for those isolates. These isolates did not exhibit any relation neither with the phage types, which were recorded in the database of *Salmonella* Reference Laboratories (Berlin, Germany) nor with the published ones. There are no available reports that study the prevalence of *S.* Typhimurium phage types in Türkiye and in some studies were reported as untypable (not react with any of the typing phages) strains (Anğ-Küçüker *et al.*, 2000). The phage types were not untypable in our study suggesting that the prevalent *S.* Typhimurium isolates in Türkiye exhibit different phage susceptibility patterns other than those in Europe. The German strains exhibited mainly DT104 phage type which showed a clonally spread in Germany and Europe (Schroeter *et al.*, 2004;

Helms *et al.*, 2005). There were also RDNC phage type strains among German origin ones, indicating the change in phage susceptibilities in strains and thus the limited differentiation capacity of phage typing method.

The use of Kado and Liu (1981) for the plasmid isolation experiments was due to the method's specificity to isolate the plasmids up to 350 MDa. The plasmids obtained in this study were in the range of <5-238 kb and the majority of the strains (58,3%) had plasmids >50 kb, so the method allowed the differentiation of related serotypes.

Some of the serovars of *Salmonella* were found not to possess any plasmids (Rychlik *et al.*, 2006). The results showed that some of the strains belonging to *S.* Montevideo, *S.* Thompson, *S.* subsp. I Roughform, *S.* Virchow, *S.* Group C1, *S.* Kentucky, *S.* Corvallis, *S.* Anatum, *S.* Telaviv and *S.* Typhimurium serotypes did not exhibit plasmid content. However, it cannot be suggested that there was a serotype specificity in having no plasmid at all. Since, most of the *S.* Virchow and *S.* Typhimurium strains possessed at least one plasmid, and there were strains of above mentioned serotypes exhibiting plasmid content.

The known plasmids found in *Salmonella* are 2-200 kb in size with a special emphasis on biological properties which they bring to host (Guerra *et al.*, 2000; Rychlik *et al.*, 2006). They control medically important properties including virulence factors, resistance to heavy metals, antibiotics and phages or utilization alternative carbon sources. Since plasmids code for genes dispensable for the functioning of the host cell, they represent genetic information under a lower selection pressure, which can be subjected to an accelerated evolution. Therefore acquisition of a plasmid allows its host to adapt to changing environments easily. Proof of this statement is the relevant appearance of R-plasmids (resistance plasmids) with *spv* or *rck* genes originating from the *Salmonella* serovar specific virulence plasmid allowing for the evolution of highly virulent and antibiotic resistant clones to *S. enterica* (Guerra *et al.*, 2002; Rychlik *et al.*, 2006). The virulence characteristics were not studied in the thesis and need to be investigated.

Plasmids are classified into incompatibility groups according to their mode of replication and maintenance in the bacterial cell. Plasmids, which confer different replication origin, are able to reside in the same bacterial cell. Moreover, plasmids, which confer the same replication origin, cannot be replicated in the same cell, thus termed as incompatible. The replication origins of two important plasmids were investigated. The plasmid carrying ESBL gene ($bla_{CTX\text{-}M3}$), pBD2006, was IncN incompatibility group. There are reports about incompatibility groups of ESBL encoding plasmids. Carattoli *et al.* (2006) demonstrated dominance of incompatibility IncA/C or IncN-related plasmids carrying some emerging resistance determinants to extended-spectrum cephalosporins and carbapenems. However, there was no observation with the plasmid carrying *qnrS1* gene, pRQ2006, with the incompatinilities analysed. The incompatibility of this plasmid should be futher investigated by cloning analysis with a set of incompatibility groups in addition to those tested.

PFGE is one of the most powerful DNA fingerprinting method that distinguishes closely related serotypes and phage types of *Salmonella* (Olive and Bean, 1999; Guerra *et al.*, 2000a; Yan *et al.*, 2003). The PFGE experiments of the strains revealed variability of the serotypes in different homology among subtypes. There were 42 subgroups out of 108 strains obtained. These subgroups exhibited two main clusters with 44,3% homology. 81% of the subgroups formed the cluster II. Mainly, the subgroups of the same serotypes were clustered together. However, it is notable that the strains belonging to different country origins were in far branches of the dendogram, especially for serotypes *S.* Enteritidis, *S.* Kentucky, and *S.* Typhimurium. The major clusters were formed below 60% homology and after that the dispersion into minor clusters was observed. These results indicated the high sensitivity of PFGE in the differentiation of *Salmonella* serotypes. Besides, when *S.* subsp.I Roughform strains were serotyped and gave reaction with all polyvalent O-group antigens, so called rough strains. The PFGE analyses allowed these strains to be assigned to a specific serotype. Similarly, the strains serotyped as *S.* Group C1 did not exhibited any reaction with polyvalent H-group antigens, although they were motile. Thus, they are named as O-group antigen that they have reacted. In our results, these strains were also assigned to a serotype by comparing the PFGE

patterns. These results support that PFGE fingerprinting is a superior technique in typing of *Salmonella* than conventional methods.

The similarity of the antimicrobial patterns and serotypes indicates again the poor sanitation conditions in food-chain in Türkiye, which resulted in clonal spread of the same strain as *S.* Infantis strain having the same antibiotic resistance pattern. However, this relation was not observed in German isolates, hence the antibiotic resistance patterns were more distinguished between these strains. In comparison of the antibiotic resistance patterns of the strains of different country origins, there was no similarity observed. This could be related to the different antibiotic regimes used in both countries.

The antibiotic resistance genes found for the isolates showed the most predominant genes reported in the literature (Schwarz and Chaslus-Dancla, 2001.). No new gene was detected conferring the antibiotic resistance phenotypes observed. PCR amplification was carried out for the presence of *sul1, sul2,* bla_{tem-1}*,* bla_{pse-1}*,* bla_{CMY2}*,* bla_{CTX-M3}*, aadA1-like, aadA2, strA, strB, aphAl, dfrA1-like, dfrA5-14, tet*(A), *tet*(B), *tet*(G), *floR, qnrS* genes. There was no serotype specific genes found except bla_{pse-1} and *floR* genes in *S.* Typhimurium. This finding is further discussed below in relation to the phage type and antimicrobial resistance pattern.

