CONJUGATIVE TRANSFER OF ANTIBIOTIC RESISTANCE GENES FROM *SALMONELLA ENTERICA* SEROVAR INFANTIS TO *ESCHERICHIA COLI*

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ABSTRACT

CONJUGATIVE TRANSFER OF ANTIBIOTIC RESISTANCE GENES FROM *SALMONELLA ENTERICA* SEROVAR INFANTIS TO *ESCHERICHIA COLI*

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The usage and misusage of antibiotics in poultry, food-producing animals and human diseases have led to transmission of conjugative plasmids carrying antibiotic resistance genes from one microorganism to another, especially to the pathogenic bacteria. Multi-drug resistant *Salmonella enterica* serovar Infantis, an emerging serotype in poultry, has been spreading all around the world in a decade. Moreover, commensal microorganisms such as commensal *Escherichia coli* in the gut microbiota, functioning as a reservoir of antimicrobial resistance, of the warm-blooded hosts acquire antibiotic resistance by plasmid from *Salmonella enterica* serovars. The aim of this study was to identify the conjugative transfer of plasmids including multi-drug resistance genes from *Salmonella enterica* serovar Infantis to commensal susceptible *E. coli* in both of phenotypic and genotypic level. *Salmonella* Infantis isolates with different antibiotic resistance profiles involving streptomycin, sulfisoxazole, ampicillin, trimethoprim/sulfamethoxazole, kanamycin, chloramphenicol, ciprofloxacin, cephalotin, nalidixic acid and tetracycline were

selected as donor bacteria (n=10), while susceptible non-pathogenic *E. coli* isolate was used as a recipient. Applying filter paper mating procedure was used for conjugation. Transconjugant *E. coli* colonies were screened by phenotypic and genotypic methods. First, yellow colonies representing transconjugant *E. coli* colonies were determined on the selective XLD agar containing 10 μg/ml tetracycline in phenotypic level. After that, conjugation genes (i.e., *traA, traE, traL, traJ, traG, traH, traI, traM, traR, traY, rfaG, rfaI, rfaJ, rfaL* and *rfbP*), and the incompatibility group of plasmids were screened by polymerase chain reaction (PCR) in genotypic level. This study contributes to recent and further studies related to the conjugative transmission of antibiotic resistance genes in *Salmonella* and *E. coli*, which is an arising serious problem worldwide.

Keywords: Antibiotic Resistance, Conjugation, Plasmid, *Salmonella, Escherichia coli*

ÖZ

ANTİBİYOTİK DİRENÇLİLİK GENLERİNİN *SALMONELLA ENTERICA* SEROVAR INFANTIS İZOLATLARINDAN *ESCHERICHIA COLI* SUŞUNA KONJUGASYONEL AKTARIM YOLU

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Kümes hayvanlarında, gıda üreten hayvanlarda ve insan hastalıklarında antibiyotiğin kullanımı ve yanlış kullanımı, antibiyotik dirençlilik genlerini taşıyan konjugasyonel plazmidlerin bir organizmadan diğerine özellikle patojen bakteriye yayılmasına neden olmuştur. Çoklu ilaç dirençli *Salmonella enterica* serovar Infantis, kümes hayvanlarında yeni görülen klon, 10 yılda tüm dünyaya yayılmış bulunmaktadır. Buna ek olarak, antimikrobiyal dirençlilik rezervuarı olarak işlev gören sıcakkanlı konakçıların bağırsak mikrobiyotasında bulunan kommensal *Escherichia coli* gibi kommensaller, *Salmonella enterica* serovarlarından antibiyotik dirençlilik genlerini taşıyan plazmidleri kazanmaktadırlar. Bu çalışmada, çoklu ilaç dirençlilik genlerini taşıyan plazmidlerin *Salmonella enterica* serovar Infantis'ten duyarlı kommensal *E. coli*'ye fenotipik ve genotipik düzeyde konjugasyonel aktarım yolu belirlenmiştir. Streptomisin, sülfisoksazol, ampisilin, trimetoprim-sülfametaksazol, kanamisin, kloramfenikol, siprofloksazin, sefalotin, nalidiksik asit ve tetrasiklin içeren farklı antibiyotik dirençlilik profillere sahip olan *Salmonella* Infantis izolatları (n=10) donör olarak seçilirken, duyarlı nonpatojenik *E. coli* izolatı alıcı olarak kullanılmıştır.

Konjugasyon için filtre kağıdıyla eşleme yöntemi uygulanmıştır. Konjuge olan *E. coli* izolatları, fenotipik ve genotipik metotlarla saptanmıştır. İlk olarak, konjuge olan *E. coli* izolatlar, 10 µg/ml tetrasiklin içeren selektif ksiloz-lizin-deoksikolat (XLD) agarda sarı kolonilere izafeten fenotipik düzeyde belirlenmişlerdir. Bundan sonra, konjugasyon genleri (*traA, traE, traL, traJ, traG, traH, traI, traM, traR, traY, rfaG, rfaI, rfaJ, rfaL* and *rfbP*) ve plazmidlerin inkompatibilite grubu, polimeraz zincir tepkimesiyle (PCR) genotipik düzeyde incelenmiştir. Yapılan bu çalışma, dünya çapında giderek artan ve ciddi bir sorun haline gelen antibiyotik dirençlilik genlerinin *Salmonella* ve *E. coli* suşlarında konjugasyonel aktarımıyla ilgili son ve ileriki çalışmalara katkıda bulunacak potansiyele sahiptir.

Anahtar kelimeler: Antibiyotik dirençlilik, Konjugasyon, Plazmid, *Salmonella, Escherichia coli*

To My Lovely Mother, Father and Sister,

and

To My Sweet Nephew

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LIST OF ABBREVIATIONS

Ak: Amikacin

Amc: Amoxicillin/clavulanic acid

Amp: Ampicillin

ARP: Antibiotic Resistance Profile

AT: Annealing Temperature

BHI: Brain Heart Infusion

bla: β-lactamase

bp: Base pair

C: Chloramphenicol

CATs: Chloramphenicol acetyltransferases

CDC: Centers for Disease Control and Prevention

Cip:Ciprofloxacin

Cn: Gentamicin

Cro: Ceftriaxone

CTX-M: Cefotaxime hydrolyzing capabilities

DHFR: Dihydrofolate reductase

DHPS: Dihydropteroate synthase

DNA: Deoxyribonucleic acid

E. coli: *Escherichia coli*

Eft: Ceftiofur

ESBL: Extended-spectrum β-lactamase

Etp: Ertapenem

Fox: Cefoxitin

Imp: Imipenem

Inc: Incompatibility group

K: Kanamycin

kb: Kilobase

Kf: Cephalotin

LB: Luria-Bertani

MLST: Multilocus Sequence Typing

ml: Milliliter

mM: Millimolar

MRSA: Methicillin-resistant *Staphylococcus aureus*

N: Nalidixic acid

^{o}C: Degree Celcius

PER: *Pseudomonas* extended resistant

PFGE: Pulsed-field Gel Electrophoresis

pH: Potential of hydrogen

RNA: Ribonucleic acid

rpm: Revolutions per minute

rRNA: Ribosomal ribonucleic acid

S: Streptomycin

Sf: Sulfisoxazole

SHV: Sulfhydryl variable

Sxt: Trimethoprim/sulfamethoxazole

T: Tetracycline

TE: Tris- EDTA

TEM: Temoneira

tra: Transfer gene

tRNA: Transfer ribonucleic acid

WGS: Whole Genome Sequencing

XLD: Xylose- Lysine- Desoxycholate

µg: Microgram

µl: Microliter

µm: Micrometer

CHAPTER 1

INTRODUCTION

Salmonella is a genus of Gram-negative, including more than 2500 different serotypes. *Salmonella* consists of two species which are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is composed of six subspecies. Moreover, *S. enterica* subspecies *enterica* includes 1531 serotypes such as *Salmonella* Infantis, Thyphimurium and Enteritidis. The basic reason for the concern about this subspecies is that *Salmonella enterica* subsp. *enterica* is the second most one leading to gastrointestinal human infections after *Campylobacter* spp. Non-typhoidal *Salmonella enterica* subsp. *enterica* (*Salmonella*) lead to salmonellosis affecting the intestinal tract with the symptoms of diarrhea, fever and abdominal cramps within 12 to 72 hours. Centers for Disease Control and Prevention (CDC) reports over a million illnesses and around 500 deaths related to nontyphoidal *Salmonella* spp. in the USA. Moreover, *Salmonella* cases have been becoming a serious threat to human health all around the world. In recent years, progressively foodborne outbreaks related to one of the *Salmonella* serovars, Infantis, have been observed. Furthermore, the majority of these isolates show antibiotic resistance the most commonly in the poultry and broiler meat in European countries. Salmonellosis targets everyone, especially the children, adults and people having weakened immune system, which are the highest risk groups. Ciprofloxacin, commonly used antibiotic in treating salmonellosis, cannot be used in children because of the unfavourable musculoskeletal effects of fluoroquinolones.

Instead of ciprofloxacin, β-lactams are chosen for the treatment of children. Different phenotypic antibiotic resistance profiles of *Salmonella* Infantis have been determined. For example, a multidrug-resistant strain of *Salmonella enterica* Infantis, which shows the resistance to nalidixic acid, streptomycin, sulphonamide and tetracycline has been spread in Hungary. In addition to this, *Salmonella enterica* serovar Infantis resistant to ampicillin, chloramphenicol, streptomycin, sulphonamide, tetracycline, kanamycin, trimethoprim and sulfamethoxazole has been collected in Italy. Multidrug-resistant strains of *Salmonella* serovars lead to more serious bacterial infections as compared with the susceptible strains. The reason of that might be because of the virulence factors on the same plasmids. Antibacterial resistant *Salmonella,* especially the ones with the extended spectrum beta-lactams might cause a problem for public health in near future. Since the misusage of antibiotics in veterinary medicine and human health has become critical, it has triggered the conjugative transfer of plasmids carrying the antimicrobial resistance genes such as for tetracycline (*tetA, tetB, tetC*), for streptomycin (*strA, strB, aadA*), for sulfonamides (*sul1*, *sul2*, *sul3*), for kanamycin and neomycin (*apha1*, *apha2*), and for beta-lactamase genes ($bla_{\text{cmy-2}}$, bla_{tem}, bla_{shv}, bla_{pse}), for kanamycin and gentamicin (*aadb, aadA1*). Furthermore, from which source *Salmonella* spp. acquire the antibacterial resistance genes and/or in what ways antibiotic resistance genes are transferred to pathogenic bacteria remains unclear because of the usage and misusage of antibiotics in poultry, food-producing animals and human diseases. At this point, the studies have revealed that the conjugative transfer of plasmids carrying antimicrobial resistance genes among Gram-negative foodborne pathogens such as *Salmonella enterica* serovars and *Escherichia coli* serovars have been increasing in recent years.

This thesis enucleates the acquisition of antibiotic resistance genes by the horizontal gene transfer among antimicrobial resistant *Salmonella enterica* Infantis and commensal *Escherichia coli* having susceptibility to antibacterials and plasmid-free. In order to determine the molecular mechanism of horizontal gene transfer, conjugation was applied. *Salmonella enterica* Infantis isolated from food origins were used as donors, while plasmid-free *Escherichia coli* was selected as recipient. The phenotypic features of antibiotic susceptibilities were examined by disc diffusion and minimal inhibitory concentration studies. The genotypic characteristics of antimicrobial resistance were screened by PCR amplification. To determine the conjugative transfer of antibiotic resistance genes, genes on plasmids regulating the conjugation process were also screened after the purification of plasmid DNA from antibiotic resistant *Escherichia coli*.

CHAPTER 2

LITERATURE SEARCH

2.1. Antibiotics and Antibiotic Resistance

Antibiotics are defined as the low molecular weight microbial metabolites which prevent the growth of microorganisms at low concentrations (Lancini et al., 1995). There are two different impacts of antibiotics on bacteria; bacteriostatic and bactericidal. Bacteriostatic components such as chloramphenicol inhibit the growth of bacterial cells, while bactericidal substances such as penicillin remove bacteria (Walsch, 2003). The main targets of antibiotics are put in order as cell wall biosynthesis, protein biosynthesis, DNA and RNA biosynthesis, and folate biosynthesis (Walsch, 2003). Gram-negative bacteria such as *Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive bacteria like *Staphylococcus aureus* and *Streptococcus pneumoniae* possess a peptidoglycan layer in order to protect themselves from the environmental stresses (Yoneyama and Katsumata, 2006). Penicillins and cephaolosporins under the group of β-lactams inactivate the functions of transglycosidase and transpeptidase, which are the main enzymes of peptidoglycan layer (Spratt and Cromie, 1988). Since protein biosynthesis consists of many complex reactions, antibiotics, aminoglycosides and tetracyclines, attack to conserved regions of the 16S rRNA of the 30S subunit, and other antibiotics, acrolides, chloramphenicol, lincosamides, and quinupristin-dalfopristin, interact with the 23S rRNA of the 50S subunit (Yoneyama et al., 2006).

Furthermore, in order to inhibit the biosynthesis of DNA and RNA, quinolones such as ciprofloxacin and nalidixic acid, and rifampicin play crucial roles, respectively (Maxwell, 1997; Spratt, 1994). Moreover, sulfamethoxazole, one of sulfonamides, and trimethoprim affect the folic acid metabolism adversely and inhibit the bacterial infections such as urinary and respiratory infections (Mascaretti, 2003).

Antibiotic resistance might be hereditary or acquired. To illustrate of intrinsic antibiotic resistance, *Pseudomonas aeruginosa* resistant to the majority of antibiotics, Gram-positive bacteria resistant to colistin and Enterobacteriaceae resistant to glycopeptides and linezolid (Yoneyama et al., 2006; MacGowan et al., 2013). On the other side, acquired resistance is originated in gain of a plasmid and/or transposon or mutational alterations in chromosome (Davies, 1994).

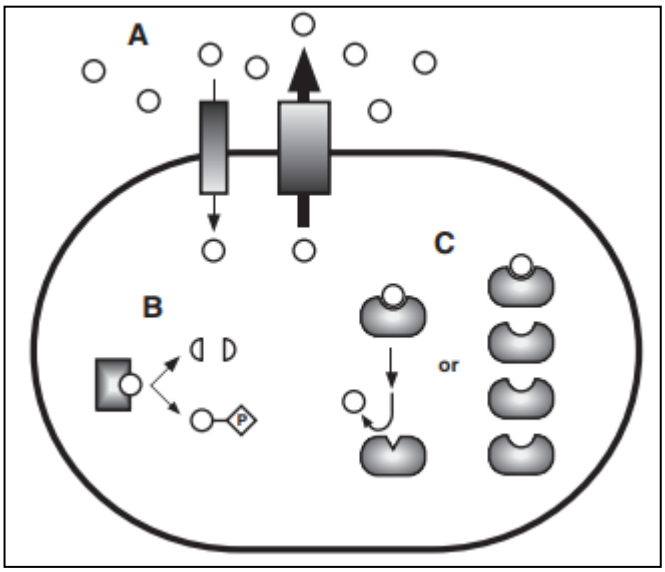

Figure 1 Main mechanisms of antibiotic resistance. Retrieved from Yoneyama, H., & Katsumata, R. (2006).

The main mechanisms of antibiotic resistance are **(A)** inhibiting the aggregation of antibiotics in bacterial cell by increasing efflux or reducing uptake, **(B)** deactivation of antibiotics by hydrolysis or phosphorylation, and **(C)** transformation of the target qualitatively in order to decrease the resemblance for the antibiotics by mutation or modification, or quantitatively by excessive production of the target as shown in Figure 1 (Spratt, 1994; Walsh, 2000).

Table 1 Most commonly used antibiotics to determine the phenotypic antibiotic resistance profile of *Salmonella* and *E. coli* isolates.

Classification	Antibiotics	Function	Reference
β-Lactams	Ampicillin Ceftiofur Cefoxitin Ceftriaxone Cephalothin Amoxicillin-clavulanic acid Ertapenem Imipenem	Interfering with the cell wall synthesis	Majiduddin et al., 2002
Quinolones	Nalidixic acid	Blocking DNA transcription	Aldred et al., 2014
Fluoroquinolones	Ciprofloxacin	Blocking DNA transcription	Aldred et al., 2014
Aminoglycosides	Amikacin Gentamicin Kanamycin Streptomycin	Inhibiting protein synthesis of 30S subunit	Fantin et al., 1991
Phenicols	Chloramphenicol	Inhibiting protein synthesis of 50S subunit	Seth et al., 2009
Tetracyclines	Tetracycline	Inhibiting protein synthesis of 30S subunit	Roberts, 2002
Sulfonamides	Trimethoprim-sulfamethoxazole Sulfisoxazole	Inhibiting folate synthesis	Van Hoek et al., 2011
DHFR inhibitors	Trimethoprim	Inhibiting folate synthesis	Sköld, 2001

2.1.1. β-lactam antibiotics have been using for various bacterial infections for a long time. They comprise of penicillins, cephalosporins, monobactams, carbapenems and β-lactamase inhibitor combinations (Watkins et al., 2017). Penicillins and some cephalosporins, β-lactam antibiotics, include a four-atom cyclic amide. β-lactam antibiotics might be reproduced from the polymerization of amino acids (Lancini et al., 1995). Gram-negative organisms, involving the majority of Enterobacteroaceae, are affected by the natural penicillin activity of aminopenicillins such as ampicillin and amoxicillin (Watkins et al., 2017). In addition to this, aerobic Gram-positive, Gram-negative and anaerobic organisms, which generate β-lactamases are more affected with the use of β-lactamase inhibitors. For cephalosporins, there are five generation types. Cefotaxime, ceftazidime and ceftriaxone, belonging to third-generation cephalosporins, have had efficient Gram-negative. Ceftaroline which is the last generation-cephalosporin has improved its efficiency against methicillin-resistant *Staphylococcus aureus* (MRSA) (Watkins et al., 2017). On the other hand, because of extended-spectrum β-lactamases and carbapenemases, resistancy to cephalosporin rises. For instance, *Salmonella* isolates resistant to third-generation cephalosporins have been found out since 1988, because of the extended-spectrum β-lactamases (ESBL) all around the world (Rhen, 2007). The extended-spectrum β-lactamases (ESBL) in *Salmonella* are found on plasmid-mediated class 1 beta-lactamases SHV, TEM, PER and CTX-M groups. An outbreak of *Salmonella enterica* Infantis producing ESBL was reported in Brazil (Pessoa-Silva et al., 2002). Non-typhoidal *Salmonella* might get resistance to extended spectrum cephalosporins due to plasmid-mediated AmpC-type beta lactamases (Rhen, 2007).