In our study, it was demonstrated that the occurrence of antibiotic resistance to NAL among Turkish isolates and antimicrobial resistance to SUL among German isolates are widespread. These results were not surprising because resistance to quinolones are emerging worldwide and SUL resistance was common since 1970s (Huovinen *et al.*, 1995). Resistance to two β-lactams, ampicillin and ceftiofur, in one strain (DMC19, *S.* Virchow) among Turkish isolates and three β-lactams ampicillin, amoxicillin/clavulonic acid and ceftiofur, in one strain (BFR3, *S.* Kentucky) of German isolates was observed. Although rare, this observation indicates the presence of ESBL producing strains in both countries, which are also emerging in worldwide in *Enterobacteriacea* (Sturenburg and Mack, 2003; Batchelor *et al.*, 2005).

DNA sequencing results exhibited that the amino acid changes observed in the QRDRs, *gyrA* and *parC*, were consistent with the previously described mutations (Malorny *et al.*, 2003). No other mutation was determined for the isolates. For *parC* mutants, the mutation was by the Thr57→Ser57 amino acid substitution. There was also a correlation between *S.* Infantis strains from both country origin isolates. The double Ser83 and Thr57 type mutation affecting both *gyrA* and *parC* was only seen in *S.* Infantis strains of all except BFR24 strain. However these strains differ in their susceptibilities and none was resistant to CIP (0,25-1 μg/mL). Our results showed no correlation between mutation number and the resistance to quinolones, especially reduced susceptibility to CIP. The strain BFR7 having three mutations two in *gyrA* (Ser83→Phe83; Asp87→Tyr87) and one in *parC* (Thr57→Ser57) also obey the fact by showing intermediate susceptibility to CIP with 0,5 μg/mL MIC value which confer only intermediate resistance. The *S.* Virchow strains from both country origins showed no mutation in *parC* region, and they differ in *gyrA* mutations. The strains from Türkiye exhibited Asp87→Tyr87 amino acid substitution, whereas Ser83→Phe83 was shown by German origin strains. All *S.* Typhimurium strains resistant to NAL had the mutation in only *gyrA* region at Asp87.

Consistent with previous reports, resistant phenotypes which the isolates are only associated with some strains of phage types, namely *S.* Typhimurium DT104. This indicates the dynamic nature of antimicrobial resistance spread. *S.*Typhimurium DT104 has spread worldwide. This *Salmonella* type has emerged within last few years as one of the most common causes of human salmomellosis in several countries. The majority of the DT104 isolates have multidrug resistance phenotype and abbreviated as ACSSuT; which means that they are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (Ahmed *et al.*, 2005). 6 strains showed typical multidrug resistant phenotype. These strains were found to have 1 and 1,2 kb integrons carrying gene cassettes bla_{pse-1} and *aadA2*, respectively. These two integrons are characteristic for the *S.* Typhimurium DT104.

The genetic makeup of many isolates of *S.* Typhimurium DT104 with ACSSuT resistance phenotype is similar, comprising *floR* and *tet*(G) genes bracketed by two class 1 integrons. These are carrying bla_{pse-1} and *aadA2* gene cassettes clustered on

14 kb region of *Salmonella* genomic island 1 (SGI1). These antibiotic resistance genes are an integral part of the chromosome (Threlfall, 1994; Briggs and Fratamico, 1999; Boyd *et al.*, 2001; Boyd *et al.*, 2002; Randall *et al.*, 2004). 6 German origin *S.* Typhimurium DT104 strains studied had the typical genetic determinants, which indicated the presence of SGI1 in these strains. Previous workers have shown that serotypes beyond *S.* Typhimurium can have integrons ranging in size from 0,65-2,7 kb and these integrons were associated with the presence of various resistance genes (White *et al.*, 2001; Orman *et al.*, 2002; Lindstedt *et al.*, 2003; Randall *et al.*, 2004).

In this study, PCR screening results of 72 antibiotic resistant strains showed class I integrons in 44 isolates (61% of the antibiotic resistant strains) of different *Salmonella* serovars, which are *S.* Infantis (21 strains), *S.* Typhimurium (17), *S.* Agona (1), *S.* Enteritidis (1), *S.* Kentucky (1), and *S.* subsp I Roughform (1). These serotypes showed 5 profiles of class I integrons. Thus, the data indicated that the gene cassettes *aadA1*, *aadB* and bla_{pse-1} are still predominant among *Salmonella* strains. The gene cassettes carried by the integrons mentioned are commonly found ones, except *dfr25* found in *S.* Agona (BFR1). This gene cassette has been published by Agerso *et al.* (2006) in *S.* Agona too and confers resistance to TMP and SXT.

The isolates carrying *qnrS1* gene were further analysed. Sequences of the PCR products of the gene were identical to *qnrS1* that confirms the result of PCR amplification (Genbank accession no. AB187515). Further, PCR amplifications which performed by *pre-qnrS* primers (Poirel *et al.*, 2006) showed the presence of the *qnrS1* gene (656 bp) found in different *Enterobacteriaceae* (Robicsek *et al.*, 2006).

Southern hybridization of plasmid DNA revealed that both the *qnrS1* and the $bla_{TEM-1-like}$ probes hybridized with a ~45 kb a plasmid, designated pRQ2006, were present in the three *qnrS1*-positive isolates. Conjugation experiments using an *E. coli* J53 rifampicin-resistant strain as recipient were performed. To avoid the selection of J53 *gyrA* mutants, selection was made on EMB-agar plates with ampicillin and rifampicin (200 and 100 mg/L, respectively). Six selected transconjugants showed low resistance to nalidixic acid (8-16 mg/L) and a decreased susceptibility to

ciprofloxacin (0.5 mg/L). They were positive for the *qnrS1* and bla_{TEM-1} genes and did not show any chromosomal mutation affecting the QRDR of the *gyrA* gene.

Restriction analysis of pRQ2006 was performed using several endonucleases. Restriction fragments generated with *Hin*cII, *Hin*dIII, *Eco*RV and *Eco*RI which were chosen for DNA- hybridisation with bla_{TEM-1} and *qnrS1* probes. Only in case of restriction with *Eco*RI, both probes hybridised to the same fragment (~20 kb).

The presence of a Tn*3*-like transposon (acc. no AB187515.1), which contained the bla_{TEM-1} gene was confirmed by partial sequencing of a *Hin*dIII (8 kb fragment) and *Eco*RV (2.7 kb fragment) restriction fragments that had been inserted into the vector pIV2 and introduced into the *E. coli* strain NEB5 (New England Biolabs, UK).