2.1.2. Quinolones and fluoroquinolones are the unnatural antimicrobial substances. They are originally reproduced from 1,8-naphthyridine constituents such as nalidixic acid. (Rubinstein et al., 2017). The inhibitory effect of naphthyridine and quinolone materials, such as nalidixic acid, was restricted to Gram-negative pathogens especially *Salmonella* and *Shigella* spp. On the other hand, adding a fluorine at position 6 has increased the activity against the Gram-negative pathogens (Rubinstein et al., 2017). The main way to acquire the quinolone resistance is the mutational alteration on the antimicrobial target site. Mutational changes on the active site of *gyrA*, which results in higher susceptibility to nalidixic acid rather than the fluoroquinolones have been found out in the majority strains of *Escherichia coli.* In addition to this, insertional *parC* mutation has led to more resistance to ciprofloxacin in *E. coli* (McDonald et al., 2001). Some studies have revealed that nontyphoidal *Salmonella* infections have shown the resistance to nalidixic acid and become less susceptible to fluoroquinolones (Poutanen et al., 2003). Another way of gaining resistance to quionolones is the efflux pumps, which generally lead to low level resistance. On the other hand, in case of overexpression of efflux pumps, it might bring out serious problems. The efflux pump system of *E. coli* is acrAB-toIC (Rubinstein et al., 2017). A number of strains of *Salmonella enterica* serovar Typhimurium have been less susceptible to fluoroquinolones, because of the overexpression of AcrAB efflux pump which might be expressed more by MarA and SoxS encoded by *marRAB* and *soxRS* in return (Giraud et al., 2000; White et al., 1997). A recent mechanism reducing susceptibility to quinolones in Enterobacteriaceae is Qnr proteins which preserve gyrase and topoisomerase IV from quinolone detention (Rubinstein et al., 2017). Another discovered mechanism is AAC(6')-lb-cr, aminoglycoside acetyltransferase, which is responsible for transferring low-level resistance to aminoglycosides and fluoroquinolones (Robicsek et al., 2006).

2.1.3. Aminoglycoside antibiotics are generated by the genera *Streptomyces, Micromonosora* and *Bacillus*. Aminoglycoside group includes streptomycin, kanamycin, gentamicin, tobramycin and neomycin. They are composed of a cyclic amino alcohol and amino sugars (Lancini et al., 1995). Aminoglycosides inhibit the protein synthesis by binding to the ribosome of bacteria (Fantin et al., 1991). Moreover, they damage the translation process of bacteria by binding to 30S ribosomal subunit reversibly, which results in gathering non-specified proteins in bacteria (Hermann, 2007). Aminoglycosides are effective against bacterial pathogens leading to serious diseases (Menashe et al., 2008). On the other hand, they are not efficient to treat intracellular bacteria such as *Staphylococcus aureus* and *Salmonella enterica* in vitro (Kihlström et al., 1985). Streptomycin was the first antibiotic treating tuberculosis (Lancini et al., 1995). Kanamycin, gentamicin, tobramycin, amikacin and netilmicin were isolated to supress bacteria, which are not susceptible to streptomycin (Lancini et al., 1995). Acquiring the resistance to aminoglycosides is runned by acetyltransferases, nucleotidyltransferases and phosphotransfeases, which results in enzymatic inactivation (Benveniste et al., 1973). *aph* genes enable bacteria to acquire the resistance to kanamycin and neomycin, while *Salmonella* isolates gain resistance to streptomycin by the help of genes, *strA*, *strB* and *aadA* (Shaw et al., 1993). Furthermore, *aadB* is responsible for the resistance to gentamicin and tobramycin. A study has revealed that *Salmonella* strains isolated from clinical source were highly resistant to kanamycin compared to amikacin, tobramycin, and gentamicin (Samadi et al., 2015). On the other side, macrophage associated *Salmonella* have been impacted by aminoglycosides based on selected concentration and exposure time of gentamicin (Menashe et al., 2008).

2.1.4. Chloramphenicol is one of the group of miscellaneous antibiotics including florfenicol. It was first isolated from *Streptomyces venezuealae* (Lancini et al., 1995). It consists of a nitrobenzene moiety (Seth et al., 2009). Chloramphenicol inhibits the protein synthesis by binding to the 50S ribosomal subunit reversibly and intercepting aminoacyl tRNA from binding to the acceptor group on the former ribosomal subunit (Seth et al., 2009). Chloramphenicol is used to treat the infections of Gram-negative bacteria because of its effective antimicrobial activity. Therefore, plasmid mediated resistance to chloramphenicol has been increasing although some strains of *Salmonella* are still susceptible to chloramphenicol. Gram-negative bacteria acquire the resistance to chloramphenicol by transferring of plasmids via conjugation. Moreover, plasmid mediated resistance might lead to multidrug resistance including chloramphenicol, tetracycline and streptomycin (Seth et al., 1995). Distinct types of chloramphenicol acetyltransferases (CATs) make ineffective chloramphenicol, thiamphenicol and azidamphenicol, while florfenicol is not affected by these enzymes mentioned before (Van Hoek et al., 2011). Furthermore, efflux mechanisms, mutational changes on the specific site and inactivation of phosphotransferases are also considered as the resistance mechanisms (Schwarz et al., 2004). There are two classified genes encoding the chloramphenicol acetyltransferases, which are *catA* and *catB*. In addition to this, *cmlA* provides resistance to chloramphenicol, while it does not have the same effect to florfenicol. On the other hand, *floR* enables the resistance to both of chloramphenicol and florfenicol (Van Hoek et al., 2011). Chloramphenicol resistance have been found out in isolates representing *Salmonella* serovars Typhi, Typhimurium and Paratyphi isolates (Lee et al., 2004; Faldynova et al., 2003; Hardjo et al., 2017).

2.1.5. Tetracyclines are composed of chlortetracycline, oxytetracycline and tetracycline. They are generated from the distinct strains of *Streptomyces.* Tetracyclines have extensive spectrum of activity against Gram-positive and Gram-negative bacteria. They inhibit the protein synthesis and the growth of bacteria (Roberts, 2002). Efflux systems connected to energy, ribosomal protection proteins and enzymatic inactivation are the mechanisms providing the resistance to tetracycline (Van Hoek et al., 2011). There are *tet* genes encoding tetracycline resistance and *otr* genes encoding oxytetracycline resistance, and *tcr* encodes for efflux pumps (Brown et al., 2008; Roberts, 2005). *tet(B)* is the most seen gene among the Gram-negative bacteria (Van Hoek et al., 2011). *tet(A), tet(B), tet(C), tet(D) and tet(G)* were also screened in *Salmonella* isolates (Chopra and Roberts, 2001).

2.1.6. Sulfonamides are the synthetic antibiotics which inhibit the dihydropteroate synthase (DHPS) taking place in thymine production and bacterial cell growth (Van Hoek et al., 2011). Sulfamethoxazole is one of the most used sulfonamides. Because of their inhibitory activity, sulfamethoazole and trimethoprim have been combined at specific concentrations. In consequence of mutational alteration in *folP* responsible for DHPS, chromosomal sulfonamide resistance arises (Sköld, 2000). *sul1*, *sul2* and *sul3* are plasmid mediated genes encoding sulfonamide resistance. *sul3* was screened in *Salmonella* serovars Typhimurium, Rissen and Agona (Antunes et al., 2005; Guerra et al., 2004). *sul1* gene is located in class 1 integrons. On the other hand, *sul2* is found on nonconjugative plasmids or large conjugative plasmids (Antunes et al., 2005). Thus, *sul1* gene is commonly screened in *Salmonella* isolates compared to *sul2*.

2.1.7. Trimethoprim is a synthetic antibiotic, which inhibits the dihydrofolate reductase (DHFR) by connecting to its active site (Van Hoek et al., 2011). DHFR has responsibility of folate biosynthetic system (Sköld, 2001). Resistance to trimethoprim, seen at low level, might be based on antibiotic resistant types of chromosomal *folA* gene producing DHFR (Sköld, 2001). On the other hand, resistance at high frequency might be transferred by plasmid mediated DHFRs. Especially in Gram-negative bacteria, this situation have become critical (Gruneberg, 1972). *dfrA* and *dfrB* genes encode DHFRs. Variations of *dfrA* gene were found in *Salmonella* serovars Typhi, acquired *dfrA7,* and Typhimurium, acquired *dfrA12.* They have shown the resistance to trimethoprim (Shanahan et al., 1978; El-Sharkawy et al., 2017).

2.2. Horizontal Gene Transfer of Antibiotic Resistance

Alterations in the genetic inheritance of a bacterium can be taken place by mutations or acquisition of new genetic materials, such as genes regulating the antimicrobial resistance (Bennett et al., 2008). The widespread usage of antibiotics in food animal production chains, human and veterinary medicine have given rise to increase the antibiotic resistant bacteria, and horizontal gene transfer of antibiotic resistance (Van Meervenne et al., 2012). The acquisition of antibacterial resistance genes by horizontal gene transfer might occur in three ways: transformation, transduction and conjugation (Bennett et al., 2004). Horizontal gene transfer takes place by at least two operations which are the physical transferring of DNA and the association with genome of the recipient in order to get consistent heredity (Stokes and Gillings, 2011). Incorporation mechanisms and autonomous replication enable DNA to sustain itself in genome of the recipient after the transfer mechanism of plasmids occurs. Moreover, transposition does the same for transposons and insertion sequence common regions, while site-specific and homologous recombination apply the same

procedure for integrative and conjugative elements, gene cassettes and integrons (Stokes et al., 2011). Bacterial horizontal gene transfer mechanisms are illustrated in Figure 2. Conjugation is paid regard to main transfer way of plasmids carrying antibiotic resistance genes and conjugative transposons (Wintersdorff et al., 2016). DNA is transferred through the genus and different species by the help of conjugation of plasmids while transformation and transduction are more restricted in transferring gene transfer agents for the same species (Mathur and Singh et al., 2005).

2.2.1. Conjugation

Conjugation is defined as the unmediated transfer of DNA from the donor to recipient bacterium by the help of pilus serving as a bridge between the two contiguous bacterial cells (Singh, 2017). Certain transfer proteins play a crucial role in the formation of pilus and transferring of DNA of the donor bacterium for conjugation. Hence, bacteria with DNA encoding transfer and mobilization genes, are eligible for conjugation. For instance, the majority of *Salmonella* plasmids have *tra* group of transfer genes (Singh, 2017).

The gut microbiota of human and animal, and environment are important reservoirs for antibiotic resistance genes on mobile genetic elements. For example, $bla_{\text{CTX–M}}$ genes have become threat in increasing antibiotic resistance plasmids because of extended-spectrum β-lactamases among Enterobacteriaceae all around the world (Hawkey et al., 2009). In addition to this, sub minimum inhibitory concentrations might be related to spread of resistance genes by promoting the conjugation (Lopez et al., 2007). Hence, the conjugative transfer of plasmids in human and foodborne pathogens has conduced toward to propagate the antimicrobial resistance genes resistant to many antibiotic agents (Huddleston, 2014).

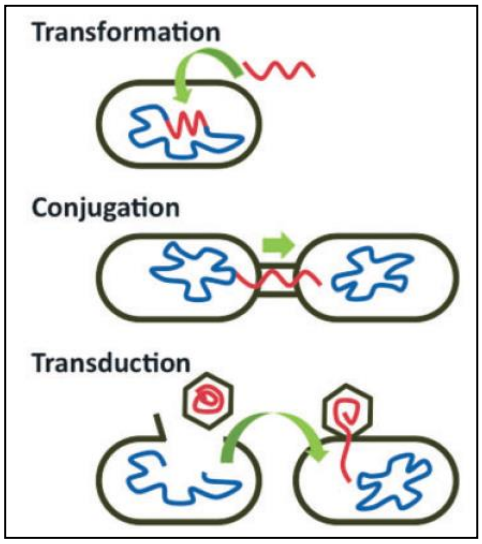

Figure 2 Bacterial horizontal gene transfer mechanisms. Retrieved from Burmeister, A. R. (2015).

2.2.2. Transformation

Griffith has discovered the transformation in which genetic exchange occurs in *Streptococcus pneumoniae* in 1928 (Griffith, 1928). Hotchkiss has exposed antibiotic sensitive strains of *Streptococcus pneumoniae* to DNA of resistant ones in order to get them acquire penicillin and streptomycin resistance (Hotchkiss, 1951). Transformation is expressed as the uptake, integration and functional expression of free extracellular DNA (Wintersdorff, 2016) as shown in Figure 2. The competent recipient bacteria attain extracellular DNA by incorporation into the its genome or recircularisation for transformation mechanism (Thomas et al., 2005).

2.2.3. Transduction

Transduction was found out first among *Salmonella* bacteria (Zinder et al., 1952). Bacteriophages, in other words bacterial viruses, function as natural vectors to move the genes of the host bacteria (Modi et al., 2013) as can be seen in Figure 2. On the other hand, two distinct ways of transduction were expressed. In *Salmonella* phage 22 and *E. coli* phage P1, some viral molecules include a part of host chromosome instead of a reproduced viral genome for generalized transduction (Arber, 2014), while transducing viral particles comprise of a segment of phage genes and some host bacterial genes for specialized transduction (Arber, 2014).

2.3. Plasmid and Integron Mediated Antibiotic Resistance

Plasmids are defined as double-stranded circular DNA elements changing in size, replicating themselves by the help of distinct molecular mechanisms, existing diversified amount of copies per cell (Tolmasky, 2013). Moreover, the host range of plasmids might vary from several genera to wide range of them. The F sex factor encoding the conjugation mechanism is found on the majority of plasmids encoding prevalent transfer (*tra*) function (Hughes and Andersson, 2001). In general, plasmids are assigned to incompatibility (Inc) groups. Incompatibility is expressed as the incapability of the plasmids to be reproduced steadily in the same cell line (Carattoli, 2003). There are four main incompatibility groups determined according to the genetic relevance and pilus structure: IncF, IncP, IncI and Ti plasmid groups. The IncF group is composed of IncF, IncS, IncC, IncD, IncJ, while the IncP group is composed of IncP, IncU, IncM, IncW, and the IncI group is composed of IncI, IncB, IncK. The last group Ti plasmid group is composed of IncX, IncH, IncN and IncT (Waters, 1999). Antimicrobial resistance genes carried by plasmids are usually found on mobile genetic elements, transposons, taking part in the transposase mechanism enabling them to incorporate into chromosome or plasmid (Carattoli, 2003).

Moreover, integrons are genetic mobile elements capturing gene cassettes including resistance genes (Hall, 2013). Integrons encode a site-specific recombinase called as IntI, and gene cassettes are merged into a particular site of integron neighboring on *intI* gene (Hall, 2013). Hence, the integrase encoded by *intI* gene and an *attI* site identified by the integrase demonstrate the two special attributes of the integrons. In addition to this, the IntI integrase might also identify the *attC* sites in the gene cassettes. Reassociation of an *attC* and *attI* site results in assembling of the gene cassette to integron (Hall, 2013). Furthermore, Pc, a promoter, performs the expression of adjoined genes (Gillings, 2017). Integrons might catch more than one gene cassette, and varying the numbers from one to hundreds of gene cassettes might be located on the integrons. Integrons are classified to four groups by taking into consideration the homology of the integrase proteins: Class 1, 2, 3 and 4. Class 1 integrons, the most commonly found, play crucial role in dissemination of antibiotic resistance. Class 1 integrons consist of *intI1* gene acquiring and expressing of gene cassettes, gene cassettes assigning antibiotic resistance, *qacE* conferring resistance to disinfectants as efflux pump, *sul1* providing resistance to sulfonamides, Tn*402* imbedding to plasmids and transposons as *res* hunting transposon, Tn*501* supplying resistance to mercury, mobile and IS elements (Gillings, 2017). Class 1 integron, taking important part in propagating antimicrobial resistance, has been found on the majority of Gram-negative bacteria such as *Salmonella, Escherichia coli* and *Shigella*. The greater part of class 1 integrons includes *aadA* gene, encoding streptomycin-spectinomycin resistance (Deng et al., 2015). In addition to this, genetic elements providing trimethoprim resistance are also found (Fluit et al., 2004). Class 2 integron is generally correlated to Tn7 transposon family involving *attI2* and Pc (Deng et al., 2015). Class 3 integron has included bla_{IMP} gene cassette providing resistance to carbapenems (Arakawa et al., 1995). In addition to this, $bla_{\text{GES-1}}$ within IncQ plasmid has been determined in *Salmonella* spp., *Esherichia coli* and *Serratia marcescens* (Deng et al., 2015; Ploy et al., 2003).

Class 4 integron has firstly been spotted in *Vibrio* isolates. It has been reported that class 4 integron carries gene cassettes encoding chloramphenicol and fosfomycin resistance (Shibata et al., 2003; Fluit et al., 2004).

Effective conjugative transfer of plasmids, genetic elements, from high-frequency recombinant (Hfr) donors demonstrates the consistent integration of F factor. The transfer of DNA starts from the F origin of transfer (*oriT*) through the circular genome from 5' to 3' direction (Gross et al., 1966). Expression of a pilus filament is required for the conjugative transfer of plasmids among Gram-negative bacteria such as *Salmonella* and *Escherichia coli* (Frost, 1993). F-like plasmids are divided to seven Inc groups by considering incompatibility, from IncFI to IncFVII, which is related to replicon(s) of a plasmid. This classifying is applied because the plasmids are located in the same Inc group in case of not being able to consistently coexist in the same host cell (Datta, 1975). In Figure 3, the phases of F-plasmid mediated conjugative transfer of DNA are demonstrated. In **A** part of Figure 3, the mechanism of intercellular contingence of DNA transfer is shown. The contiguity between donor and recipient cells are constructed between F pilus and the surface of recipient cell (Helmuth et al., 1978). The surfaces of donor and recipient cells are connected by the help of depolymerization of filament subunits in order to transfer DNA.

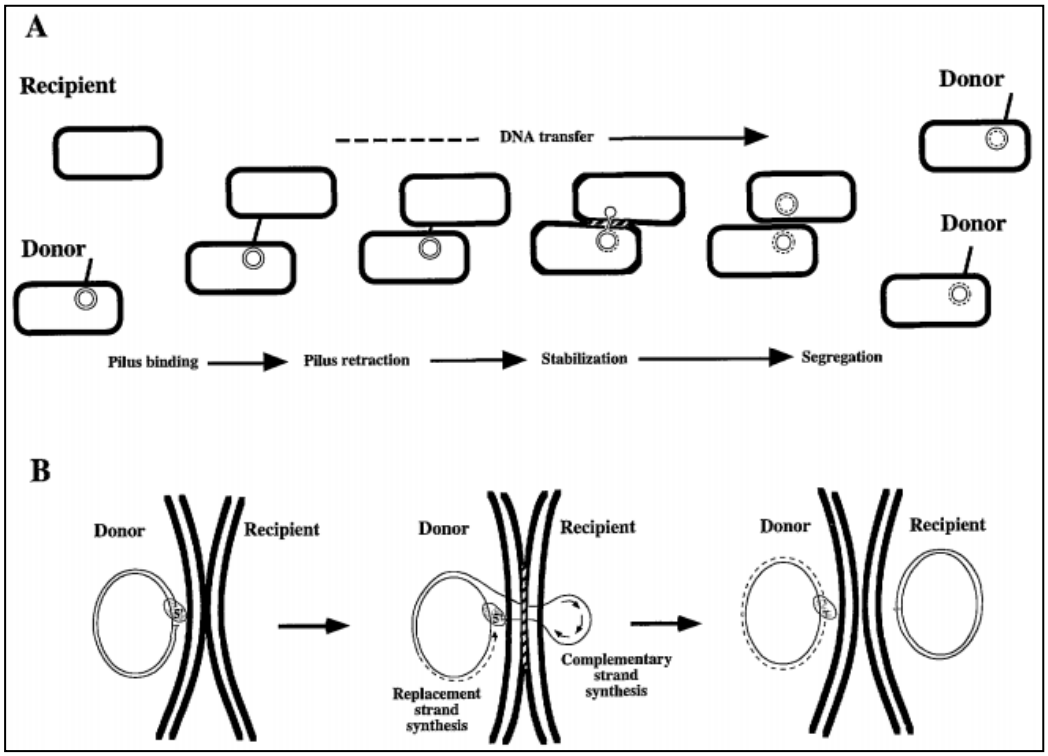

Figure 3 Thedifferentphases of F-plasmid mediated conjugative transfer of DNA. Retrieved from Firth, N., K. Ippen-Ihler, and R. A. Skurray. (1996).

After electron-compacted conjugative joint part is formed, plasmid is transferred following segregation (Firth et al., 1996). On the other side, in **B** part of Figure 3, the contact surfaces of donor and recipient cells are formed by a mating signal transmitted from the pilus to a protein composite involving TraI linked to F-*oriT* site. A single strand of F mediated plasmid DNA is transferred to the recipient cell. F-TraI relaxase/helicase keeps connected to 5' *oriT* end by considering its location at the intercellular interaction site, after nicking. Replacement strand synthesis in the donor cell and complementary strand synthesis in the recipient cell take place while the strand is unstuck from *oriT.* After the recircularization of transferred strand at *oriT*, conjugative transfer mechanism stops.

33.3 kb F *tra*region, regulating gene expression and protein synthesis, play an important role in conjugal DNA transfer and employment as receptor (Firth et al., 1996). For example, *traA* regulates pilus biogenesis in the inner membrane and the extracellulary inner membrane; *traE and traL* regulate pilus biogenesis in inner membrane; *traJ* take part in the regulation of conjugative transfer of plasmids in cytoplasm; *traI* regulates DNA metabolism in cytoplasm (Firth et al., 1996; Frost et al., 1984; Cuozzo et al., 1986; Bradshaw et al., 1990).

2.3.1. Plasmid-Mediated Antibiotic Resistance in *Salmonella* Serovars

Salmonella serovars from food animals and humans have been showing antibiotic resistance to four or more antimicrobials incrementally (Carattoli, 2003). Fluoroquinolones, ampicillin, trimethoprim-sulfamethoxazole or third-generation cephalosporins are effectively used antibiotics for *Salmonella* infections. On the other hand, trimethoprim-sulfamethoxazole and ampicillin are inefficient because of the accelerating resistance. Moreover, in salmonellosis cases of children, fluoroquinolones are not used. Thus, extended-spectrum cephalosporins have been currently used in children infected with *Salmonella* (Acheson and Hohmann, 2001). On the contrary, resistance of *Salmonella* species to extended-spectrum cephalosporins have been emerging all around the world (Threlfall et al., 1997). Plasmid-mediated CMY-2, one of the plasmid-mediated AmpC β-lactamases, was firstly found out on a conjugative plasmid of *Salmonella* serovar Senftenberg (Koeck et al., 1997).Furthermore, *Salmonella* Typhimurium isolated from human and cattle hosts were detected within CMY-2 encoding plasmids (Fey et al., 2000). Plasmids having *cmy-2* gene were classified according to restriction patterns and its hybridisation models. Type A and C plasmids provided resistance to streptomycin, chloramphenicol, tetracycline, sulphonamides, and ceftriaxone provided by CMY-2 β-lactamase.

In addition to this, resistance to β-lactam antibiotics by CMY-2 was acquired by type B plasmids (Carattoli et al., 2002). Resistance to extended spectrum cephalosporins by plasmid-mediated CMY-2 β-lactamase has been found in *Salmonella* serovar Bredeney isolate in Canada (Allen et al., 2002), *Salmonella* serovars Mikawasima and Montevideo isolates in Spain (Navarro et al., 2001), and *Salmonella* serovar Typhimurium isolate in Taiwan and South Korea (Yan et al., 2003; Lee et al., 2014), and *Salmonella* serovar Heidelberg isolate in South America (Cejas et a., 2014). Since ceftiofur, an extended-spectrum cephalosporin, is used in veterinary medicine, cephalosporin resistance in *Salmonella enterica* strains especially isolated from food animals are frequently seen. IncI, IncH and IncF plasmids conferring antibiotic resistance have been mostly determined in *Salmonella* isolates (Rychlik et al., 2006). Resistance of *Salmonella enterica* serovar Typhimurium isolates carrying IncFI plasmids within two class 1 integrons; Int1 including *aadB* and *catB3* genes providing kanamycin and chloramphenicol resistance, and Int2 involving *oxa1* and *aadA1* genes acquiring β-lactams and streptomycin-spectinomycin resistance, to ampicillin, chloramphenicol, kanamycin, streptomycin, spectinomycin, sulfonamides, tetracycline and trimethoprim has been identified in Albania (Tosini et al., 1998). Moreover, *Salmonella* serovars Typhimurium and 4,5,12:i:- carrying IncFIC plasmid comprising *spv*CD genes and antibiotic resistance genes coding ACSSuT (Ampicillin, Chloramphenicol, Streptomycin, Sulphonamides and Tetracycline) have been identified (Llanes et al., 1999; Guerra et al., 2001). On the other side, *Salmonella* Typhimurium phage or definitive type (DT) 104 is an emerging multidrug-resistant pathogen all around the world (Poppe et al., 1998). Furthermore, *Salmonella* Typhimurium definitive type 204c has shown the resistance to gentamicin and apramycin by conjugative transfer of plasmids of I1 incompatibility group (Threlfall et al., 1986).

Unlike the conjugative transfer of plasmids belonging to Typhimurium DT204c and encoding ACSSuT, chromosomally integration of antibiotic resistance genes in Typhimurium DT104 has been determined in despite of the possibility of plasmid origin (Threlfall et al., 1994). In addition to this, acquiring resistance to trimethoprim of Typhimurium DT104 strains has been occured by nonconjugative but mobilizable plasmid which also provide resistance to sulfonamides (Threlfall et al., 1996). The recent study related to global and local epidemiology of *Salmonella* serovar Typhimurium DT104 has revealed details by the help of whole-genome sequencing (WGS). Multidrug-resistance profile of DT104 strains has been formed by the horizontal transfer of the 13- kb *Salmonella* genomic island 1 (SGI1) multidrug-resistance region (Leekitcharoenphon et al., 2016).The majority of *Salmonella enterica* serotype Typhimurium DT104 strains have shown multidrug resistance to streptomycin and spectinomycin, sulfonamides, chloramphenicol and florfenicol, tetracyclines, and β-lactams because of *aadA2*, *sul1*, *floR*, *tetA*(G), and *blaP1*(alternative form $bla_{\text{PSE-1}}$ or $bla_{\text{CARB-2}}$) in return (Levings et al., 2005). Furthermore, SGI1, an integrative mobilizable element, was also determined in *Salmonella enterica* serovars Paratyphi B, DT 120, Agona, Cerro, Derby, Dusseldorf, Infantis, Kiambu (Doublet et al., 2005; Meunier, 2002; Doublet et al., 2004; Levings et al., 2005). *Salmonella enterica* serovar Enteriditis isolates resistant to extended-spectrum cephalosporins by the $bla_{\text{SHV-12}}$ gene found on conjugative plasmids have been observed (Villa et al., 2002). Moreover, pACM1 plasmid from *Klebsiella oxytoca* and pSEM plasmid from *Salmonella* Typhimurium pertain to IncL/M group and acquire resistance to extended-spectrum cephalosporins by $bla_{\text{SHV-5}}$ gene. These plasmids include class 1 integron providing resistance to aminoglycoside, and play a crucial role in spreading the antibiotic resistance among Gram-negative pathogens.

In addition to these, an emerging serotype observed over the last ten years, *Salmonella* Infantis, has showed an extraordinary antibiotic resistance all around the world. For example, *Salmonella* Infantis has indicated plasmid-mediated resistance to third-generation cephalosporins in Belgium (Ceyssens et al., 2015). Moreover, in Hungary, multidrug-resistant *Salmonella* Infantis with the large-sized conjugative plasmid isolated from broiler chickens has been determined (Nógrády et al., 2007). Furthermore, *Salmonella* Infantis isolated from human clinical sources in China has demonstrated antibiotic resistance (Liang et al., 2015).

2.4. The Gut Microbiota As a Reservoir for Antibiotic Resistance Determinants

The human intestine includes various and abundant microbial habitat composed of around 1000 distinct bacterial species and more than 7000 distinct serovars (Bäckhed et al., 2005). These bacteria participate in important biological functions such as physiological processes and immune system (Jernberg et al., 2010). However, antibiotics used for a long time have undesirable effects on normal gut microbiota complex. For example, colonization resistance of the commensal microbiota has been impaired, because of the usage of antibiotics (Jernberg et al., 2010). Dysbiotic gut microbiota might not implement life-sustaining functions such as supplying nutrient, protecting from pathogens and producing vitamin (Guarner et al., 2003). In addition to this, dysbiosis of the gut microbiota has been correlated to metabolic, immunological, developmental diseases and increasing susceptibility to infectious diseases (Langdon et al., 2016). On the other hand, microbiome enables horizontal transfer of antibiotic resistance genes to the latent pathogens while the gut microbiota performs viral functions for the human health (Penders et al., 2013). The intestine possesses moist and warm medium which is rich in regard to nutrients. Thus, this leads to high copy numbers of potential bacterial cells for acquiring resistance, and creates a great gene pool for theresistance factors (Jernberg et al., 2010).

Antibiotic resistance genes might be transferred to pathogens from commensals after commensal bacteria acquire themin the gut microbiota. For instance, transmission of plasmid keeping β-lactamase gene from a resistant *Escherichia coli* serovar to a previously susceptible same strain has been determined in a child cured with ampicillin (Karami et al., 2007). In addition to this, when commensal microbiota not been exposed to antibiotics for minimum one year has been examined by the help of metagenomic way, unique resistance determinants have been found out (Sommer et al., 2009). Hence, new resistant pathogens might occur because of the multifarious gene pool in the gut microbiota of healthy people. Additionally, the recent research has demonstrated that bacteria generating extended-spectrum β-lactamases, and *Escherichia coli* having CTX-M-15 gene have been determined by the shotgun metagenomic sequencing in the gut microbiota of the study individuals not been exposed to antibiotic treatment (Bengtsson-Palme et al., 2015). Moreover, class 1 integrons have been found out in the microbiota of human and food animals at high level, due to selective pressure of antibiotics (Gillings, 2017).

2.5. The Aim of the Thesis

Emerging pathogens, gaining antibiotic resistance, have been threating the human health, veterinary and agriculture all around the world. Plasmids play an important role in the horizontal gene transfer of antimicrobial resistance genes. At this point, the acquisition route of these determinants, and conjugative transfer of plasmids among the Gram-negative pathogens have been uncertain. Furthermore, the effects of antibiotics and resistant pathogens on the gut microbiota are the issue of concern recently. The goals of this thesis were to investigate the horizontal gene transfer based upon plasmids from resistant *Salmonella enterica* subsp. *enterica* serovar Infantis to susceptible commensal *Escherichia coli*, and make contribution to the subsequent studies such as the sequencing of the plasmid metagenome, which targets to find the antibiotic resistance genes in the gut microbiota.

CHAPTER 3

MATERIALS AND METHODS

3.1. Donor *Salmonella enterica* Serovar Infantis Strains and Recipient *Escherichia coli* Strain

Donor bacterial strains isolated from chicken meat were selected according to their antibiotic resistance profiles (ARP) and antibiotic resistance genes located on plasmids. In other words, the compatibilities of screening antibiotic resistance genes on plasmids, and observing their relevant antibiotic targets were the main criteria for the selection of isolates. 10 donor isolates out of 70 *Salmonella* Infantis isolates were selected from the data bank of Food Safety Laboratory, Department of Food Engineering, Middle East Technical University. On the other hand, recipient strain was chosen with regard to antibiotic susceptibility and plasmid-free. PFGE and MLST types of *Salmonella* Infantis isolates were analyzed in the former project (Acar et al., 2017).

Table 2 Phenotypic and genotypic antibiotic resistance profiles of donor strains.

***Salmonella* Infantis Isolates**	**Source**	**Brand**	**Isolation Date**	**PFGE Type**	**MLST Type**	**Antibiotic Resistance**	**Antimicrobial Resistance Genes**
MET-S1-669	Chicken wing meat	A	December, 2012	PT07	ST32	SAmpKfNT	*aadA1*,$bla_{\text{TEM-1}}$, *sul1*
MET-S1-774	Chicken rib meat	B	February, 2015	PT08	ST32	SfSxtKSNT	*sul1*, *aphA1-lab*, *aadA1*, *tetA*
MET-S1-777	Chicken thigh meat	C	February, 2015	PT50	ST32	SfSxtSCipNT	*sul1*, *aadA1*
MET-S1-782	Chicken wing meat	C	February, 2015	PT08	ST32	SfSxtKSNT	*sul1*, *aphA1-lab*, *aadA1*, *tetA*
MET-S1-785	Chicken thigh meat	C	February, 2015	PT08	ST32	SfSxtCSNT	*sul1*, *aadA1*, *tetA*
MET-S1-788	Chicken breast meat	D	February, 2015	PT08	ST32	SfSxtCSCipNT	*sul1*,*aadA1*, *tetA*
MET-S1-792	Chicken heart meat	E	February, 2015	PT08	ST32	SfSxtSNT	*sul1*, *aadA1*, *tetA*
MET-S1-798	Chicken heart meat	E	February, 2015	PT08	ST32	SfSxtCSNT	*sul1*, *aadA1*, *tetA*
MET-S1-801	Chicken breast meat	E	February, 2015	PT08	ST32	SfSxtCKSNT	*sul1*, *aphA1-lab*, *aadA1*, *tetA*
MET-S1-804	Chicken wing meat	E	February, 2015	PT08	ST32	SfSxtCKNT	*sul1*, *aphA1-lab*, *aadA1*, *tetA*

S: Streptomycin, Sf: Sulfisoxazole, Amp: Ampicillin, Sxt: Trimethoprim/sulfamethoxazole, K: Kanamycin, S: Streptomycin, C: Chloramphenicol, Cip:Ciprofloxacin, Kf: Cephalotin, N: Nalidixic acid, T: Tetracycline.

Table 3 Phenotypic and genotypic antibiotic resistance profiles of recipient strain.

Recipient *Escherichia coli* Strain	Source	Isolation Date	Antibiotic Resistance	Antimicrobial Resistance Genes
MET-A1-018	Chicken meat	February, 2015	*Susceptible	$bla_{\text{TEM-1}}$, *aphA1-lab*

*Susceptible: Recipient strain showed susceptibility to all antibiotics used in this study. Antibiotics were given below.

S: Streptomycin, Sf: Sulfisoxazole, Amp: Ampicillin, Sxt: Trimethoprim/sulfamethoxazole, K: Kanamycin, S: Streptomycin, C: Chloramphenicol, Cip:Ciprofloxacin, Kf: Cephalotin, N: Nalidixic acid, T: Tetracycline, Cro: Ceftriaxone, Eft: Ceftiofur, Imp: Imipenem, Ak: Amikacin, Cn: Gentamicin, Amc: Amoxicillin/clavulanic acid, Fox: Cefoxitin, Etp: Ertapenem.

3.2. Chemicals

Chemicals used in this study were listed in Appendix A.

3.3. Buffers and Solutions

Preparation of buffers and solutions was explained in Appendix B.

3.4. Disc Diffusion Method

In order to determine the antibiotic susceptibilities of *Salmonella* and *E.coli* isolates, disc diffusion method was done. The cultures of *Salmonella* and *E. coli* were incubated individually in 4 ml of Mueller-Hinton broth at 37^{o}C for 18 hours. In proportion to 1:100, dilution was done after the incubation process. Diluted cultures were spread onto the Mueller-Hinton agars. Paper discs including antibiotics were placed to the surface of Mueller-Hinton agar. They were incubated at 37^{o}C between 16 and 18 hours. Antibiotics specified in Table 4 were used for the disc diffusion method. *E. coli* ATCC 25922 was used as a quality control strain. According to the Clinical Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST), limits given in Table 4 were followed.

Table 4 Zone diameter standards to determine antibiotic susceptibility of *Salmonella* and *E. coli* by disc diffusion method.