Only a few *qnrS1*-carrying plasmids have been described so far. They differ in their molecular sizes, resistance genes (i.e. for ß-lactamases) and bacterial host. The 47 kb plasmid pAH0376 (acc. no. AB187515.1) was the first *qnrS1*-carrier plasmid described. It was found in a human clinical isolate of *Shigella flexneri* in Japan and carries a Tn*3*-like transposon (Hata *et al.*, 2005). The 58 kb pINF5 plasmid (acc. no. AM234722.1) also carries the Tn*3*-like transposon, and was isolated from *Salmonella* Infantis recovered from poultry in Germany (Kehrenberg *et al.*, 2006). The pK245 plasmid (acc. no. JQ449578.1) is a 98 kb plasmid, which carries a bla_{SHV-2} (ampicillin-resistance) gene found in a clinical isolate of *Klebsiella pneumoniae* (Chen *et al.*, 2006). In *Enterobacter cloacae* isolated in France and Vietnam, several plasmids with sizes between 50 kb (p287) and 100 kb (ie. pS3-5) have been described and some of them carry a new bla_{LAP-1} gene (Poirel *et al.*, 2007). Recently, Hopkins *et al.* (2007) identified in the UK several *qnrS1*-plasmids, including the 44 kb IncN plasmid TPqnrS-2a/b, which also carries the bla_{TEM-1} gene, in *S.* Virchow PT8 isolated from Thai cooked chicken.

The plasmid pRQ2006 was compared to pINF5 from *S.* Infantis and pAH0376 from *S. flexneri* by primer walking and/or amplification using primers deduced from the published sequences of pINF5 (13,389 bp) and pAH0376 (11,002 bp), as well as by comparison of RFLP- hybridisation patterns.

First, a region of about 3,430 bp of pRQ2006 was sequenced. This region matched with nucleotides 915-3,093 from pINF5 and 2,114-5,545 from pAH0376 and contained the bla_{TEM-1} gene, the *tnpR* gene (encoding the resolvase) and part of the *tnpA* gene (encoding the transposase) of the Tn*3*-like transposon. The region located upstream of Tn*3* only matched with pAH0376 from *S. flexneri* (nucleotides 2,114 to 2,909). This region included a TAAAA direct repeat at the boundaries of the Tn*3* element, which differs from the TTATT repeat part of an IS*26* relic found in pINF5.

Secondly, a pRQ2600 region of about 1,677 bp containing *qnrS1* was sequenced. This sequence was similar to the sequences described for pINF5 and pAH0376 (nucleotides 6,871-8,547 from pINF5 and 9,661-10,132 from pAH0376). Based on the sequence of pINF5, amplification experiments targeting other genes/sequences located downstream of *qnrS1* were carried out. Using primers designed for the CS12 fimbrial gene cluster of *E. coli* present in pINF5 (nucleotides 9,661 to 10,143) a 100% homologous PCR product of 482 bp was amplified (Kehrenberg *et al.*, 2006). In contrast, no product targeting the *tnp* gene encoding the transposase of IS26 of pINF5 (expected size of 429 bp corresponding to nucleotides 11,591-12,020) was obtained, indicating the lack of the IS*26* in pRQ2006.

Kehrenberg *et al.* (2006) reported that pINF5 and pAH0376 have different *Hin*dIII restriction patterns. RFLP-hybridisation with *qnrS1* and bla_{TEM-1}-probes had been carried out. The results are similar to those obtained with pRQ2006: *qnrS1* is located on a 2.6 kb *Hin*dIII-fragment of pRQ2006 (as described for pINF5 and pAH0376) and the bla_{TEM-1} gene is located on an 8.4 kb fragment (as described for pAH0376). Furthermore, both genes are located on a ~20 kb *Eco*RI fragment (as described for pAH0376).

The pRQ2006 *qnrS1*-plasmid differs from the other described plasmids found in *Salmonella*, and seems to be related to the plasmid pAH0376 found in *S. flexneri* isolated from human clinical samples in Japan. The presence of these plasmids in *Salmonella* isolates from food producing animals is another threatening step in the increasingly observed fluoroquinolone-resistance.

CHAPTER 6

RECOMMENDATIONS

Epidemiological studies for *Salmonella* are very important, since it is the causative agent of food-borne salmonellosis and the majority of *Salmonella* are acquiring resistance to one or more antibiotics. This study has limitations from epidemiological point of view to track *Salmonella* strains in Türkiye as a whole. Besides, there is lack of information in this era in both in scientific researches and governmental attempts. Thus, it is highly recommended that there must be more studies conducted on *Salmonella*, its prevalence and antibiotic resistance. Moreover, there must be a centralized governmental institution which perform the surveillance of *Salmonella* like in Europe or other countries. The prevalence of serotypes and phage types of *Salmonella* should be studied in detail and a national database must be built to allow the comparison with those databases from other countries.

Antimicrobial resistance studies exhibit that the acquired resistance among pathogens are increasing by resistance against not only one antibiotic but also more than one antibiotic. This is worrisome in that the treatments of infectious diseases are becoming more difficult. For this reason, antimicrobial drug usage should be carefully regulated in countries. Unfortunately, in the developing countries like Türkiye regulations are not enough to prevent uncontrolled use of antibiotics. Antibiotic resistance studies at the molecular biology level also revealed the dynamic nature of antimicrobial resistance. The frequent mutations and lateral transfer of resistance genes accelerate the change in resistance mechanisms, which should be taken into account in fighting with the resistance.

CHAPTER 7

CONCLUSION

Contamination of foods in Turkish markets is high with *Salmonella* serotypes by *S.* Enteritidis being the most prevalent. Multidrug resistant strains of *S.* Infantis are clonally distributed in foods indicating the cross-contaminations in the food-chain. Considerable amount of the strains were confirmed to be antibiotic resistant to one or more antibiotics. It was revealed that quinolone resistance is emerging in both countries. However, in the case of the antimicrobial drug showing the highest resistance, the strains differed in according to their country origin. It can be concluded that *S.* Typhimurium DT104 with penta-resistance phenoype is still a problem in Germany. The presented thesis also gives new information about the molecular basis of *Salmonella* isolates from foods in Türkiye with comparative approach from Germany. There were no correlation between the plasmid profiles except *S.* Infantis strains from both countries. The differences in PFGE patterns of the strains revealed that isolates of the two countries formed different subgroups. On the other hand, antibiotic resistance genes and the mutations contributed to resistance does not differ neither in both countries nor with those in the literature. Finally, lateral gene transfer by mobile genetic elements such as integrons, transposons and plasmids was presented in the study and concluded that they were common and found not only for *S.* Typhimurium but also for *S.* Enteritidis, *S.* Infantis, *S.* Kentucky, and *S.* Agona. Similar studies has to be done regularly in order to establish a control system to pursue the changes in drug resistance pattern before the cases become epidemic even panepidemic.

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APPENDIX I

Table A.I.1. Antimicrobial disks and their contents used in the study (Oxoid Ltd., UK).