Classification	Antibiotic	Disc Content	Zone diameter (mm)		
		(µg)	Susceptible	Intermediate	Resistant
Aminoglycosides	Amikacin [1]	30	≥17	15-16	≤14
	Gentamicin [1]	10	≥15	13-14	≤12
	Kanamycin [1]	30	≥18	14-17	≤13
	Streptomycin [1]	10	≥15	12-14	≤11
β-lactams	Ampicillin [1]	10	≥17	14-16	≤13
	Ceftiofur[2]	30	≥21	18-20	≤17
	Cefoxitin [1]	30	≥18	15-17	≤14
	Ceftriaxone [1]	30	≥23	20-22	≤19
	Cephalothin [1]	30	≥18	15-17	≤14
	Amoxicillin-clavulanic acid [1]	20/10	≥18	14-17	≤13
	Ertapenem [1]	10	≥23	20-22	≤19
	Imipenem [1]	10	≥23	20-22	≤19
Phenicols	Chloramphenicol[1]	30	≥18	13-17	≤12
Quinolones	Nalidixic acid [1]	30	≥19	14-18	≤13
Fluoroquinolones	Ciprofloxacin [1]	5	≥21	16-20	≤15
Tetracyclines	Tetracycline [1]	30	≥15	12-14	≤11
Sulfonamides	Trimethoprim-sulfamethoxazole[1]	1.25/23.75	≥16	11-15	≤10
	Sulfisoxazole [1]	300	≥17	13-16	≤12

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

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

3.5. Confirmation of Antibiotic Resistance Genes on *Salmonella* and *E. coli* Isolates

After the antibiotic resistance profiles of *Salmonella* and *E. coli* isolates were determined at phenotypic level, their genotypic antibiotic resistance profiles were analyzed by using purified *Salmonella* and *E. coli* DNA (Aydin, 2018). PCR master mix was prepared by using double distilled water, 10X PCR buffer, $MgCI_2$, dNTPs, forward and reverse primers (shown in Table 5), and Tsg DNA polymerase (Lamda Biotech) with reference to required volumes shown in Table 5. 98 µl of PCR master mix was transferred from 1.5 ml Eppendorf tube to 0.2 ml PCR tube. Thereafter, for each *Salmonella* and *Escherichia coli* isolate, 2 µl of purified DNA was added individually including negative and positive control. Prepared PCR tubes were put into thermocycler (US and T100 Thermal Cycler, Bio-Rad, CA, US) by considering the procedure specified in Table 8. After 5 µl of each DNA sample was mixed with 1 µl of 6X Loading dye, they were inserted into the 1.7% agarose gel, and plasmid DNA samples were run by 100 Volts for 45 minutes.

Table 5 PCR master mix.

PCR Substances (Concentration)	Volume (µl)
ddH_2O	71.50
10X PCR Buffer	10.00
$MgCI_2$ (25mM)	6.00
dNTPs (10 mM)	2.00
*Forward Primer (12.5µM)	4.00
*Reverse Primer (12.5µM)	4.00
Tsg DNA Polymerase	0.50
Total	98.00

*: The primer sequences were given in Table 7.

Table 6 Locations and antibiotic targets of the antibiotic resistance genes used in this study.

Genes	Location	Resistance to Antibiotic	Reference
Resistance to β-lactams			
blaTEM-1	Plasmid	Class A β-lactam	Chen et al., 2004
blaPS1E-1	Chromosome	Class A β-lactam	Chen et al., 2004
blaCMY-2	Plasmid	Ceftiofur, Ceftriaxone	Chen et al., 2004
ampC	Plasmid	β-lactams	Pérez-Pérez et al., 2002
Resistance to Chloramphenicol			
cat1	Plasmid	Chloramphenicol	Chen et al., 2004
cat2	Plasmid	Chloramphenicol	Chen et al., 2004
flo	Chromosome	Chloramphenicol	Chen et al., 2004
cmlA	Plasmid	Chloramphenicol	Chen et al., 2004
Resistance to Aminoglycosides			
aadA1	Plasmid	Streptomycin	Randall et al., 2004
aadA2	Chromosome	Streptomycin	Randall et al., 2004
strA	Plasmid	Streptomycin	Gebreyes et al., 2002
strB	Plasmid	Streptomycin	Gebreyes et al., 2002
aacC2	Plasmid	Gentamicin, Kanamycin	Chen et al., 2004
aphA1-Iab	Plasmid	Kanamycin	Frana et al., 2001
Resistance to Trimethoprim			
dhfrI	Plasmid	Trimethoprim	Chen et al., 2004
dhfrXII	Plasmid	Trimethoprim	Chen et al., 2004
Resistance to Sulfonamide			
sul1	Chromosome	Sulfonamide	Chen et al., 2004
sul2	Plasmid	Sulfonamide	Chen et al., 2004
Resistance to Tetracycline			
tetA	Plasmid	Tetracycline	Chen et al., 2004
tetB	Plasmid	Tetracycline	Chen et al., 2004
tetG	Chromosome	Tetracycline	Chen et al., 2004

Table 7 The sequences and binding temperatures of primers.

Gene	Primer Sequence	Binding Temperature (°C)	Reference
blaTEM-1	F: CAGCGGTAAGATCCTTGAGA	53.9	Chen et al., 2004
	R: ACTCCCCGTCGTGTAGATAA		
blaPS1E-1	F: TGCTTCGCAACTATGACTAC	52.4	Chen et al., 2004
	R: AGCCTGTGTTTGAGCTAGAT		
blaCMY-2	F: TGGCCGTTGCCGTTATCTAC	60.8	Chen et al., 2004
	R: CCCGTTTTATGCACCCATGA		
ampC	F: AACACACTGATTGCGTCTGAC	60	Pérez-Pérez et al., 2002
	R: CTGGGCCTCATCGTCAGTTA		
cat1	F: CTTGTCGCCTTGCGTATAAT	Touchdown 55-45	Chen et al., 2004
	R: ATCCCAATGGCATCGTAAAG		
cat2	F: AACGGCATGATGAACCTGAA	60	Chen et al., 2004
	R: ATCCCAATGGCATCGTAAAG		
flo	F: CTGAGGGTGTCGTCATCTAC	54.4	Chen et al., 2004
	R: GCTCCGACAATGCTGACTAT		
cmlA	F: CGCCACGGTGTTGTTGTTAT	58.5	Chen et al., 2004
	R: GCGACCTGCGTAAATGTCAC		
aadA1	F: TATCAGAGGTAGTTGGCGTCAT	53.6	Randall et al., 2004
	R: GTTCCATAGCGTTAAGGTTTCATT		
aadA2	F: TGTTGGTTACTGTGGCCGTA	57.3	Randall et al., 2004
	R: GATCTCGCCTTTCACAAAGC		
strA	F: CTTGGTGATAACGGCAATTC	51.8	Gebreyes et al., 2002
	R: CCAATCGCAGATAGAAGGC		
strB	F: ATCGTCAAGGGATTGAAACC	57	Gebreyes et al., 2002
	R: GGATCGTAGAACATATTGGC		
aacC2	F: GGCAATAACGGAGGCAATTCGA	57.9	Chen et al., 2004
	R: CTCGATGGCGACCGAGCTTCA		
aphA1-Iab	F: AAACGTCTTGCTCGAGGC	54	Frana et al., 2001
	R: CAAACCGTTATTCATTCGTGA		
dhfrI	F: CGGTCGTAACACGTTCAAGT	51.7	Chen et al., 2004
	R: CTGGGGATTTCAGGAAAGTA		
dhfrXII	F: AAATTCCGGGTGAGCAGAAG	57.9	Chen et al., 2004
	R: CCCGTTGACGGAATGGTTAG		
sul1	F: TCACCGAGGACTCCTTCTTC	55.6	Chen et al., 2004
	R: CAGTCCGCCTCAGCAATATC		
sul2	F:CCTGTTTCGTCCGACACAGA	56	Chen et al., 2004
	R: GAAGCGCAGCCGCAATTCAT		
tetA	F: GCGCCTTTCCTTTGGGTTCT	57.7	Chen et al., 2004
	R: CCACCCGTTCCACGTTGTTA		
tetB	F: CCCAGTGCTGTTGTTGTCAT	58.4	Chen et al., 2004
	R: CCACCACCAGCCAATAAAAT		
tetG	F: AGCAGGTCGCTGGACACTAT	60	Chen et al., 2004
	R: CGCGGTGTTCCACTGAAAAC		

F: Forward primer., R: Reverse primer.

Table 8 Polymerase chain reaction (PCR) amplification conditions.

One hold at 94.0°C for 8 minutes	**(**1 cycle)
.....	
35 cycles of the following:	
At 94.0°C for 30 seconds	
At X*°C for 30 seconds	(30 cycles)
At 72.0°C for 30 seconds	
.....	
One hold at 72.0°C for 5 minutes	**(**1 cycle)
One hold at 4.0°C	**(**∞)

X*: Annealing temperature changes with regard to the each primer binding temperature given in Table 7.

3.6. Plasmid Isolation from *Salmonella* and *Escherichia coli*

Plasmid isolation from *Salmonella* and conjugated *E. coli* isolates was performed by using QIAGEN Plasmid Mini Kit. *Salmonella* colonies reflect red with black centres, while *Escherichia coli* colonies reflect yellow color on XLD agar. Plasmid isolation from *Salmonella* isolates was done following the same steps of the plasmid isolation from *E. coli*.

Each single colony of *E. coli* was selected from XLD agar containing 10 µg/ml tetracycline, and incubated in 3 ml of LB broth involving 10 µg/ml tetracycline at 300 rpm and 37ºC for 8 hours. 6 µl of the inoculated culture was put on 3 ml of LB broth comprised of tetracycline for 16 hours at 37ºC at 300 rpm. Henceforth, bacterial cells were centrifuged at 8700 rpm and 4ºC for 15 minutes. Each bacterial pellet was resuspended with 300 µl of Buffer P1 (Qiagen Plasmid Mini Kit) by vortexing. After that, 300 µl of Buffer P2 (Qiagen Plasmid Mini Kit) was supplemented to former ones by vigorously inverting at 22ºC for 5 minutes. Refrigerated Buffer P3 (Qiagen Plasmid Mini Kit) at 4ºC was added, and then the solutions were instantly inverted and incubated on ice for 5 minutes. In order to get supernatants involving plasmid DNA, they were centrifuged for 10 minutes at 15000 rpm. The supernatants were individually applied to QIAGEN-tip 20 equilibrated with 1 ml of Buffer QBT (Qiagen Plasmid Mini Kit) before by gravity flow. DNA samples of each mated bacterial cell were eluted using 800 µl of Buffer QF (Qiagen Plasmid Mini Kit) heated to 65ºC previously, after the QIAGEN-tip 20 were washed with 4 ml of Buffer QC (Qiagen Plasmid Mini Kit) by gravity flow. DNA samples of each cell were precipitated by adding 560 µl of isopropanol (615080, Sigma-Aldrich) at 22ºC and instantly centrifuged at 15000 rpm and 4ºC for 30 minutes. Next, DNA pellets were washed with 1 ml of 70% ethanol (16368, Sigma-Aldrich) and centrifuged at 13700 rpm for 10 minutes. The pellets were dried at 22ºC for 8 minutes and dissolved in 100 µl of pH 8.0 Tris-EDTA (TE) buffer solution, after the supernatants were removed with caution.

3.7. Determination of the Plasmid Incompatibility Group

After the plasmid isolation of the donor *Salmonella* Infantis and recipient strain *E. coli* isolates, plasmid incompatibility groups of them were screened by PCR following conditions in Table 11. In order to screen plasmid incompatibility groups, PCR master mix was prepared by using double distilled water, MyTaq Red Mix, 2x, forward and reverse primers, and Tsg DNA polymerase (Lamda Biotech) with reference to required volumes shown in Table 9. 49 µl of PCR master mix was transferred from 1.5 ml Eppendorf tube to 0.2 ml PCR tube. After that, 1 µl of *Salmonella* Infantis, and *Escherichia coli* plasmid DNA was added individually including negative and positive control. Prepared PCR tubes were put into thermocycler (US and T100 Thermal Cycler, Bio-Rad, CA, US) by considering the procedure. 5 µl of each DNA sample was inserted into the 1.7% agarose gel, and the samples were run by 100 Volts for 45 minutes.

Table 9 PCR master mix.

PCR Substances (Concentration)	**Volume (µl)**
ddH_2O	36.50
MyTaq Red Mix, 2x	10.00
*Forward Primer (20 mM)	1.00
*Reverse Primer (20 mM)	1.00
Tsg DNA Polymerase	0.50
Total	49.00

*: The sequences of the primers were given in Table 10.

Table 10 Primer sequences, product sizes in bp, and the annealing temperatures of the different types of plasmids (Carattoli et al., 2005).

*IG	Primer Sequences	Product Size (bp)	Annealing Temperature (ºC)
A/C	F: GAGAACCAAAGACAAAGACCTGGA R: ACGACAAACCTGAATTGCCTCCTT	465	60
B/O	F: GCGGTCCGGAAAGCCAGAAAAC R: TCTGCGTTCCGCCAAGTTCGA	159	60
FIA	F: CCATGCTGGTTCTAGAGAAGGTG R: GTATATCCTTACTGGCTTCCGCAG	462	60
FIB	F: GGAGTTCTGACACACGATTTTCTG R: CTCCCGTCGCTTCAGGGCATT	702	60
FIC	F: GTGAACTGGCAGATGAGGAAGG R: TTCTCCTCGTCGCCAAACTAGAT	262	60
FII_S	F: CTGTCGTAAGCTGATGGC R: CTCTGCCACAAACTTCAGC	270	60
F_{repB}	F: TGATCGTTTAAGGAATTTTG R: GAAGATCAGTCACACCATCC	270	52
HI1	F: GGAGCGATGGATTACTTCAGTAC R: TGCCGTTTCACCTCGTGAGTA	471	60
HI2	F: TTTCTCCTGAGTCACCTGTTAACAC R: GGCTCACTACCGTTGTCATCCT	644	60
I1	F: CGAAAGCCGGACGGCAGAA R: TCGTCGTTCCGCCAAGTTCGT	139	60
K/B	F: GCGGTCCGGAAAGCCAGAAAAC R: TCTTTCACGAGCCCGCCAAA	160	60
L/M	F: GGATGAAAACTATCAGCATCTGAAG R: CTGCAGGGGCGATTCTTTAGG	785	60
N	F: GTCTAACGAGCTTACCGAAG R: GTTTCAACTCTGCCAAGTTC	559	60
P	F: CTATGGCCCTGCAAACGCGCCAGAAA R: TCACGCGCCAGGGCGCAGCC	534	60
T	F: TTGGCCTGTTTGTGCCTAAACCAT R: CGTTGATTACACTTAGCTTTGGAC	750	60
W	F: CCTAAGAACAACAAAGCCCCCG R: GGTGCGCGGCATAGAACCGT	242	60
X	F: AACCTTAGAGGCTATTTAAGTTGCTGAT R: GAGAGTCAATTTTTATCTCATGTTTTAGC	376	60
Y	F: AATTCAAACAACACTGTGCAGCCTG R: GCGAGAATGGACGATTACAAAACTTT	765	60

*IG: Incompatibility Group, F: Forward primer, R: Reverse primer.

Table 11 Polymerase chain reaction (PCR) amplification conditions.

One hold at 95.0^{o}C for 1 minute	**(**1 cycle)
.....	
30 cycles of the following:	
At 95.0^{o}C for 15 seconds	
At X*^{o}C for 15 seconds	(30 cycles)
At 72.0^{o}C for 10 seconds	
.....	
One hold at 72.0^{o}C for 5 minutes	**(**1 cycle)
One hold at 4.0^{o}C	**(**∞)

X*: Annealing temperature changes with regard to the each primer binding temperature given in Table 10.

3.8. Conjugation Procedure

Stored donor and recipient cells were taken from -80ºC freezer (Thermo Fisher Scientific, US), and each isolate was streaked on BHI agar plate. They were incubated for 24 hours at 37ºC. Then, one colony of each isolate was picked. Picked donor *Salmonella enterica* Infantis strains and recipient *Escherichia coli* were incubated in brain heart infusion (BHI) broth (CM1136, Oxoid, Thermo Fisher Scientific Inc.) at 150 revolutions per minute (rpm) at 37ºC. Each donor *Salmonella* Infantis and recipient *E. coli* were mated in proportion to 1:10 in the unit of milliliter (ml) respectively. Each mated bacterial culture was filtrated through sterile mixed cellulose ester filters (0.45μm) (HAWP04700, MF-Millipore membrane filter, Merck) by using vacuum filter. Afterwards, 3 ml of sterilised buffer peptone water was applied to filtrated mated culture in order to provide the cells to attach the filter. After this stage, the membrane filters were incubated on Luria-Bertani (LB) agar for 24 hours at 37ºC by taking into consideration the optimal growth conditions of the recipient cell. The incubated mated bacterial cells were elutriated by the help of 3 ml of sterilised buffer peptone water. Obtained bacterial cell solutions were diluted. Dilutions of each mating culture were spread onto xylose-lysine-desoxycholate (XLD) agar (CM0469, Oxoid, Thermo Fisher Scientific Inc.) plates including 10 μg/ml tetracycline, and incubated for 48 hours at 37ºC. Moreover, the donor and recipient bacterial cells were individually spread onto selective XLD agar containing tetracycline as the control groups.

3.9. Confirmation of Conjugation Genes Located on Plasmids

In order to confirm the genes participating in conjugative transfer, PCR master mix was confected by using double distilled water, 10X PCR buffer, $MgCI_2$, dNTPs, forward and reverse primers (shown in Table 12), and Tsg DNA polymerase (Lamda Biotech) with reference to required volumes illustrated in Table 12. 49 µl of PCR master mix was transferred from 1.5 ml Eppendorf tube to 0.2 ml PCR tube. Thereafter, for each *Salmonella* and conjugated *Escherichia coli* isolates, 1 µl of plasmid DNA was added individually including negative and positive control. Prepared PCR tubes were put into thermocycler (US and T100 Thermal Cycler, Bio-Rad, CA, US) by considering the procedure specified in Table 14. After 5 µl of each DNA sample was mixed with 1 µl of 6X Loading dye, they were inserted into the 1.7% agarose gel, and plasmid DNA samples were run by 100 Volts for 45 minutes.

Table 12 PCR master mix.

PCR Substances (Concentration)	Volume (µl)
ddH_2O	30.75
10X PCR Buffer	10.00
$MgCI_2$ (25mM)	3.00
dNTPs (10 mM)	1.00
*Forward Primer (20 mM)	2.00
*Reverse Primer (20 mM)	2.00
Tsg DNA Polymerase	0.25
Total	49.00

*: The sequences of the primers were given in Table 13.

Table 13 Genes taking part in conjugative transfer, sequences of primers, functions of the conjugative genes, and annealing temperatures of the genes.

Genes	Primer Sequences	Functions of the genes	*AT (ºC)	References
traA	F: TGCCCCCTGCGGCGTTAGTA R: TTGCCAACGCAGCCGCTGAT	Pilus biogenesis		
traE	F: TGATGCGGCAAGTGCGACAA R: AGCGCGGCGAGATGCTTGAG	Pilus biogenesis	65.0	Wang et al., 2011
traL	F: AGCGCATCGCTACAAGCACA R: ACGACAACCCCACCGAGGCT	Pilus biogenesis		
traJ	F: GCTTTACGACCACCGTCATT R: CCTGTCATCAGGGATTCGAT	Regulation of conjugative transfer	55.0	Peterson et al., 2011
traG	F:CTGTCCATAACGACGGGTTC R: TCGGATAAAAGCGGAATCAC	Pilus biogenesis and aggregate stabilization	55.0	Peterson et al., 2011
traH	F: GGACGTGAAGGTTGACTGGT R: GACTGGGAAGGTGATGCAAT	Pilus biogenesis	55.0	Peterson et al., 2011
traI	F: TTGTCTTCCTTCCTGCCATC R: TGAACGCTTTGTCAGCAATC	DNA metabolism	55.0	Peterson et al., 2011
traM	F: AATATTCGCGCTCCACATTC R: AACAGCGGGCAAATAATGTC	DNA metabolism	55.0	Peterson et al., 2011
traR	F: TCGACATTGCGAACCATATC R: GCCGGAGCAAACTGACTAAG	Unknown function	55.0	Peterson et al., 2011
traY	F: TGCGACGAAACTCAGTATGC R: GGAAGCATGTTCTGGGTGTT	DNA metabolism	55.0	Peterson et al., 2011
korA	F: GGAACGTTTGTAYCTTGTATTG R: ACTCACTATCTTCTGTTGATTG	Regulation of conjugation	60.0	García-Fernández et al., 2011
rfaG	F: GGATGCTATTGCACGCGGCTG R:TCACCTGCCTGCAGGCTCATAGC AGGCTGTCCAAA	Regulation of the structure of lipopolysaccharide	63.5	Kong et al., 2011
rfaI	F: GTATGGTTGGCAAAGCGCGC R:TATGGGTTCCTGCAGGAGTGATC ACTTTTGTAATTTC	Regulation of the structure of lipopolysaccharide	58.0	Kong et al., 2011
rfaJ	F:AGTGAATAAAGCGCGTTTTG R:GAGGGGAACCTGCAGGTACATC ACCTATGGGTTTTAT	Regulation of the structure of lipopolysaccharide	54.0	Kong et al., 2011
rfbP	F:CAACTGATAAAAGTCAATCC R:GTAAGCTTACCTGCAGGTTAATC CTCACCCTCTGA	Regulation of the structure of lipopolysaccharide	60.0	Kong et al., 2011
rfaL	F:GTGGGCAACACCGGATTACGG R:GCGTTTTTTTACTTTTCTCCACAA TAGGTTTGG	Regulation of the structure of lipopolysaccharide	56.0	Kong et al., 2011

*AT: Annealing Temperature, F: Forward Primer, R: Reverse Primer.

Table 14. Polymerase chain reaction (PCR) amplification conditions.

One hold at 94.0°C for 8 minutes	**(**1 cycle)
.....	
35 cycles of the following:	
At 94.0°C for 30 seconds	
At X*°C for 30seconds	(35 cycles)
At 72.0°C for 30 seconds	
.....	
One hold at 72.0°C for 5 minutes	**(**1 cycle)
One hold at 4.0°C	**(**∞)

X*: Annealing temperature changes with regard to the each primer binding temperature given in Table 13.

3.10. Counting Colonies of *Salmonella* and Conjugated *E. coli*

After the conjugation procedure, *Salmonella* and conjugated *E. coli* colonies on XLD agars containing 10^{-5} diluted mating solutions for each isolate were counted in order to calculate the conjugation frequency, which is from 0 to 1. Conjugation frequency 0 means that there is no conjugation between donor and recipient, while 1 means that all recipient cells require the plasmid. Conjugation efficiency was calculated as the proportion of transconjugants (*E. coli*) to the total colony forming units of the bacterial cells on the selective XLD agar by using the information given in Table 15.

3.11. Statistical Analysis

Two-way ANOVA was applied to the replicates given in Table 15 to find whether there is a significant difference or not between replicates of transconjugant *Escherichia coli*and *Salmonella enterica* serovar Infantis isolates . In addition to this, one-way ANOVA was used to determine the standard error in the conjugation frequency of each transconjugant *Escherichia coli* isolate.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Phenotypic Conjugation Results of *Escherichia coli* Isolates

Salmonella spp. are observed as red colonies with black centres, while *Escherichia coli* spp. are seen as yellow colonies on Xylose-Lysine-Desoxycholate (XLD) agar. Conjugated *E. coli* isolates were seen phenotypically on XLD agars as yellow colonies after the incubation for 48 hours at 37ºC. *Salmonella enterica* serovar Infantis colonies were observed as red colonies with black centers. In Figure 4, *E. coli* conjugated with MET-S1-792 *Salmonella* Infantis isolate was shown. Other transconjugant *E. coli* isolates were illustrated in Appendix C.

Figure 4 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-792+MET-A1-018).

Two-way ANOVA with replication was done to find out whether there is any significant difference or not among the replications consisting of two independent variables. The results have demonstrated that there were not any significant differences in samples and interactions ($F<F_{CRITICAL}$ and *p*-value>0.05). In order to find the conjugation efficiency, colony forming units (CFUs) of donors (*Salmonella enterica* serovar Infantis) and recipients (*Escherichia coli*) were counted with the replicates as can be seen in Table 15.

In addition to this, conjugation frequency of each mated bacterial cell has been demonstrated with standard errors by applying one-way ANOVA in Figure 5 with the information supplied in Table 15.

Table 15 The counts of donor (*Salmonella enterica* serovar Infantis) bacterial cells and transconjugant recipient (*Escherichia coli*) bacterial cells on XLD agar including 10 µg/ ml tetracycline.

Mated Bacterial Cells (*Salmonella* Infantis + *E. coli*)	1st Replicate, CFUs of *Salmonella* × 10^5	1st Replicate, CFUs of *E. coli* × 10^5	2nd Replicate, CFUs of *Salmonella* × 10^5	2nd Replicate, CFUs of *E. coli* × 10^5	3rd Replicate, CFUs of *Salmonella* × 10^5	3rd Replicate, CFUs of *E. coli* × 10^5	4th Replicate, CFUs of *Salmonella* × 10^5	4th Replicate, CFUs of *E. coli* × 10^5
MET-S1-669+MET-A1+018	30	10	33	15	38	17	42	20
MET-S1-774+MET-A1+018	100	80	80	63	96	64	85	70
MET-S1-777+MET-A1+018	100	50	72	17	85	31	80	37
MET-S1-782+MET-A1+018	65	9	32	8	54	9	70	12
MET-S1-785+MET-A1+018	50	20	40	10	45	12	48	13
MET-S1-788+MET-A1+018	50	1	30	3	44	8	38	7
MET-S1-792+MET-A1+018	40	62	30	16	26	10	35	17
MET-S1-798+MET-A1+018	50	60	27	15	46	32	39	22
MET-S1-801+MET-A1+018	20	25	40	34	28	18	32	24
MET-S1-804+MET-A1+018	70	10	30	2	45	6	60	11

*CFUs: Colony Forming Units.

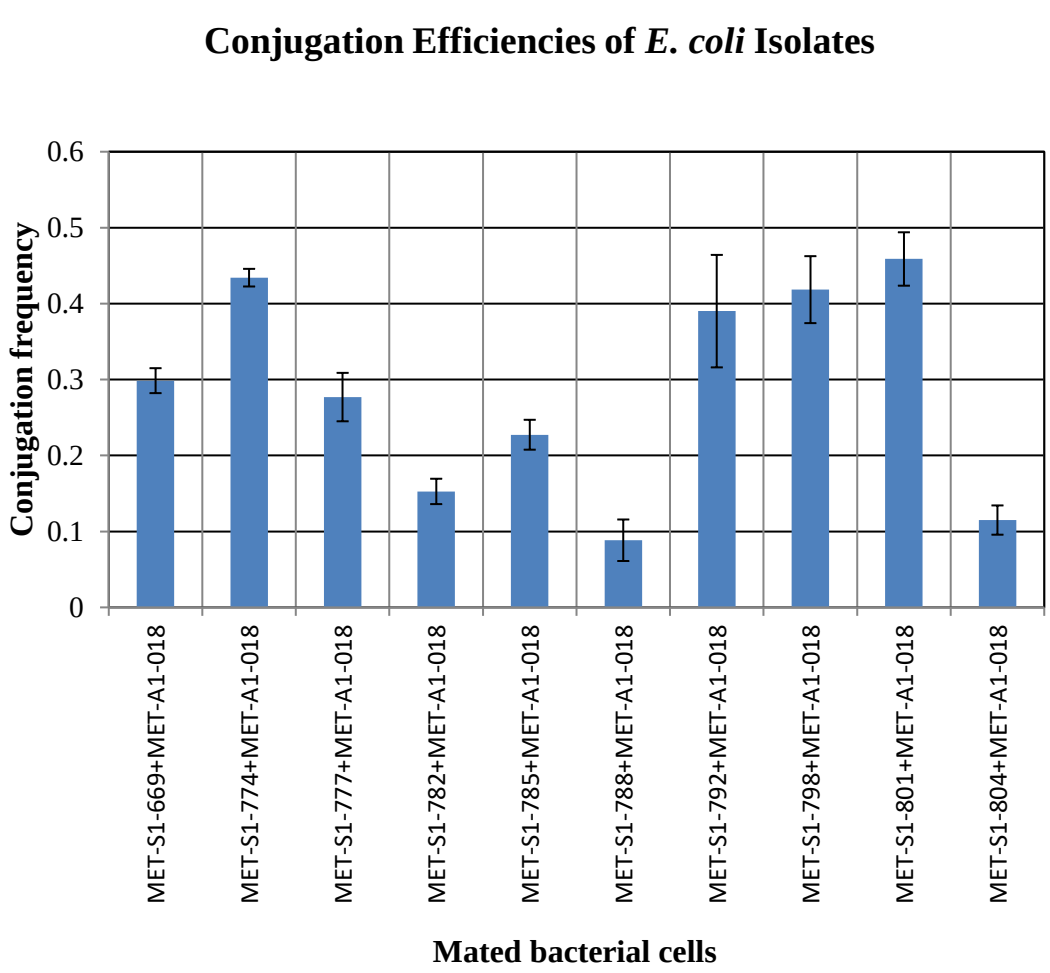

Figure 5 Conjugation frequency values of transconjugant *E. coli* isolates with each mated *Salmonella enterica* serovar Infantis strain.

Table 16 Conjugation frequency values with standard errors of each mated bacterial cell.

***Salmonella* Infantis Isolates Used for Mating**	**Conjugation Frequency ± SE**
MET-S1-669	0.2986 ± 0.0164
MET-S1-774	0.4341 ± 0.0116
MET-S1-777	0.2769 ± 0.0319
MET-S1-782	0.1527 ± 0.0167
MET-S1-785	0.2273 ± 0.0197
MET-S1-788	0.0884 ± 0.0273
MET-S1-792	0.3901 ± 0.0740
MET-S1-798	0.4184 ± 0.0441
MET-S1-801	0.4588 ± 0.0352
MET-S1-804	0.1150 ± 0.0193

Conjugative transfer of antibiotic resistance genes from *Salmonella enterica* serovar Infantis to commensal *Escherichia coli* has been observed in both of phenotypic and genotypic ways given in the result part of the thesis. Moreover, the conjugation efficiencies have differed from each mated bacterial cells. For example, the transformation frequencies of *E. coli* mated with MET-S1-801, 774, 798 and 792 have been observed higher compared to *E. coli* mated with MET-S1-669, 777, 785 in Table 16. On the other hand, the least conjugation frequencies have been determined in *E. coli* mated with MET-S1-782, 788, 804. The reasons of the differences in transfer frequencies of transconjugant *E. coli* samples might be based on physical, chemical and biological conditions (Andrup and Andersen, 1999). In other words, conjugative transfer of genetic element in *Escherichia coli* is not reciprocal (Griffiths et al., 2000), so at this point, *Salmonella* Infantis isolates acting as donor cells play important roles in affecting conjugative transfer frequency.

The highest conjugation frequency was determined in *E. coli* mated with MET-S1-801, while the least one was detected in *E. coli* mated with MET-S1-804. Although, the genotypic antibiotic resistance profiles of MET-S1-801 and MET-S1-804 are the same, MET-S1-801 has showed extra phenotypic resistance to streptomycin (S) differently from the former isolate. At this point, streptomycin might significantly affect the conjugation frequency. Even though MET-S1-782 has the same genotypic antibiotic resistance profile and has lacked resistance to chloramphenicol (C) compared to MET-S1-801, there is a huge difference in conjugation frequencies. Furthermore, finding high conjugation frequency between an emerging strain, *Salmonella* Infantis, and commensal *E. coli* means higher spreading frequency of multidrug- resistance genes among the bacteria. Thus, serious health problems might occur in humans and animals infected with *Salmonella* Infantis. In order to understand in detail why conjugation frequency considerably varies, more molecular studies are required.

4.2. Genotypic Screening of Conjugation In *Escherichia coli* Isolates

Transfer genes, including *traA, traE, traL, traJ, traG, traH, traI, traM, traR, traY, korA, rfaG, rfaI, rfaJ, rfbP* and *rfaL*, were screened on all of the transconjugant *Escherichia coli* isolates mated with *Salmonella enterica* serovar Infantis strains. Except *traA, traE, traL*, and *rfbP*, all studied genes were observed in the transconjugant *Escherichia coli* isolates (Table 17; Figures 7-18). In Figure 6, the plasmid sizes acquired by transconjugant *Escherichia coli* isolates were indicated. The plasmid sizes of the transconjugant *E. coli* mated with MET-S1-669 were determined as 40 kb and ~20 kb, while in the transconjugant *E. coli* mated with MET-S1-785, 798, 801, 804 they were found as 47 kb and ~20 kb. On the other side, the transconjugant *E. coli* mated with MET-S1-788 acquired 45 kb and ~20 kb plasmids. The plasmid sizes of transconjugant *E. coli* mated with MET-S1-774, 777, 782 and 792 were confirmed as ~20 kb as can be seen in Figure 6.

E. coli 39R861 was used as a positive control because of the different sizes of plasmids which are 7 kb, 20 kb, 36 kb, and 63 kb in order to adjust the size of plasmids. Furthermore, the plasmid profiles acquired by transconjugant *E. coli* isolates were found as exactly same with the plasmid profiles of donor *Salmonella* Infantis isolates.

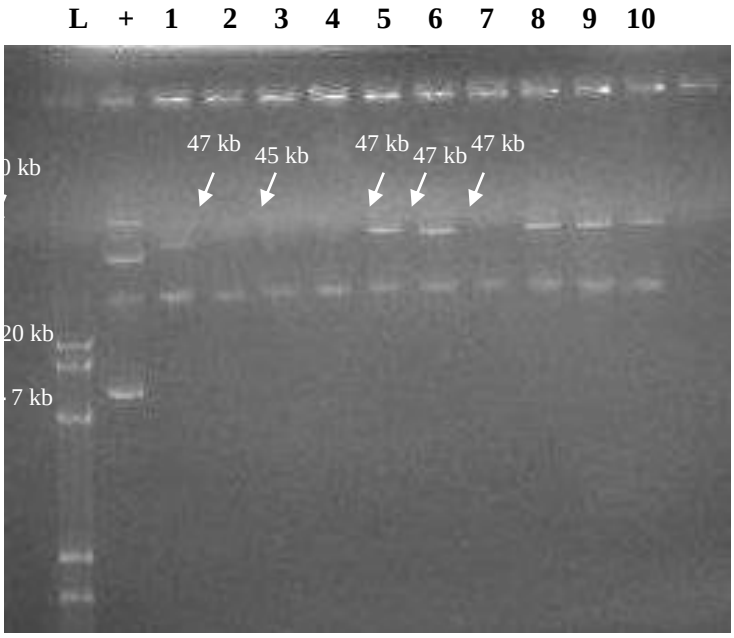

Figure 6 Gel photograph of the plasmid profiles acquired by transconjugant *E. coli* isolates.

L: PerfectSize 1 kb XL Ladder, +: *E. coli* 39R861 (7 kb, 20 kb, 36 kb, 63 kb), 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

Table 17 Results of the conjugation genes screened on the transconjugant *Escherichia coli* mated with *Salmonella* Infantis isolates.

Salmonella Infantis Isolates	*traA*	*traE*	*traL*	*traJ*	*traG*	*traH*	*traI*	*traM*	*traR*	*traY*	*korA*	*rfaG*	*rfaI*	*rfaJ*	*rfbP*	*rfaL*
MET-S1-669	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-774	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-777	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-782	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-785	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-788	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-792	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-798	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-801	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+
MET-S1-804	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+

+: Found, -: Not Found.

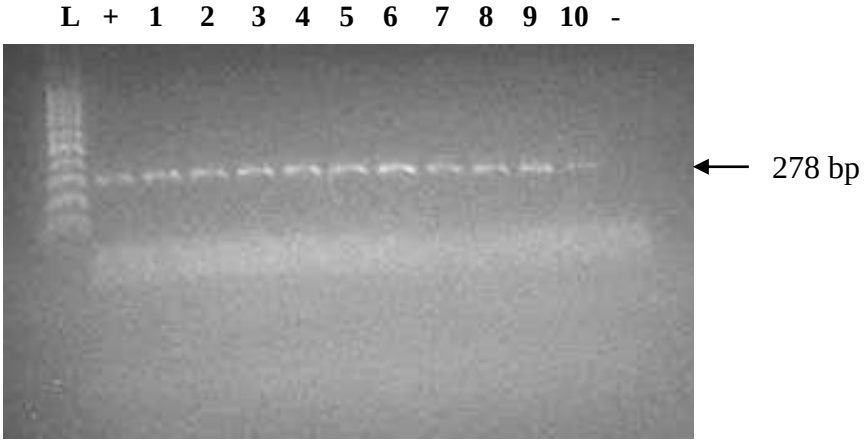

Figure 7 Gel photograph of *korA* gene of transconjugant *E. coli* isolates.