Antibiotic (Symbol)	**Disk Content (µg)**
Ampicillin (AMP)	10
Amoxicillin/clavulanic acid (AMC)	30
Chloramphenicol (CHL)	30
Florfenicol (FFC)	30
Ceftiofur (EFT)	30
Kanamycin (KAN)	30
Neomycin (NEO)	10
Gentamicin (GEN)	10
Nalidixic acid (NAL)	30
Ciprofloxacin (CIP)	5
Tetracycline (TET)	30
Spectinomycin (SPT)	10
Sulfonamides (SUL)	300
Trimethoprim (TMP)	5
Trimethoprim/sulfamethoksazol 1:19 (SXT)	25
Streptomycin (STR)	10
Ceftazidime (CAZ)	10
Cefotaxime (CTX)	30
Cefoxitine (FOX)	30
Ceftriaxone (CRO)	5
Cefuroxime (CXM)	30
Aztreonam (ATM)	30
Imipenem (IMP)	10
Cefpodoxime (CPD)	10

Table A.I.2. Antimicrobial content of microtiter plates used in the analyses of minimal inhibitory concentration.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**CIP 4µg/mL**	**SPE 128µg/mL**	**NAL 128µg/mL**	**AMP 32µg/mL**	**CHL 64µg/mL**	**FFC 64µg/mL**	**GEN 32µg/mL**	**NEO 32µg/mL**	**AMC 32/16µg/mL**	**TET 32µg/mL**	**STR 64µg/mL**	**SUL 512µg/mL**
B	**CIP 2µg/mL**	SPE 64µg/mL	**NAL 64µg/mL**	AMP 16µg/mL	**CHL 32µg/mL**	**FFC 32µg/mL**	**GEN 16µg/mL**	**NEO 16µg/mL**	AMC 16/8µg/mL	**TET 16µg/mL**	**STR 32µg/mL**	SUL 256µg/mL
C	CIP 1µg/mL	SPE 32µg/mL	**NAL 32µg/mL**	AMP 8µg/mL	CHL 16µg/mL	FFC 16µg/mL	GEN 8µg/mL	NEO 8µg/mL	AMC 8/4µg/mL	TET 8µg/mL	STR 16µg/mL	SUL 128µg/mL
D	CIP 0.5µg/mL	SPE 16µg/mL	NAL 16µg/mL	AMP 4µg/mL	CHL 8µg/mL	FFC 8µg/mL	GEN 4µg/mL	NEO 4µg/mL	AMC 4/2µg/mL	TET 4µg/mL	STR 8µg/mL	SUL 64µg/mL
E	CIP 0.25µg/mL	SPE 8µg/mL	NAL 8µg/mL	AMP 2µg/mL	CHL 4µg/mL	FFC 4µg/mL	GEN 2µg/mL	NEO 2µg/mL	AMC 2/1µg/mL	TET 2µg/mL	STR 4µg/mL	SUL 32µg/mL
F	CIP 0.12µg/mL	SPE 4µg/mL	NAL 4µg/mL	AMP 1µg/mL	CHL 2µg/mL	FFC 2µg/mL	GEN 1µg/mL	TMP 4µg/mL	TMP 8µg/mL	**TMP 16µg/mL**	**TMP 32µg/mL**	POS 0
G	CIP 0.06µg/mL	SPE 2µg/mL	COL 4µg/mL	COL 8µg/mL	**COL 16µg/mL**	**COL 32µg/mL**	**COL 64µg/mL**	SXT 1/19µg/mL	SXT 2/38µg/mL	**SXT 4/76µg/mL**	**SXT 8/152µg/mL**	POS 0
H	CIP 0.03µg/mL	EFT 0.5µg/mL	EFT 1µg/mL	EFT 2µg/mL	EFT 4µg/mL	**EFT 8µg/mL**	KAN 4µg/mL	KAN 8µg/mL	KAN 16µg/mL	KAN 32µg/mL	**KAN 64µg/mL**	NEG 0

The cells written in bold face letters indicate Resistance, underlined letters Intermediate, and normal typing letters Susceptible concentrations of antimicrobials according to CLSI breakpoints.

AMP: Ampicillin; AMC: Amoxicillin/clavulonic acid; CIP: Ciprofloxacin; COL: Colistin; CHL: Chloramphenicol; EFT: Ceftiofur; FFC: Florfenicol; GEN: Gentamicin; KAN: Kanamycin; NEO: Neomycin; NAL: Nalidixic acid; STR Streptomycin; SPE: Spectinomycin; SUL: Sulfonamides; TET: Tetracycline; TMP: Trimethoprim; SXT: Sulfamethoxazol/Trimethoprim; POS: Positive control; NEG: Negative control.

Table A.I.3. Isolation material, date, place and results.

Sample Code	Isolation Place	Isolation Date	Isolation Material	Result
DC1	Uşak	07.11.2005	Calf liver	-
UTT2	Ankara-Ulus	19.10.2005	Chicken gizzard	+
UTT1	Ankara-Ulus	19.10.2005	Chicken gizzard	+
UTT5	Ankara-Dikmen	29.10.2005	Chicken gizzard	-
UTT6	Ankara-Balgat	29.10.2005	Chicken gizzard	-
UTT7	Ankara-Ulus	15.11.2005	Chicken gizzard	+
UTT8	Ankara-Ulus	15.11.2005	Chicken gizzard	+
UTT10	Ankara-Ulus	25.01.2006	Chicken gizzard	+
UTT9	Ankara-Ulus	25.01.2006	Chicken gizzard	+
UTT11	Ankara-Ulus	28.01.2006	Chicken gizzard	-
UTT12	Ankara-Ulus	28.01.2006	Chicken gizzard	-
UTT4	Ankara-Ulus	19.10.2005	Chicken gizzard	+
TB3	Ankara-Kızılay	09.11.2005	Chicken leg	+
TB7	Ankara-Maltepe	11.11.2005	Chicken leg	+
TB4	Ankara-Balgat	09.11.2005	Chicken leg	+
TB5	Ankara-Balgat	11.11.2005	Chicken leg	+
TB6	Ankara-Maltepe	11.11.2005	Chicken leg	+
TB8	Ankara-Ulus	15.11.2005	Chicken leg	+
TB12	Ankara-Ulus	25.01.2006	Chicken leg	-
TB13	Ankara-Ulus	25.01.2006	Chicken leg	-
TB9	Ankara-Ulus	15.11.2005	Chicken leg	+
TB10	Ankara-Kızılay	20.01.2006	Chicken leg	-
TB11	Ankara-Kızılay	20.01.2006	Chicken leg	-
UC10	Ankara-Maltepe	11.11.2005	Chicken liver	+
UC14	Ankara-Ulus	25.01.2006	Chicken liver	+
UC1	Ankara-Ulus	12.09.2005	Chicken liver	+
UC2	Ankara-Ulus	04.10.2005	Chicken liver	+
UC3	Ankara-Ulus	04.10.2005	Chicken liver	+
UC4	Ankara-Ulus	15.10.2005	Chicken liver	+
UC5	Ankara-Ulus	15.10.2005	Chicken liver	+
UC6	Ankara-Dikmen	29.10.2005	Chicken liver	-
UC7	Ankara-Dikmen	29.10.2005	Chicken liver	-
UC8	Uşak	07.11.2005	Chicken liver	+
UC11	Ankara-Ulus	15.11.2005	Chicken liver	-
UC12	Ankara-Ulus	15.11.2005	Chicken liver	-
UC13	Ankara-Ulus	25.01.2006	Chicken liver	+
UH1	Ankara-Ulus	23.11.2005	Chicken meat (breast)	+
UTG1	Ankara-Söğütözü	01.11.2005	Chicken meat (breast)	+
UTG3	Ankara-Söğütözü	01.11.2005	Chicken meat (breast)	+
UYT1	Ankara-Ulus	05.09.2005	Chicken meat (mixed)	+
UYT2	Ankara-Ulus	05.09.2005	Chicken meat (mixed)	+
UTB2	Ankara-Ulus	19.10.2005	Chicken neck	+
UB7	Ankara-Ulus	28.01.2006	Chicken neck	+
TB15	Ankara-Ulus	28.01.2006	Chicken neck	+
UB8	Ankara-Ulus	28.01.2006	Chicken neck	+