L: 100 bp -1000 bp Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

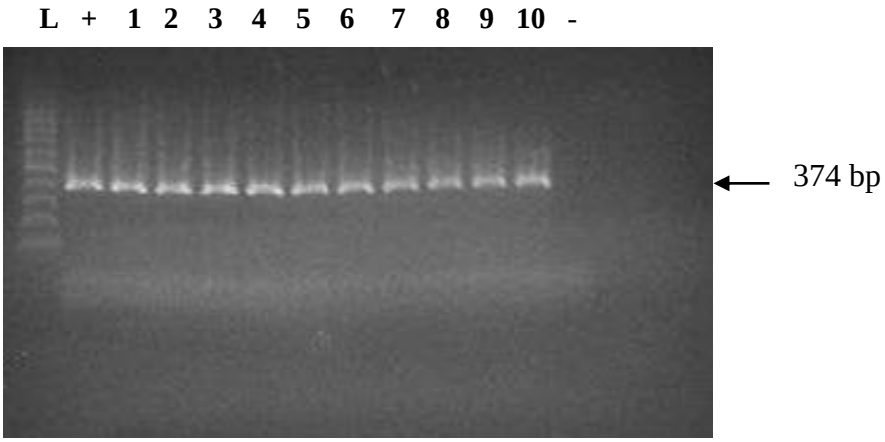

Figure 8 Gel photograph of *rfaG* gene of transconjugant *E. coli* isolates.

L: 100 bp -1000 bp Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

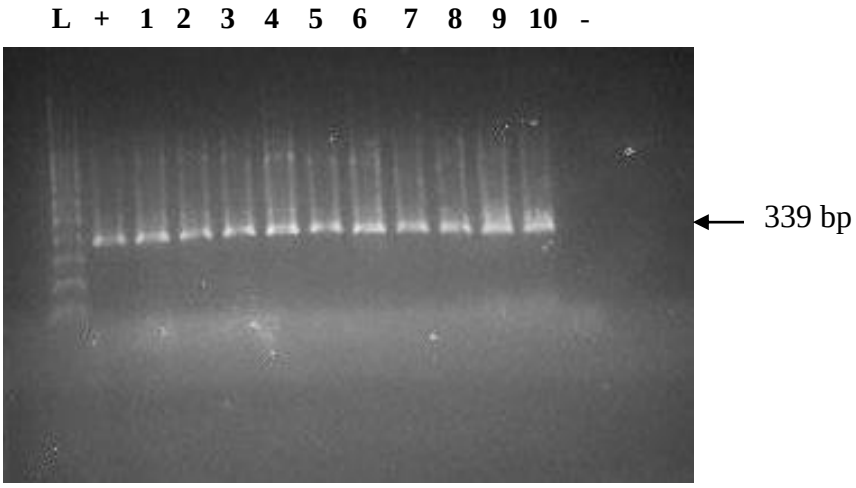

Figure 9 Gel photograph of *rfaI* gene of transconjugant *E. coli* isolates.

L: 100 bp -1000 bp Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

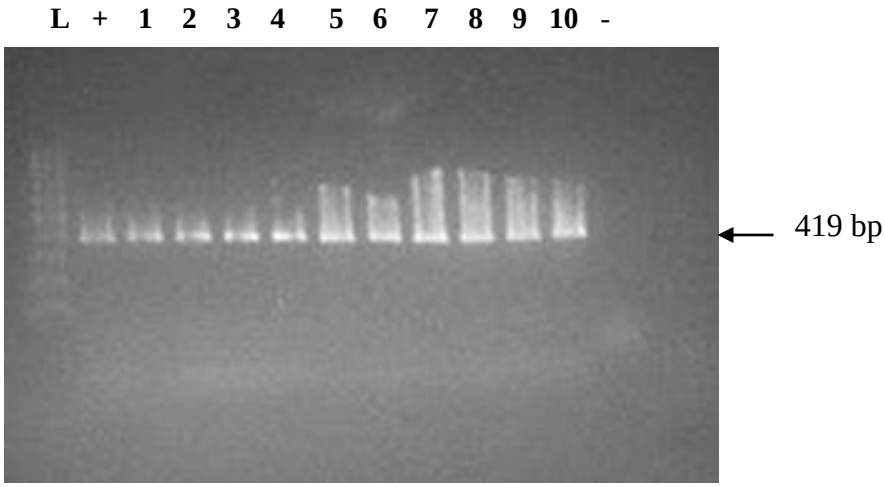

Figure 10 Gel photograph of *rfaL* gene of transconjugant *E. coli* isolates.

L: 100 bp -1000 bp Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

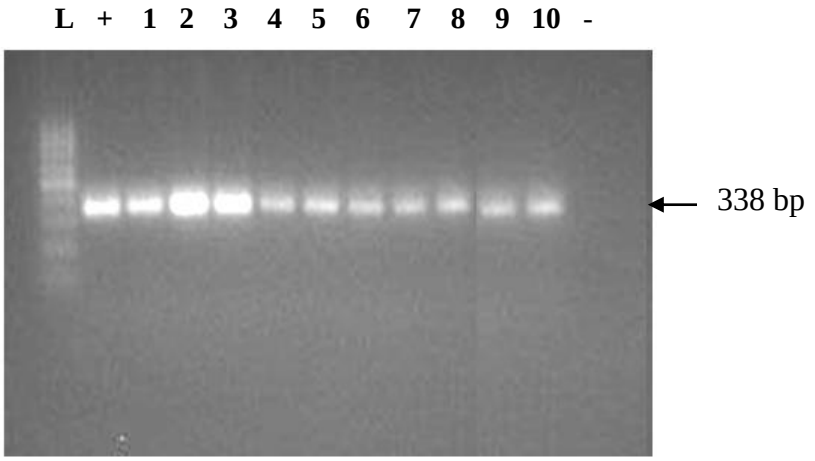

Figure 11 Gel photograph of *rfaJ* gene of transconjugant *E. coli* isolates.

L: 100 bp -1000 bp Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

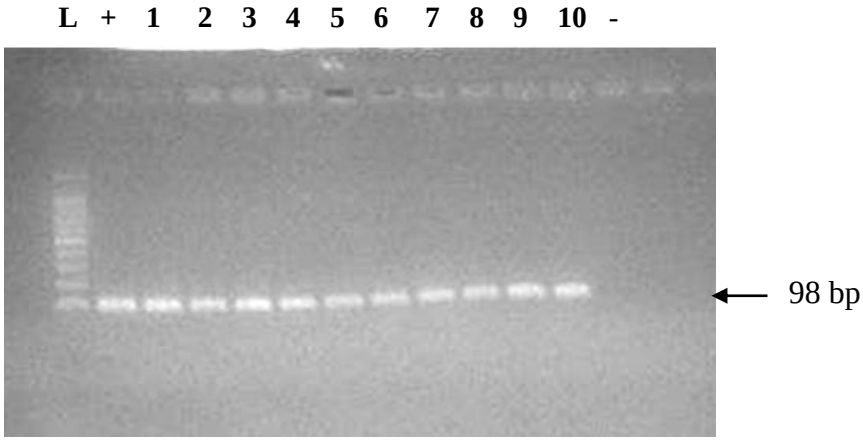

Figure 12 Gel photograph of *traJ* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

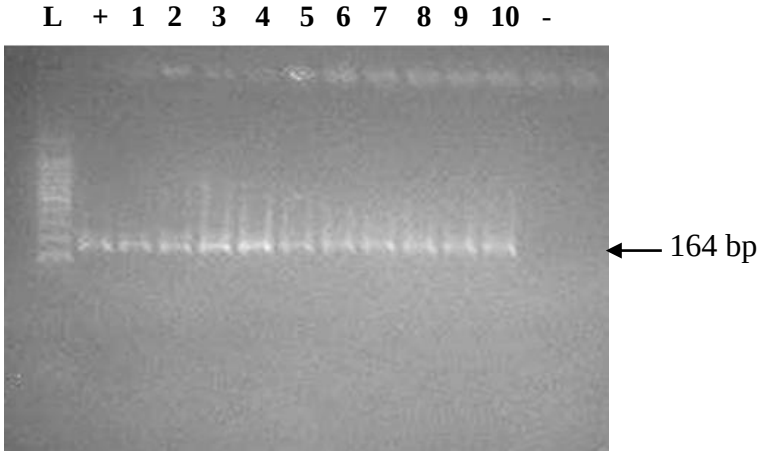

Figure 13 Gel photograph of *traG* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

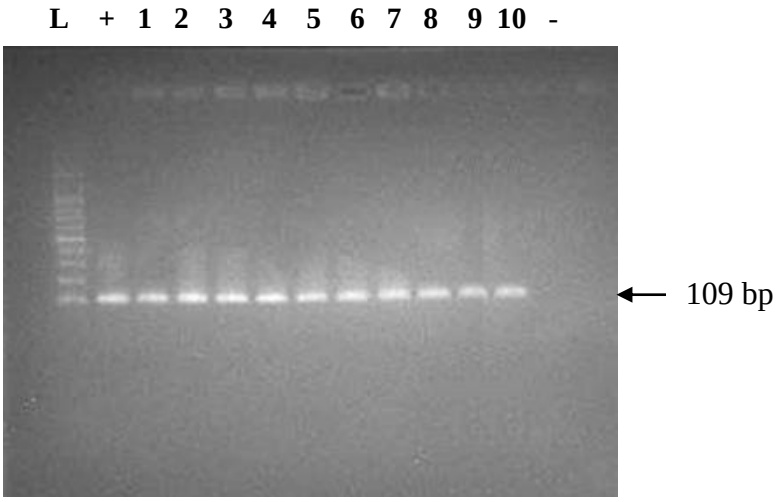

Figure 14 Gel photograph of *traH* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

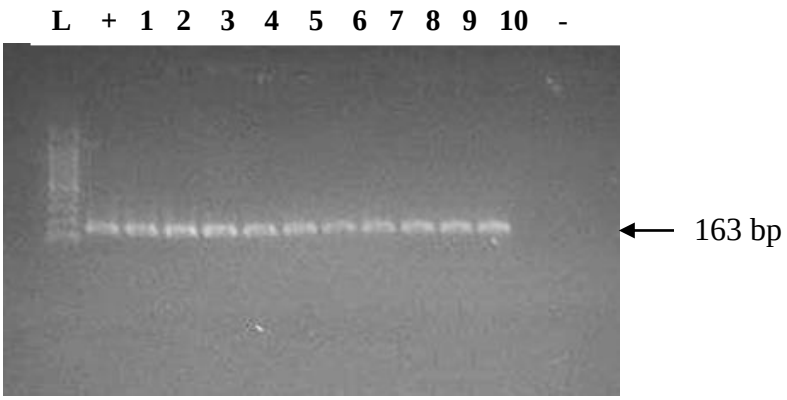

Figure 15 Gel photograph of *traI* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

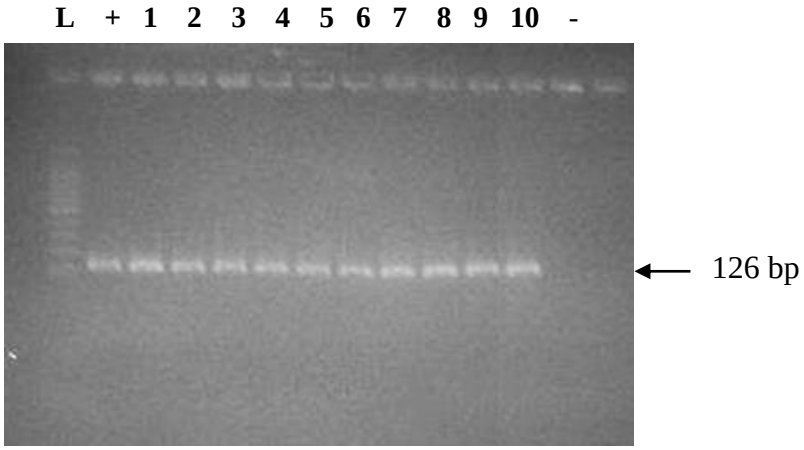

Figure 16 Gel photograph of *traM* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

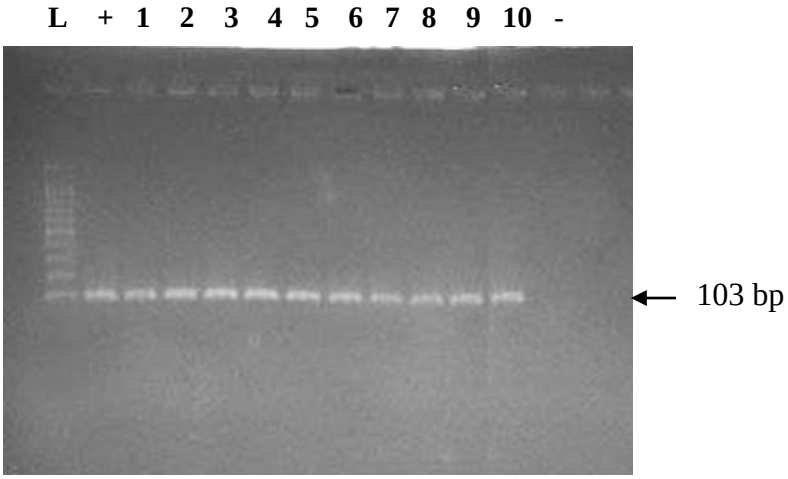

Figure 17 Gel photograph of *traR* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

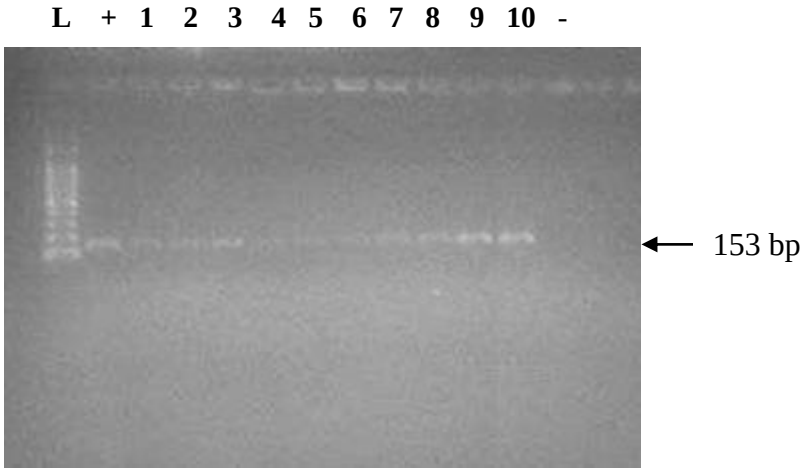

Figure 18 Gel photograph of *traY* gene of transconjugant *E. coli* isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, -: Negative control, 1: Transconjugant *E. coli* mated with MET-S1-669, 2: Transconjugant *E. coli* mated with MET-S1-774, 3: Transconjugant *E. coli* mated with MET-S1-777, 4: Transconjugant *E. coli* mated with MET-S1-782, 5: Transconjugant *E. coli* mated with MET-S1-785, 6: Transconjugant *E. coli* mated with MET-S1-788, 7: Transconjugant *E. coli* mated with MET-S1-792, 8: Transconjugant *E. coli* mated with MET-S1-798, 9: Transconjugant *E. coli* mated with MET-S1-801, 10: Transconjugant *E. coli* mated with MET-S1-804.

Three of transfer genes (*traA, traE* and *traL)* ,not found in the conjugated in *E. coli* isolates in our study, were found in F plasmids that have roles inregulating gene expression and protein synthesis. In other words, *traA* expresses the pioneer of the pilus subunit propilin and is maturated by the gene products of *traQ* and *traX*, while *traE* and *traL* take part in pilus assembly in the F plasmid (Firth et al., 1996). Moreover, when TraQ is absence, *traA* is quickly degraded (Maneewannakul et al., 1993). Thus, in this study, *traA, traE* and *traL* might not be screened. Furthermore, other *tra* genes following *traJ, traH, traG, traI, traM, traR* and *traY* were determined in all transconjugant *E. coli* isolates. On the other hand, conjugative pilus formation and conjugative transfer of F plasmid from donor, *Salmonella* Infantis, to recipient, commensal *Escherichia coli*, have been in need of the proteins expressed by 33.3 kb *tra* operon located in F plasmid (Gubbins et al., 2002). Transfer gene expression has been controlled by the negative loop drived by FinOP (fertility inhibition system) and positive loop composed of TraM, TraJ and TraY. Furthermore, *traM, traJ* expression has been suppressed by FinP and FinO, responsible for the fertility inhibition (Zatyka and Thomas, 1998). In the fertility inhibition system (FinOP system), *finP* expresses antisense RNA specified to a plasmid, while the output of *finO* is interchangeable polypeptide among many different plasmids such as F plasmid (Zatyka and Thomas, 1998). Hence, FinP adhering to *traJ* mRNA interferes with the entry of ribosomes and restrains the translation of *traJ* (Koraimann et al, 1991). On the other side, core lipopolysaccharide is essential to the recipient for the effective conjugation system despite the fact that the O (somatic) side chains of the lipopolysaccharide decrease the conjugation frequency (Watanabe et al., 1970). The outer membrane of the Gram-negative bacteria is composed of minimum two lipids including lipopolysaccharide (LPS) and phospholipids, and jointly some specific proteins (Roncero and Casadaban, 1992). LPS involves lipid A region, core oligosaccharide and O-antigen polysaccharide (Kong et al., 2011).

The genes responsible for the synthesis and modification of lipopolysaccharide core are clustered in the *rfa* region aparting from 2-keto-3-deoxyoctulosonic acid (KDO) biosynthesis (Roncero et al., 1992). In addition to this, *rfaK* and *rfaL,* the genes located in this cluster, are assigned in the binding of O antigen to the core (Schnaitman and Klena, 1993). *rfaG* adding the first backbone hexose (Glc I, glucose I) to Hep II (heptose II) in *E. coli, rfaI* adding the second backbone hexose (Glc II, glucose II) to core in *E. coli* (Pradel et al., 1992), *rfaJ* adding the third (terminal) backbone hexose (Glc III, glucose III) to core in *E. coli* (Pradel et al., 1992), and *rfaL* playing a crucial role in the supplement of O antigen to core, and *rfe*-based insertion of GlcNAc (N-Acetylglucosamine) to terminal Glc III in *E. coli* (Klena et al., 1992). Moreover, *rfbBCAD* cluster participates in expression of LOS (lipooligosaccharide) form of core in *E. coli* (Klena et al., 1992). By taking into consideration the effects of lipopolysaccharide core system on the conjugative transfer, it showed that all *E. coli* isolates mated with *Salmonella enterica* serovar Infantis of this research modified their core system, during the conjugative transfer of genetic elements, such as plasmid. On the other hand, since *rfaL* responsible for ligation of O antigen to the core, the conjugation frequency of *E. coli* mated with MET-S1-782, 788, 804 might be influenced negatively as well as the lipopolysaccharide core features of *Salmonella* Infantis. On the contrary, in *E. coli* isolates mated with MET-S1-801, 774, 798 and 792, the expression of O side chains of the lipopolysaccharide might be repressed during the conjugation. In order to understand the mechanisms underlying conjugation frequency affected adversely, the more researches are required.