Table A.I.3. Isolation material, date, place and results (continued).

Sample Code	Isolation Place	Isolation Date	Isolation Material	Result
UB1	Ankara-Ulus	12.09.2005	Chicken neck	+
UB2	Ankara-Ulus	12.09.2005	Chicken neck	+
UB3	Ankara-Ulus	15.10.2005	Chicken neck	-
UB4	Ankara-Ulus	15.10.2005	Chicken neck	-
UTB1	Ankara-Ulus	19.10.2005	Chicken neck	+
TB1	Ankara-Söğütözü	01.11.2005	Chicken neck	+
UB5	Ankara-Ulus	15.11.2005	Chicken neck	+
TB14	Ankara-Ulus	28.01.2006	Chicken neck	+
TB2	Ankara-Söğütözü	01.11.2005	Chicken neck	+
UK2	Ankara-Ulus	05.09.2005	Chicken wing	+
UK5	Ankara-Ulus	27.09.2005	Chicken wing	+
UK7	Ankara-Ulus	27.09.2005	Chicken wing	+
BK1	Ankara-Blagat	26.08.2005	Chicken wing	-
UK1	Ankara-Ulus	05.09.2005	Chicken wing	+
UK3	Ankara-Ulus	15.09.2005	Chicken wing	-
UK4	Ankara-Ulus	15.09.2005	Chicken wing	-
UK8	Ankara-Ulus	04.10.2005	Chicken wing	+
UK10	Ankara-Tandoğan	10.10.2005	Chicken wing	+
UK12	Ankara-Ulus	15.10.2005	Chicken wing	-
UK13	Ankara-Balgat	29.10.2005	Chicken wing	+
UK14	Ankara-Söğütözü	01.11.2005	Chicken wing	+
UK15	Ankara-Söğütözü	01.11.2005	Chicken wing	+
UK17	Ankara-Kızılay	09.11.2005	Chicken wing	+
UK16	Ankara-Balgat	09.11.2005	Chicken wing	+
UK18	Ankara-Maltepe	11.11.2005	Chicken wing	+
UK20	Ankara-Ulus	15.11.2005	Chicken wing	+
UK19	Ankara-Ulus	15.11.2005	Chicken wing	+
UK22	Ankara-Ulus	23.11.2005	Chicken wing	-
UK21	Ankara-Maltepe	24.11.2005	Chicken wing	-
UK23	Ankara-Ulus	25.01.2006	Chicken wing	-
UK24	Ankara-Ulus	25.01.2006	Chicken wing	-
UK9	Ankara-Ulus	04.10.2005	Chicken wing	+
AS51	Ankara-Ulus	14.12.2005	Fresh cheese	-
AS52	Ankara-Ulus	14.12.2005	Fresh cheese	-
AS53	Ankara-Ulus	23.12.2005	Fresh cheese	-
UDK1	Ankara-Ulus	05.09.2005	Minced meat	+
UDK5	Ankara-Ulus	15.10.2005	Minced meat	+
UDK6	Ankara-Ulus	15.10.2005	Minced meat	+
UDK15	Ankara-Ulus	15.11.2005	Minced meat	+
UDK16	Ankara-Ulus	15.11.2005	Minced meat	+
UDK17	Ankara-Ulus	15.11.2005	Minced meat	+

Table A.I.3. Isolation material, date, place and results (continued).

Sample Code	Isolation Place	Isolation Date	Isolation Material	Result
UDK18	Ankara-Ulus	15.11.2005	Minced meat	+
UDK26	Ankara-Ulus	14.12.2005	Minced meat	+
UDK27	Ankara-Ulus	14.12.2005	Minced meat	+
UDK43	Ankara-Ulus	07.02.2006	Minced meat	+
UDK29	Ankara-Ulus	14.12.2005	Minced meat	+
UDK44	Ankara-Ulus	07.02.2006	Minced meat	+
UDK45	Ankara-Ulus	07.02.2006	Minced meat	+
UDK35	Ankara-Ulus	23.12.2005	Minced meat	+
UDK37	Ankara-Ulus	25.01.2006	Minced meat	+
UDK2	Ankara-Ulus	05.09.2005	Minced meat	+
UDK3	Ankara-Tandoğan	10.10.2005	Minced meat	+
UDK4	Ankara-Tandoğan	10.10.2005	Minced meat	+
UDK7	Ankara-Dikmen	29.10.2005	Minced meat	-
UDK8	Ankara-Dikmen	29.10.2005	Minced meat	-
UDK9	Uşak	07.11.2005	Minced meat	-
UDK10	Ankara-Balgat	09.11.2005	Minced meat	+
UDK11	Ankara-Kızılay	09.11.2005	Minced meat	-
UDK12	Ankara-Balgat	11.11.2005	Minced meat	+
UDK13	Ankara-Balgat	11.11.2005	Minced meat	-
UDK14	Ankara-Ulus	15.11.2005	Minced meat	-
UDK19	Ankara-Ulus	29.11.2005	Minced meat	-
UDK20	Ankara-Ulus	29.11.2005	Minced meat	+
UDK21	Ankara-Ulus	29.11.2005	Minced meat	-
UDK22	Ankara-Ulus	01.12.2005	Minced meat	-
UDK23	Ankara-Ulus	01.12.2005	Minced meat	-
UDK24	Ankara-Ulus	01.12.2005	Minced meat	+
UDK25	Ankara-Ulus	01.12.2005	Minced meat	-
UDK28	Ankara-Ulus	14.12.2005	Minced meat	-
UDK30	Ankara-Ulus	23.12.2005	Minced meat	-
UDK31	Ankara-Ulus	23.12.2005	Minced meat	-
UDK32	Ankara-Ulus	23.12.2005	Minced meat	-
UDK33	Ankara-Ulus	23.12.2005	Minced meat	+
UDK34	Ankara-Ulus	23.12.2005	Minced meat	-
UDK36	Ankara-Kızılay	20.01.2006	Minced meat	-
UDK38	Ankara-Ulus	25.01.2006	Minced meat	+
UDK39	Ankara-Ulus	28.01.2006	Minced meat	-
UDK40	Ankara-Ulus	28.01.2006	Minced meat	-
UDK41	Ankara-Ulus	02.02.2006	Minced meat	+
UDK42	Ankara-Ulus	02.02.2006	Minced meat	+
UDK46	Ankara-Ulus	07.02.2006	Minced meat	+
KY1	Ankara-Tandoğan	10.10.2005	Ram's testicles	+
KY2	Ankara-Tandoğan	10.10.2005	Ram's testicles	+
AS9	Nevşehir-Avanos	15.09.2005	Raw Milk	+
AS13	Burdur-Varollar	22.09.2005	Raw Milk	+
AS20	Nevşehir-Acıgöl	13.10.2005	Raw Milk	+

Table A.I.3. Isolation material, date, place and results (continued).

Sample Code	Isolation Place	Isolation Date	Isolation Material	Result
AS45	Nevşehir-Avanos	29.11.2005	Raw Milk	+
AS1	Nevşehir-Avanos	05.09.2005	Raw Milk	-
AS2	Nevşehir-Acıgöl	05.09.2005	Raw Milk	+
AS3	Burdur-Varollar	05.09.2005	Raw Milk	-
AS4	Nevşehir-Avanos	08.09.2005	Raw Milk	-
AS5	Nevşehir-Acıgöl	08.09.2005	Raw Milk	-
AS6	Antalya-Bozova	08.09.2005	Raw Milk	-
AS7	Nevşehir-Avanos	11.09.2005	Raw Milk	-
AS8	Nevşehir-Acıgöl	11.09.2005	Raw Milk	-
AS10	Nevşehir-Acıgöl	15.09.2005	Raw Milk	-
AS11	Ankara	15.09.2005	Raw Milk	+
AS12	Kayseri	15.09.2005	Raw Milk	+
AS14	Nevşehir-Avanos	22.09.2005	Raw Milk	-
AS15	Nevşehir-Avanos	03.10.2005	Raw Milk	+
AS16	Nevşehir-Acıgöl	03.10.2005	Raw Milk	+
AS17	Nevşehir-Avanos	10.10.2005	Raw Milk	-
AS18	Nevşehir-Acıgöl	10.10.2005	Raw Milk	-
AS19	Nevşehir-Avanos	13.10.2005	Raw Milk	-
AS21	Burdur-Varollar	13.10.2005	Raw Milk	-
AS22	Nevşehir-Avanos	19.10.2005	Raw Milk	-
AS23	Nevşehir-Acıgöl	19.10.2005	Raw Milk	-
AS24	Nevşehir-Avanos	20.10.2005	Raw Milk	-
AS25	Nevşehir-Acıgöl	20.10.2005	Raw Milk	-
AS26	Ankara	20.10.2005	Raw Milk	-
AS27	Burdur-Varollar	20.10.2005	Raw Milk	-
AS28	Ankara-Ayaş	20.10.2005	Raw Milk	-
AS29	Nevşehir-Avanos	24.10.2005	Raw Milk	-
AS30	Nevşehir-Acıgöl	24.10.2005	Raw Milk	-
AS31	Ankara	24.10.2005	Raw Milk	+
AS32	Nevşehir-Avanos	27.10.2005	Raw Milk	-
AS33	Nevşehir-Acıgöl	27.10.2005	Raw Milk	-
AS34	Burdur-Varollar	27.10.2005	Raw Milk	+
AS35	Nevşehir-Avanos	07.11.2005	Raw Milk	-
AS36	Nevşehir-Avanos	07.11.2005	Raw Milk	+
AS37	Nevşehir-Avanos	22.11.2005	Raw Milk	-
AS38	Antalya-Bozova	22.11.2005	Raw Milk	-
AS39	Ankara	22.11.2005	Raw Milk	-
AS40	Nevşehir-Acıgöl	22.11.2005	Raw Milk	+
AS41	Nevşehir-Acıgöl	24.11.2005	Raw Milk	-
AS42	Nevşehir-Avanos	24.11.2005	Raw Milk	-
AS43	Burdur-Varollar	24.11.2005	Raw Milk	-
AS44	Ankara	24.11.2005	Raw Milk	-
AS46	Nevşehir-Avanos	29.11.2005	Raw Milk	+
AS47	Nevşehir-Avanos	01.12.2005	Raw Milk	-

Table A.I.3. Isolation material, date, place and results (continued).

Sample Code	Isolation Place	Isolation Date	Isolation Material	Result
AS48	Nevşehir-Acıgöl	01.12.2005	Raw Milk	+
AS49	Antalya-Bozova	01.12.2005	Raw Milk	-
AS50	Burdur-Varollar	01.12.2005	Raw Milk	+
RS5	Ankara-Kızılay	09.11.2005	Salad	-
RS8	Ankara-Kızılay	14.11.2005	Salad	-
RS9	Ankara-Kızılay	14.11.2005	Salad	-
RS15	Ankara-Kızılay	20.01.2006	Salad	-
RS1	Ankara-Kızılay	07.10.2005	Salad	-
RS2	Ankara-Dikmen	29.10.2005	Salad	+
RS3	Ankara-Kızılay	09.11.2005	Salad	-
RS4	Ankara-Balgat	09.11.2005	Salad	-
RS6	Ankara-Maltepe	11.11.2005	Salad	-
RS7	Ankara-Kızılay	14.11.2005	Salad	-
RS10	Ankara-Kızılay	14.11.2005	Salad	-
RS11	Ankara-Kızılay	14.11.2005	Salad	+
RS12	Ankara-Ulus	14.11.2005	Salad	-
RS13	Ankara-Ulus	14.11.2005	Salad	-
RS14	Ankara-Ulus	23.12.2005	Salad	-
RS16	Ankara-Kızılay	20.01.2006	Salad	-
RS17	Ankara-Ulus	28.01.2006	Salad	-
RS18	Ankara-Ulus	28.01.2006	Salad	-
KE4	Ankara-Söğütözü	01.11.2005	Sheep kidney	-
KE1	Ankara-Söğütözü	01.11.2005	Sheep meet	+
KE2	Ankara-Söğütözü	01.11.2005	Sheep meet	+
KE3	Ankara-Söğütözü	01.11.2005	Sheep meet	+