4.3. Results of the Plasmid Incompatibility Group

As can be seen in Table 18, only incompatibility group P was determined in donor *Salmonella enterica* serovar Infantis isolates and transconjugant *E. coli* isolates mated with them in Figure 19. IncP plasmids involve IncPα (RK2, RP1, RP4, R68 and R18) and IncPβ (R751) (Thomas et al., 1987). The schema of the conjugative transfer of IncP genes was illustrated in Figure 20. The major control is carried out by KorA, KorB and TrbA which are the three global regulators. The transfer genes are suppressed by KorB and TrbA, while KorA expressed by *korA* is required to repress the expression of *trbA*, which enables to stop the transcription of *tra* and *trb* genes when the conjugative plasmid is formed (Zatyka et al., 1998). TraJ and TraK binding to *oriT* and suppressing the promoters in this region shown in Figure 20 check over the relaxosome. Regulatory genes are illustrated as black; genes functioning in DNA processing as light grey; surface exclusion genes as dark grey; forming mating pair as diagonal hatching; unknown function as white; disassociated genes between the two regions as vertical hatching are shown in Figure 20. Moreover, the black arrowhead at *oriT* represents the direction of transfer, while horizontal arrows mean recommended transcriptional units (Zatyka et al., 1998). Interlocking regulons in IncP plasmids are controlled by repressors such as KorA and KorB expressed by the autogenously regulated central control operon (Zatyka et al., 1998). Moreover, this operon stabilizes independently unstable plasmids (Motallebi-Veshareh et al., 1990). Although the transfer frequencies of IncP plasmids are high, their conjugation genes are not expressed as a basis in order to abstain from the high metabolic complex comprising many genes. To control this complex system, global regulators and local autoregulators take place. The conjugation genes in plasmid RK2 are located in Tra1 expressing *tra* genes, and Tra2 expressing *trb* genes (Pansegrau et al., 1994). *korA* gene was determined in all transconjugant *Escherichia coli* isolates. Furthermore, *korA* protects *E. coli* from the lethal effects of *kilA* in plasmid RK2 (Bechhofer et al., 1983).

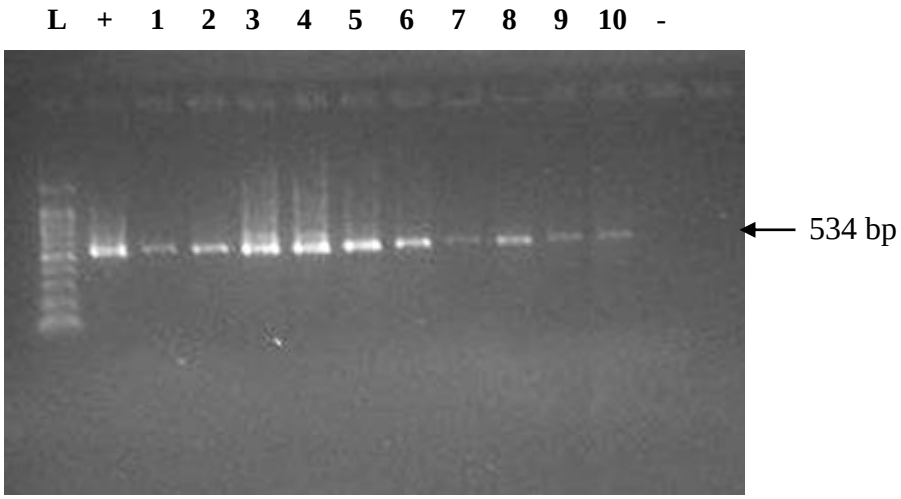

Figure 19 Gel photograph of the incompatibility group P gene of donor *Salmonella enterica* serovar Infantis isolates.

L: PerfectSize 100 bp XL Ladder, +: Positive control, 1: MET-S1-669, 2: MET-S1-774, 3: MET-S1-777, 4: MET-S1-782, 5: MET-S1-785, 6: MET-S1-788, 7: MET-S1-792, 8: MET-S1-798, 9: MET-S1-801, 10: MET-S1-804, -: Negative control.

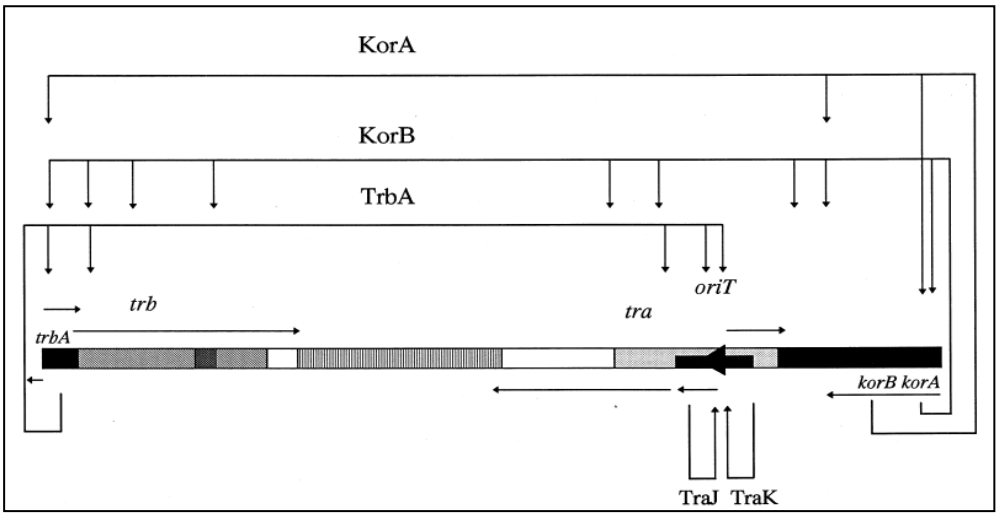

Figure 20 The IncP transfer system. Retrieved from Zatyka, M., & Thomas, C. M. (1998).

Table 18 Results of the plasmid incompatibility groups screened on donor *Salmonella enterica* Infantis isolates and transconjugant *Escherichia coli* mated with *Salmonella* Infantis isolates.

Salmonella **Infantis Isolates**	A/C	B/O	FIA	FIB	FIC	FII_s	F_{repB}	HI1	HI2	I1	K/B	L/M	N	P	T	W	X	Y
MET-S1-669	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-774	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-777	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-782	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-785	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-788	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-792	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-798	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-801	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
MET-S1-804	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

+: Found., -: Not Found.

Tra1 region is composed of *oriT*, transfer origin, and genes taking place in DNA processing and transfer. In addition to this, *traF, traG, traH, traI, traJ* and *traK* which are Tra1 genes play crucial roles in mating for intraspecific *Escherichia coli.* The relaxosome is formed by TraI, TraJ and TraK binding to *oriT*. The first phase of relaxosome generation is suggested by TraJ binding to 19 bp inverted repeat sequence in *oriT* (Ziegelin et al., 1989). The other Tra1 genes including *traC* expressing primase are not required for mating among *E. coli* strains. On the other hand, *traM* enhances the conjugative transfer performance (Lanka and Wilkins, 1995). In *Salmonella* strains, plasmids of IncI, IncH and IncF incompatibility groups are mostly encountered, while IncN, IncP and IncQ are less often observed compared to them (Rychlik et al., 2006). Plasmids from the pre-antibiotic period pertained to IncI, IncX and IncF. The studies have revealed that current plasmids expressing antibiotic resistance were determined among plasmids of similar incompatibility groups from the pre-antibiotic period (Rychlik et al., 2006). Thus, it might be presumed that plasmids of IncP incompatibility group found in this study transformed from the former plasmids from the pre-antibiotic period by acquiring new genetic factors. A recent research has found an IncP plasmid in colistin resistant *Salmonella enterica* serovar Typhimurium isolated from a healthy person (Lu et al., 2017). Moreover, conjugative transfer of plasmids carrying antibiotic resistance and virulence from *Salmonella enterica* serovar Infantis to commensal *E. coli* strain was observed (Aviv et al., 2016). In addition to this, the conjugative transfer of large ~100 kb plasmid from *Salmonella* Infantis to *E. coli* was determined (Gal-Mor et al., 2010). All in all, commensal *Escherichia coli* sensitive to antimicrobial agents also acquired the antibiotic resistance genes from multidrug resistant *Salmonella enterica* serovar Infantis by the way of conjugative transfer of plasmid(s), 20, 40, 45 and 47 kb. It might be concluded that conjugative transfer of plasmids of IncP incompatibility group carrying multidrug resistance genes spread in Gram-negative bacteria recently.

CHAPTER 5

CONCLUSION

Recently emerged *Salmonella* serotype Infantis with multidrug resistancy was focused and how the microbial resistancy is transfered from these strains to commencial *E. coli* isolates was investigated. For this aim, commensal *E. coli* mated with each *Salmonella enterica* serovar Infantis isolated from chicken and showing different phenotypic antibiotic resistance profiles including streptomycin, sulfisoxazole, ampicillin, trimethoprim/sulfamethoxazole, kanamycin, streptomycin, chloramphenicol, ciprofloxacin, cephalotin, nalidixic acid and tetracycline acquired the antibiotic resistance by the conjugative transfer of plasmid(s). Moreover, distinct conjugation frequencies were obtained and evaluated by applying both of one-way and two-way ANOVA statistical analysis. We observed that all *Salmonella* isolates used in here carried P plasmids. After conjugation, the native transfer genes for P plasmids were found also in transconjugant *Escherichia coli* mated with all *Salmonella* Infantis isolates. In addition to this, plasmids of IncP incompatibility group were determined in all transconjugant *E. coli* strains.

In conjuction with the environmental selective pressure induced by the usage of antimicrobial agents, antibiotic resistance genes have spread all around the world. At this point, the recent studies have remarked that *Salmonella enterica* serovar Infantis, emerging strain in livestock, broiler chicken and human, have been acquiring multi-drug resistance genes via conjugative plasmids, and transferring the antibiotic resistance genes located on plasmids to the Gram-negative bacteria and/or

Salmonella species. Hence, the usage of antibiotics in food animal production should be reduced in order to prevent these bacteria from acquiring the antibiotic resistance.

This study set sight on the mechanism of conjugative transfer between *E. coli* and *Salmonella enterica* serovar Infantis in order to make contribution for the further studies, and to comprehend how antibiotic resistance genes are acquired.

CHAPTER 6

RECOMMENDATIONS

Salmonella enterica serovar Infantis is one of the prevalent salmonellae leading to serious human health problems, foodborne infections and veterinary infectious diseases around the world. Furthermore, these problems mentioned above have been spreading among Gram-negative bacteria such as *Escherichia coli* because of the conjugative transfer system of multidrug resistance genes found on plasmids. In spite of the limitations in this study, important information about the conjugative transfer of plasmid(s) carrying antibiotic resistance genes between emerging clone *Salmonella* Infantis isolated from chicken meat in Turkey and commensal *E. coli* is given.

In addition to this, transmission and reservoirs of these plasmids among Gram-negative bacteria and Gram-positive bacteria play crucial role in the gut microbiota of warm-blooded hosts. In other words, while the usage and misusage of antibiotics on humans, veterinary and animal foods damages the normal flora of gut microbiota, the environment of the warm-blooded hosts' intestines enables favorable reservoir for the antibiotic resistance at the same time. For further studies, in order to find the reliable solutions for these, widescale research takes an important place. To illustrate, by the help of whole genome sequencing (WGS) technology, the source and origin of antibiotic resistance genes on transmissible plasmids, and some still unclear mechanisms of conjugation might be revealed.

REFERENCES

Acar, S., Bulut, E., Durul, B., Uner, I., Kur, M., Avsaroglu, M. D., … Soyer, Y. (2017). Phenotyping and genetic characterization of Salmonella enterica isolates from Turkey revealing arise of different features specific to geography. *International Journal of Food Microbiology*, *241*, 98–107. https://doi.org/10.1016/j.ijfoodmicro.2016.09.031

Acheson, D., & Hohmann, E. L. (2001). Nontyphoidal Salmonellosis. *Clinical Infectious Diseases*, *32*(2), 263–269. https://doi.org/10.1086/318457

Aldred, K. J., Kerns, R. J., & Osheroff, N. (2014). Mechanism of quinolone action and resistance. *Biochemistry*. https://doi.org/10.1021/bi5000564

Allen, K. J., & Poppe, C. (2002). Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by β-lactamase CMY-2 in Salmonella isolated from food-producing animals in Canada. *Canadian Journal of Veterinary Research*, *66*(3), 137–144.

Andrup, L., & Andersen, K. (1999). A comparison of the kinetics of plasmid transfer in the conjugation systems encoded by the F plasmid from Escherichia coli and plasmid pCF10 from Enterococcus faecalis. *Microbiology*, *145*(8), 2001–2009. https://doi.org/10.1099/13500872-145-8-2001

Antunes, P., Machado, J., Sousa, J. C., & Peixe, L. (2005). Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese Salmonella enterica strains and relation with integrons. *Antimicrobial Agents and Chemotherapy*, *49*(2), 836–839. https://doi.org/10.1128/AAC.49.2.836-839.2005

Arakawa, Y., Murakami, M., Suzuki, K., Ito, H., Wacharotayankun, R., Ohsuka, S., … Ohta, M. (1995). A novel integron-like element carrying the metallo-β-lactamase gene bla(IMP). *Antimicrobial Agents and Chemotherapy*. https://doi.org/10.1128/AAC.39.7.1612

Arber, W. (2014). Horizontal Gene Transfer among Bacteria and Its Role in Biological Evolution. *Life (Basel, Switzerland)*, *4*(2), 217–24. https://doi.org/10.3390/life4020217

Aviv, G., Rahav, G., & Gal-Mor, O. (2016). Horizontal transfer of the Salmonella enterica serovar infantis resistance and virulence plasmid pESI to the gut microbiota of warm-blooded hosts. *mBio*, *7*(5). https://doi.org/10.1128/mBio.01395-16

Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*. https://doi.org/10.1126/science.1104816

Bechhofer, D. H., & Figurski, D. H. (1983). Map location and nucleotide sequence of korA, a key regulatory gene of promiscuous ptasmid RK2. *Nucleic Acids Research, 11*(21), 7453–7469. https://doi.org/10.1093/nar/11.21.7453

Bengtsson-Palme, J., Angelin, M., Huss, M., Kjellqvist, S., Kristiansson, E., Palmgren, H., … Johansson, A. (2015). The human gut microbiome as a transporter of antibiotic resistance genes between continents. *Antimicrobial Agents and Chemotherapy*, *59*(10), 6551–6560. https://doi.org/10.1128/AAC.00933-15

Bennett, P. M. (2004). An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy, 53(3),* 418-431. doi: 10.1093/jac/dkh087

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. In *British Journal of Pharmacology* (Vol. 153). https://doi.org/10.1038/sj.bjp.0707607

Benveniste, R., and J. Davies. (1973). Mechanisms of antibiotic resistance in bacteria. Annu. Rev. Biochem. 42:471-506.

Bradshaw, H. D., Traxler, B. A., Minkley, E. G., Nester, E. W., & Gordon, M. P. (1990). Nucleotide sequence of the traI (Helicase I) gene from the sex factor F. *Journal of Bacteriology*, *172*(7), 4127–4131. https://doi.org/10.1128/jb.172.7.4127-4131.1990

Brown, M. G., Mitchell, E. H., & Balkwill, D. L. (2008). Tet 42, a novel tetracycline resistance determinant isolated from deep terrestrial subsurface bacteria. *Antimicrobial Agents and Chemotherapy*, *52*(12), 4518–4521. https://doi.org/10.1128/AAC.00640-08

Burmeister, A. R. (2015). Horizontal Gene Transfer definition and background. *Evolution, Medicine, and Public Health*, *2015*(1), 193–194. https://doi.org/10.1093/emph/eov018

Carattoli, A. (2003). Plasmid-mediated antimicrobial resistance in Salmonella enterica. *Current Issues in Molecular Biology*.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K. L., & Threlfall, E. J. (2005). Identification of plasmids by PCR-based replicon typing. *Journal of Microbiological Methods*, *63*(3), 219–228. https://doi.org/10.1016/j.mimet.2005.03.018

Carattoli, A., Tosini, F., Giles, W. P., Rupp, M. E., Hinrichs, S. H., Angulo, F. J., … Fey, P. D. (2002). Characterization of plasmids carrying CMY-2 from expanded-spectrum cephalosporin-resistant Salmonella strains isolated in the United States between 1996 and 1998. *Antimicrobial Agents and Chemotherapy*, *46*(5), 1269–1272. https://doi.org/10.1128/AAC.46.5.1269-1272.2002

Cejas, D., Vignoli, R., Quinteros, M., Marino, R., Callejo, R., Betancor, L., … Radice, M. A. (2014). First detection of CMY-2 plasmid mediated β-lactamase in Salmonella Heidelberg in South America. *Rev Argent Microbiol*, *46*(1), 30–33. https://doi.org/http://dx.doi.org/10.1016/S0325-7541(14)70044-6

Ceyssens, P. J., Mattheus, W., Vanhoof, R., & Bertrand, S. (2015). Trends in serotype distribution and antimicrobial susceptibility in salmonella enterica isolates from humans in Belgium, 2009 to 2013. *Antimicrobial Agents and Chemotherapy*, *59*(1), 544–552. https://doi.org/10.1128/AAC.04203-14

Chen, S., Zhao, S., White, D. G., Schroeder, C. M., Lu, R., Yang, H., … Meng, J. (2004). Characterization of Multiple-Antimicrobial-Resistant Salmonella Serovars Isolated from Retail Meats. *Applied and Environmental Microbiology*, *70*(1), 1–7. https://doi.org/10.1128/AEM.70.1.1-7.2004

Chopra, I., & Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Cuozzo, M., & Silverman, P. M. (1986). Characterization of the F plasmid TraJ protein synthesized in F' and Hfr strains of Escherichia coli K-12. *Journal of Biological Chemistry*, *261*(11), 5175–5179.