0,25 N HCl Solution

Formula	mL
5 N HCl	12,5
Sterile dH_2O	247,5

0,5 M EDTA, pH 8

Formula	
$Na_2EDTA.2H_2O$	37.22 g
Sterile dH_2O	200 mL

pH was adjusted by using 5 M NaOH and the solution was autoclaved (121^oC/15 min).

0,5 N NaOH Solution

Formula	mL
5 N NaOH	25
Sterile dH_2O	225

0,5X Tris-Borat-EDTA (TBE) Solution

Formula	
10X TBE stock solution	100 mL
Sterile dH_2O	1900 mL

1 M Tris-HCl, pH 8

Formula	
Trizma-base	24,22 g
Sterile dH_2O	200 mL

pH was adjusted by using 5 M HCl and the solution was autoclaved (121^oC/15 min).

1X Blocking Reagent (Roche GmbH., Germany)

Formula	
10X Blocking Reagent	5 mL
Maleic acid buffer solution	45 mL

3 M Sodium Acetate, pH 5,2

Formula	
Sodyum asetat.$3H_2O$	40,8 g
H_2O	100 mL

pH was adjusted by using glacial acetic acid to 5,2 and the solution was sterilized in the autoclave (121°C / 15 min).

10X Tris-Borat-EDTA (TBE) Stock Solution

Formula	
(0.9 M Trizma-base, 0.9 M Boric acid, 0.02 M EDTA)	
Tris-Base	109 g
$Na_2EDTA.2H_2O$	9.3 g
Boric acid	55.6 g

Solution was prepared in 1000 mL distilled water and sterilized in autoclave at 121^{o}C for 15 min.

20X SSC Stock Solution

Formula	g/L
NaCl	175,3
Sodium citrate.2H2O	88,2

Solution sterilized in autoclave at 121^{o}C for 15 min.

20% SDS Solution

Formula	
SDS	2 g
Sterile dH_2O	10 mL

Bismuth Sulfite Agar (BSA) (Merck KGaA., Germany)

Formula	g/L
Meat extract	5,0
Peptone from meat	10,0
D(+)glucose	5,0
Di-sodium hydrogen phosphate	4,0
Iron (III) sulfate	0,3
Bismuth sulfite indicator	8,0
Brilliant green	0,025
Agar	15,0

pH 7,6 ± 0,2 (before sterilization)

19,0 g dehydrated media was suspended in 400 mL sterile distilled water and agar in the content was melted by heating in boiling waterbath. Later, it was cooled down to 55^{o}C and poured 25 mL into sterile petri plates to form a thick layer. This media was not sterilized in autoclave.

Buffered Peptone Water (BPW) (Oxoid Ltd., UK)

Formula	g/L
Enzymatic digest of casein	10,0
NaCl	5,0
Disodium hydrogen phosphate (anhydrous)	3,5
Potassium dihydrogen phosphate	1,5

pH 7,0 ± 0,2 (before sterilization)

Add 4,5 g of BPW was dissolved in 225 mL of distilled water. It was mixed well and sterilized by autoclaving at 121°C for 15 min.

Cell Lysis Buffer Solution

Formula	
1 M Tris-HCl, pH 8	25 mL
0,5 M EDTA, pH 8	50 mL
Sarcosyl	5 g
Sterile dH_2O	425 mL
Proteinase K (20 mg/mL)	2,5 mL

Cell Suspension Buffer Solution

Formula	
(100 mM Tris-HCl, 100 mM EDTA, pH 8)	
1 M Tris-HCl, pH 8	10 mL
0,5 M EDTA, pH 8	20 mL
Sterile dH_2O	97 mL

Solution sterilized in autoclave at 121^{o}C for 15 min.

Detection Buffer Solution (Roche GmbH., Germany)

Formula	
1M Tris/HCl (pH 9.5)	5 mL
5M NaCl	1 mL
1M $MgCl_2$	2,5 mL
Sterile dH_2O	41,5 mL

Detection-Wash Solution (Roche GmbH., Germany)

Formula	
Maleic acid buffer solution	100 mL
Tween20	200 µL

DNA-Sample-Buffer

Formula	mL
15% Ficoll	10
5% Bromphenolblue in bdH_2O	0,25

Double Strength Bacto-Nutrient Broth and Agar (Difco, USA)

Formula	g/L
Beef extract	6
Peptone	10
NaCl	8,5
Agar	13

pH 6,8 ± 0,2 (before strilization)

8 g dehydrated media for broth and 23 g dehydrated media for solid media were suspended in 1000 mL distilled water. The broth was dispensed into standard glass tubes and autoclaved at 115°C for 10 min. The solid media was sterilized the same before dispensing into sterile petri dishes at 55°C.

Eosin Methylene Blue (EMB) Agar

Formula	g/L
Peptone	10
Di-potassium hydrogen phosphate	2
Lactose	5
Sucrose	5
Eosin Y, yellowish	0,4
Methylene blue	0,07
Agar	13,5

pH 7,1 ± 0,2 (before sterilization)

36 g dehydtrated media was dissolved in 1000 mL distilled water and sterilized at 121 °C for 15 min in autoclave. Then it was cooled down to 55^{o}C and poured into sterile petri plates approximately 15-20 mL.

Gassner Agar (Merck KGaA., Germany)

Formula	g/L
Peptone	14,0
NaCl	5,0
Laktose	43,0
Water blue	0,62
Metachrome yellow	1,25
Agar	13,0

pH 7,2 ± 0,2 (before sterilization)

77,0 g dehydrated media was dissolved in 1000 mL distilled water. It was autoclaved (121°C / 15 min) and transferred 15-20 mL into sterile petri plates at 55°C.

Hybridization Solution (Roche GmbH., Germany)

Formula	
20X SSC	50 mL
10% N-Laurylsarcosine	2 mL
10% SDS	0,4 mL
10% 10X Blocking Solution	20 mL

Iodine-Iodide Solution

Formula	g/100mL
Potassium iodide	25,0
Iodine	20,0

25,0 g potassium iodide was dissolved in 10 mL sterile distilled water. 20,0 g iodine was added and the volume was completed to 100 mL with sterile distilled water.

KADO-Buffer Solution

Formula	g/100mL
(50 mM Tris / 1 mM EDTA, pH 8)	
Trizma-base	0,6
EDTA	0,03

Luria Bertani (LB) Broth and Agar

Formula	g/L
Tryptone	10,0
Yeast extract	5,0
NaCl	10,0
Agar	13,0

pH 7,0± 0,2 (before strailization)

The content was mixed and suspended in 1000 mL distilled water. When the liquid media was dispensed into tubes and when agar was added to prepare agar media, they were sterilized in autoclave (121°C / 15 min). Agar was poured 15-20 mL into sterile petri plates when it was cooled down to 55°C.

Lysis Mix Solution

Formula	mL
Steril, bdH_2O	11,5
15% SDS	4
250mM Tris	4
5N NaOH	0,3

This solution was freshly prepared in the course of plasmid analyses.

Maleic Acid Buffer Solution, pH 7,5 (Roche GmbH., Germany)

Formula	
Maleic acid	23,2 g
NaCl	17,5 g
Sterile dH_2O	2000 mL

McFarland Solution

Formula	mL
$BaCl_2.2H_2O$ (1,175% w/v)	0,5
H_2SO_4 (0,36 N)	99,5

Müller-Hinton Agar (Oxoid Ltd., UK)

Formula	g/L
Meat extract	300
Casein hydrolysate	17,5
Starch	1,5
Agar	17,0

pH 7,3 ± 0,1 (before sterilization)

38 gram dehydrated media was dissolved in 1000 mL distilled water and starilized at 121°C for 15 min in autoclave. After cooled down to 55°C, 10-15 mL was dispensed into sterile petri dishes and stored at +4^{o}C.

Müller-Kauffmann Tetrathionate/Novobiocin Broth (MKTTn)
(Oxoid Ltd., UK)

Formula	g/L
Meat extract	4,3
Enzymatic digest of casein	8,6
NaCl	2,6
Calcium carbonate	38,7
Sodium thiosulphate (anhydrous)	30,5
Ox bile	4,78
Brilliant green	0,0096
Novobiocin solution (0,8%)	5,0 mL
Iodine-Iodide solution	20,0 mL

pH 8,0 ± 0,2 (before sterilization)

89,5 g of dehydrated media was suspended in 1 L of sterile distilled water. The suspension mixed well and brought to boil with frequent agitation. When it was completely dissolved, iodine-iodide and novobiocin (Novobiocin Selective Supplement, Oxoid Ltd., UK) solutions were added and dispensed into 10 mL sterile tubes. This media was not sterilized in the autoclave.

Phenol/Chloroform Solution

Formula	mL
Fenol	1
Chloroform	1

This solution was freshly prepared in the course of plasmid analyses.

Post-Hybridization-Wash Solution-I

Formula	
20X SSC	5 mL
10% SDS	50 µL
Sterile dH_2O	44,95 mL

Post-Hybridization-Wash Solution-II

Formula	
20X SSC	0,5 mL
10% SDS	100 µL
Sterile dH_2O	99,4 mL

Rappaport Vassiliadis Broth (RVB) (Merck KGaA., Germany)

Formula	g/L
Peptone from soymeal	4,5
Magnesium chloride hexahydrate	29,0
NaCl	8,0
Di-potassium hydrogen phosphate	0,4
Potassium di-hydrogen phosphate	0,6
Malachite-green	0,036

pH 5,2 ± 0,2 (before sterilization)

42,5 g of dehydrated media was suspended in 1 L distilled water. Then it was dispensed into 10 mL test tubes and sterilized in autoclave (115°C / 15 min).

Seakem Agarose (1%)-SDS

Formula	
Seakem Agarose (Biozyme, USA)	0,25 g
Tris-EDTA solution (TE)	23,5 mL
20% SDS solution	1,25 mL

Seakem agarose was molten in TE solution in microwave. Then, in the waterbath (GFL 1002, GFL GmbH., Germany), it was cooled down to 50^{o}C and mixed with 1,25 mL of prewarmed (50^{o}C) 20% SDS solution.

Seakem Agarose (1%)-TBE

Formula	
Seakem Agarose	1 g
0.5X TBE solution	100 mL

Seakem agarose was molten in 0,5X TBE solution in microwave and cooled down to 50^{o}C in waterbath (GFL 1002, GFL GmbH., Germany) before poring into the gel tray.

Semi-Solid Agar (Swarm-Agar)

Formula	g/L
Meat extract	7,0
Peptone	10,0
NaCl	3,0
Di-sodium hydrogen phosphate.12 hydrate	2,0
Glucose	1,0
Agar	5,0

pH 7,5 ± 0,2 (before sterilizaiton)

The content was suspended in 1000 mL distilled water and autoclaved (121°C / 15 min). When it was cooled down to 55°C, it was poured into sterile 15mm petri plates.

SOC Medium

Formula	g/L
Trypton	20 g
Yeast extract	5 g
5M NaCl	2 mL
1M KCl	2,5 mL
1M $MgCl_2$	10 mL
1M $MgSO_4$	10 mL
1M Glucose	20 mL

The media contents were dissolved in 1000mL sterile water and autoclaved at 121°C for 15 min.

Tris-EDTA (TE) Buffer Solution

Formula	
(10 mM Tris-HCl, 1 mM EDTA, pH 8)	
1 M Tris-HCl, pH 8	10 mL
0,5 M EDTA, pH 8	2 mL
Sterile dH_2O	988 mL

Solution sterilized in autoclave at 121^{o}C for 15 min.

Xylose-Lysin Desoxycholate (XLD) Agar (Oxoid Ltd., UK)

Formula	g/L
Yeast extract	3,0
L-Lysine HCl	5,0
Xylose	3,75
Lactose	7,5
Sucrose	7,5
Sodium desoxycholate	1,0
NaCl	5,0
Sodium thiosulphate	6,8
Ferric ammonium citrate	0,8
Fenol red	0,08
Agar	12,5

pH 7,4 ± 0,2 (before sterilization)

26,5 g of dehydrated media was suspended in 500 mL distilled water. The agar in the composition was melted in the boiling waterbath by avoiding overheating. Subsequently, it was cooled down to 55^{o}C and transferred 15-20 mL in sterile petri plates. This media was not sterilized in autoclave.

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