Datta, N. (1975). Epidemiology and classification of plasmids. p. 9-15. In D. Schlessinger (ed.), Microbiology-1974. American Society for Microbiology, Washington, D.C.

Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science*, *264*(5157), 375–382. https://doi.org/10.1126/science.8153624

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., … Yu, G. (2015). Resistance integrons: Class 1, 2 and 3 integrons. *Annals of Clinical Microbiology and Antimicrobials*. https://doi.org/10.1186/s12941-015-0100-6

Doublet, B., Boyd, D., Mulvey, M. R., & Cloeckaert, A. (2005). The Salmonella genomic island 1 is an integrative mobilizable element. *Molecular Microbiology*, *55*(6), 1911–1924. https://doi.org/10.1111/j.1365-2958.2005.04520.x

Doublet, B., Butaye, P., Imberechts, H., Boyd, D., Mulvey, M. R., Chaslus-Dancla, E., & Cloeckaert, A. (2004). Salmonella genomic island 1 multidrug resistance gene clusters in Salmonella enterica serovar agona isolated in Belgium in 1992 to 2002. *Antimicrobial Agents and Chemotherapy*, *48*(7), 2510–2517. https://doi.org/10.1128/AAC.48.7.2510-2517.2004

El-Sharkawy, H., Tahoun, A., El-Gohary, A. E.-G. A., El-Abasy, M., El-Khayat, F., Gillespie, T., … El-Adawy, H. (2017). Epidemiological, molecular characterization and antibiotic resistance of Salmonella enterica serovars isolated from chicken farms in Egypt. *Gut Pathogens*, *9*(1), 8. https://doi.org/10.1186/s13099-017-0157-1

Faldynova, M., Pravcova, M., Sisak, F., Havlickova, H., Kolackova, I., Cizek, A., … Rychlik, I. (2003). Evolution of antibiotic resistance in Salmonella enterica serovar Typhimurium strains isolated in the Czech Republic between 1984 and 2002. *Antimicrobial Agents and Chemotherapy*, *47*(6), 2002–2005. https://doi.org/10.1128/AAC.47.6.2002-2005.2003

Fantin, B., Ebert, S., Leggett, J., Vogelman, B., & Craig, W. A. (1991). Factors affecting duration of in-vivo postantibiotic effect for aminoglycosides against gram-negative bacilli. *Journal of Antimicrobial Chemotherapy*, *27*(6), 829–836. https://doi.org/10.1093/jac/27.6.829

Fey, P. D., Safranek, T. J., Rupp, M. E., Dunne, E. F., Ribot, E., Iwen, P. C., … Hinrichs, S. H. (2000). Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle. *New England Journal of Medicine*, *342*(17), 1242–1249. https://doi.org/10.1056/NEJM200004273421703

Firth, N., Ippen-ihler, K., & Skurray, R. a. (1996). Structure and Function of the F Factor and Mechanism of Conjugation. *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*, 2377–2401.

Fluit, A. C., & Schmitz, F. J. (2004). Resistance integrons and super-integrons. *Clinical Microbiology and Infection*. https://doi.org/10.1111/j.1198-743X.2004.00858.x

Frana, T. S., Carlson, S. A., & Griffith, R. W. (2001). Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in Salmonella enterica serotype typhimurium phage type DT104. *Applied and Environmental Microbiology*, *67*(1), 445–448. https://doi.org/10.1128/AEM.67.1.445-448.2001

Frost, L. S. (1993). Conjugative Pili and Pilus-Specific Phages. In *Bacterial Conjugation* (pp. 189–221). https://doi.org/10.1007/978-1-4757-9357-4_7

Frost, L. S., Paranchych, W., & Willetts, N. S. (1984). The complete sequence of the F traALE region that includes the gene for F pilin. *Journal of Bacteriology*.

Gal-Mor, O., Valinsky, L., Weinberger, M., Guy, S., Jaffe, J., Schorr, Y. I., … Nissan, I. (2010). Multidrug-resistant Salmonella enterica serovar Infantis, Israel. *Emerging Infectious Diseases*, *16*(11), 1754–7. https://doi.org/10.3201/eid1611.100100

García-Fernández, A., Villa, L., Moodley, A., Hasman, H., Miriagou, V., Guardabassi, L., & Carattoli, A. (2011). Multilocus sequence typing of IncN plasmids. *Journal of Antimicrobial Chemotherapy*, *66*(9), 1987–1991. https://doi.org/10.1093/jac/dkr225

Gebreyes, W. A., & Altier, C. (2002). Molecular Characterization of Multidrug-Resistant Salmonella enterica subsp. enterica Serovar Typhimurium Isolates from Swine. *Journal of Clinical Microbiology*, *40*(8), 2813–2822. https://doi.org/10.1128/JCM.40.8.2813-2822.2002

Gillings, M. R. (2017). Class 1 integrons as invasive species. *Current Opinion in Microbiology*. https://doi.org/10.1016/j.mib.2017.03.002

Giraud, E., Cloeckaert, A., Kerboeuf, D., & Chaslus-Dancla, E. (2000). Evidence for Active Efflux as the Primary Mechanism of Resistance to Ciprofloxacin in Salmonella enterica Serovar Typhimurium. *Antimicrobial Agents and Chemotherapy*, *44*(5), 1223–1228. https://doi.org/10.1128/AAC.44.5.1223-1228.2000

Griffith, F. (1928). The Significance of Pneumococcal Types. *Journal of Hygiene*, *27*(2), 113–159. https://doi.org/10.1017/S0022172400031879

Griffiths, A., Wessler, S. R., Carroll, S. B., & Doebly, J. (2000). Introduction To Genetic Analysis. *Aneuploidy*, 800. https://doi.org/10.1007/s13398-014-0173-7.2

Gross, J. D., and L. G. Caro. (1966). DNA transfer in bacterial conjugation. J. Mol. Biol. 16:269-284.

Gruneberg, R. N. (1972). Trimethoprim Resistance Determined by R Factors. *British Medical Journal*, *1*(5802), 726–728. https://doi.org/10.1136/bmj.1.5802.726

Guarner, F., & Malagelada, J. R. (2003). Gut flora in health and disease. *Lancet*. https://doi.org/10.1016/S0140-6736(03)12489-0

Gubbins, M. J., Lau, I., Will, W. R., Manchak, J. M., Raivio, T. L., & Frost, L. S. (2002). The positive regulator, TraJ, of the Escherichia coli F plasmid is unstable in a cpxA* background. *Journal of Bacteriology*, *184*(20), 5781–5788. https://doi.org/10.1128/JB.184.20.5781-5788.2002

Guerra, B., Junker, E., & Helmuth, R. (2004). Incidence of the recently described sulfonamide resistance gene sul3 among German Salmonella enterica strains isolated from livestock and food. *Antimicrobial Agents and Chemotherapy*, *48*(7), 2712–2715. https://doi.org/10.1128/AAC.48.7.2712-2715.2004

Guerra, B., Soto, S. M., Arg??elles, J. M., & Mendoza, M. C. (2001). Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent Salmonella enterica serotype [4,5,12:i:-]. *Antimicrobial Agents and Chemotherapy*, *45*(4), 1305–1308. https://doi.org/10.1128/AAC.45.4.1305-1308.2001

Hall, R. M. (2013). Integrons. In Brenner's Encyclopedia of Genetics (Second Edition). 107-109. https://doi.org/10.1016/B978-0-12-374984-0.00804-4.

Hardjo Lugito, N. P., & Cucunawangsih. (2017). Antimicrobial resistance of salmonella enterica serovars Typhi and paratyphi isolates from a general Hospital in Karawaci, Tangerang, Indonesia: A five-year review. *International Journal of Microbiology*, *2017*. https://doi.org/10.1155/2017/6215136

Hawkey, P. M., & Jones, A. M. (2009). The changing epidemiology of resistance. *Journal of Antimicrobial Chemotherapy*, *64*(SUPPL.1). https://doi.org/10.1093/jac/dkp256

Helmuth, R., & Achtman, M. (1978). Cell-cell interactions in conjugating Escherichia coli: purification of F pili with biological activity. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(3), 1237–1241. https://doi.org/10.1073/pnas.75.3.1237

Hermann, T. (2007). Aminoglycoside antibiotics: old drugs and new therapeutic approaches. *Cell Mol Life Sci*, *64*(14), 1841–1852. https://doi.org/10.1007/s00018-007-7034-x

HOTCHKISS, R. D. (1951). Transfer of penicillin resistance in pneumococci by the desoxyribonucleate derived from resistant cultures. *Cold Spring Harbor Symposia on Quantitative Biology*, *16*, 457–461.

Huddleston, J. R. (2014). Horizontal gene transfer in the human gastrointestinal tract: Potential spread of antibiotic resistance genes. *Infection and Drug Resistance*. https://doi.org/10.2147/IDR.S48820

Hughes, D., & Andersson, D. I. (2001). Antibiotic development and resistance. London: Taylor & Francis.

Jernberg, C., Löfmark, S., Edlund, C., & Jansson, J. K. (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology*. https://doi.org/10.1099/mic.0.040618-0

Karami, N., Martner, A., Enne, V. I., Swerkersson, S., Adlerberth, I., & Wold, A. E. (2007). Transfer of an ampicillin resistance gene between two Escherichia coli strains in the bowel microbiota of an infant treated with antibiotics. *Journal of Antimicrobial Chemotherapy*, *60*(5), 1142–1145. https://doi.org/10.1093/jac/dkm327

Kehrenberg, C., Friederichs, S., de Jong, A., Michael, G. B., & Schwarz, S. (2006). Identification of the plasmid-borne quinolone resistance gene qnrS in Salmonella enterica serovar Infantis. *Journal of Antimicrobial Chemotherapy*, *58*(1), 18–22. https://doi.org/10.1093/jac/dkl213

Kihlström, E., & Andåker, L. (1985). Inability of gentamicin and fosfomycin to eliminate intracellular enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, *15*(6), 723–728. https://doi.org/10.1093/jac/15.6.723

Klena, J. D., Ashford, R. S., & Schnaitman, C. A. (1992). Role of Escherichia coli K-12 rfa genes and the rfp gene of Shigella dysenteriae 1 in generation of lipopolysaccharide core heterogeneity and attachment of O antigen. *Journal of Bacteriology*, *174*(22), 7297–7307. https://doi.org/10.1128/jb.174.22.7297-7307.1992

Koeck, J. L., Arlet, G., Philippon, A., Basmaciogullari, S., Thien, H. V., Buisson, Y., & Cavallo, J. D. (1997). A plasmid-mediated CMY-2 β-lactamase from an Algerian clinical isolate of Salmonella senftenberg. *FEMS Microbiology Letters*, *152*(2), 255–260. https://doi.org/10.1016/S0378-1097(97)00206-1

Kong, Q., Yang, J., Liu, Q., Alamuri, P., Roland, K. L., & Curtiss, R. (2011). Effect of deletion of genes involved in lipopolysaccharide core and O-antigen synthesis on virulence and immunogenicity of Salmonella enterica serovar Typhimurium. *Infection and Immunity*, *79*(10), 4227–4239. https://doi.org/10.1128/IAI.05398-11

Koraimann, G., Koraimann, C., Koronakis, V., Schlager, S., & Högenauer, G. (1991). Repression and derepression of conjugation of plasmid R1 by wild-type and mutated finP antisense RNA. *Molecular Microbiology*, *5*(1), 77–87. https://doi.org/10.1111/j.1365-2958.1991.tb01828.x

Lancini, G., Parenti, F., & Gallo G. G. (1995). *Antibiotics: a multidisciplinary approach*. New York: Plenum Press.

Langdon, A., Crook, N., & Dantas, G. (2016). The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Medicine*. https://doi.org/10.1186/s13073-016-0294-z

Lanka, E., & Wilkins, B. M. (1995). DNA Processing Reactions in Bacterial Conjugation. *Annual Review of Biochemistry*, *64*(1), 141–169. https://doi.org/10.1146/annurev.bi.64.070195.001041

Lee, K. E., Lim, S. I., Choi, H. W., Lim, S. K., Song, J. Y., & An, D. J. (2014). Plasmid-mediated AmpC ??-lactamase (CMY-2) gene in Salmonella typhimurium isolated from diarrheic pigs in South Korea. *BMC Research Notes*, *7*(1). https://doi.org/10.1186/1756-0500-7-329

Lee, K., Yong, D., Yum, J. H., Lim, Y. S., Kim, H. S., Lee, B. K., & Chong, Y. (2004). Emergence of multidrug-resistant Salmonella enterica serovar Typhi in Korea. *Antimicrobial Agents and Chemotherapy*, *48*(11), 4130–4135. https://doi.org/10.1128/AAC.48.11.4130-4135.2004

Leekitcharoenphon, P., Hendriksen, R. S., Le Hello, S., Weill, F.-X., Baggesen, D. L., Jun, S.-R., … Aarestrup, F. M. (2016). Global Genomic Epidemiology of Salmonella enterica Serovar Typhimurium DT104. *Applied and Environmental Microbiology*, *82*(8), 2516–2526. https://doi.org/10.1128/AEM.03821-15

Levings, R. S., Lightfoot, D., Partridge, S. R., Hall, R. M., & Djordjevic, S. P. (2005). The genomic island SGI1, containing the multiple antibiotic resistance region of Salmonella enterica serovar typhimurium DT104 or variants of it, is widely distributed in other S. enterica serovars. *Journal of Bacteriology*, *187*(13), 4401–4409. https://doi.org/10.1128/JB.187.13.4401-4409.2005

Liang, Z., Ke, B., Deng, X., Liang, J., Ran, L., Lu, L., … Wu, S. (2015). Serotypes, seasonal trends, and antibiotic resistance of non-typhoidal Salmonella from human patients in Guangdong Province, China, 2009–2012. *BMC Infectious Diseases*, *15*(1), 53. https://doi.org/10.1186/s12879-015-0784-4

Llanes, C., Kirchgesner, V., & Plesiat, P. (1999). Propagation of TEM- and PSE-type beta-lactamases among amoxicillin-resistant Salmonella spp. isolated in France. *Antimicrobial Agents and Chemotherapy*, *43*(10), 2430–2436.

López, E., Elez, M., Matic, I., & Blázquez, J. (2007). Antibiotic-mediated recombination: Ciprofloxacin stimulates SOS-independent recombination of divergent sequences in Escherichia coli. *Molecular Microbiology*, *64*(1), 83–93. https://doi.org/10.1111/j.1365-2958.2007.05642.x

Lu, X., Hu, Y., Luo, M., Zhou, H., Wang, X., Du, Y., … Kan, B. (2017). MCR-1.6, a new MCR variant carried by an IncP plasmid in a colistin-resistant Salmonella enterica serovar Typhimurium isolate from a healthy individual. *Antimicrobial Agents and Chemotherapy*, *61*(5). https://doi.org/10.1128/AAC.02632-16

MacGowan, A., Macnaughton, E. (2013). Antibiotic resistance. In Medicine, volume 41, issue 11. 642-648. https://doi.org/10.1016/j.mpmed.2013.08.002

Majiduddin, F. K., Materon, I. C., & Palzkill, T. G. (2002). Molecular analysis of beta-lactamase structure and function. *International Journal of Medical Microbiology*, *292*(2), 127–137. https://doi.org/10.1078/1438-4221-00198

Maneewannakul, K., Maneewannakul, S., & Ippen-Ihler, K. (1993). Synthesis of F pilin. *Journal of Bacteriology*, *175*(5), 1384–1391. https://doi.org/10.1128/jb.175.5.1384-1391.1993

Mascaretti, O. A. (2003). *Bacteria versus antibacterial agents: an integrated approach.* Washington, D.C.: ASM.

Mathur, S., & Singh, R. (2005). Antibiotic resistance in food lactic acid bacteria - A review. *International Journal of Food Microbiology*. https://doi.org/10.1016/j.ijfoodmicro.2005.03.008

Maxwell, A. (1997). DNA gyrase as a drug target. *Trends Microbiol*, *5*(3), 102–109. https://doi.org/S0966-842X(96)10085-8 [pii]\r10.1016/S0966-842X(96)10085-8

McDonald, L. C., Chen, F. J., Lo, H. J., Yin, H. C., Lu, P. L., Huang, C. H., … Ho, M. (2001). Emergence of reduced susceptibility and resistance to fluoroquinolones in Escherichia coli in Taiwan and contributions of distinct selective pressures. *Antimicrobial Agents and Chemotherapy*, *45*(11), 3084–3091. https://doi.org/10.1128/AAC.45.11.3084-3091.2001

Menashe, O., Kaganskaya, E., Baasov, T., & Yaron, S. (2008). Aminoglycosides affect intracellular Salmonella enterica serovars typhimurium and virchow. *Antimicrobial Agents and Chemotherapy*, *52*(3), 920–926. https://doi.org/10.1128/AAC.00382-07

Meunier, D., Boyd, D., Mulvey, M. R., Baucheron, S., Mammina, C., Nastasi, A., … Cloeckaert, A. (2002). Salmonella enterica serotype Typhimurium DT 104 antibiotic resistance genomic island I in serotype Paratyphi B. *Emerging Infectious Diseases*, *8*(4), 430–433. https://doi.org/10.3201/eid0804.010213

Modi, S. R., Lee, H. H., Spina, C. S., & Collins, J. J. (2013). Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature*, *499*(7457), 219–222. https://doi.org/10.1038/nature12212

Motallebi-Veshareh, M., Rouch, D. A., & Thomas, C. M. (1990). A family of ATPases involved in active partitioning of diverse bacterial plasmids. *Molecular Microbiology*, *4*(9), 1455–1463. https://doi.org/10.1111/j.1365-2958.1990.tb02056.x

Navarro, F., Perez-Trallero, E., JM, M., Aliaga, R., Gomariz, M., Mirelis, B., … Mirelis, B. (2001). CMY-2-producing Salmonella enterica, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis and Escherichia coli strains isolated in Spain (October 1999-December 2000). *Journal of Antimicrobial Chemotherapy*, *48*(3), 383–389. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11533003

Neidhardt, F. (1996). *Escherichia coli and Salmonella: Cellular and Molecular Biology*. *Escherichia coli and Salmonella: cellular and molecular biology*. Retrieved from http://scholar.google.com/scholar_lookup?title=Escherichia coli and Salmonella%3A Cellular and Molecular Biology&author=R Meganathan&publication_year=1996#0

Nógrády, N., Tóth, Á., Kostyák, Á., Pászti, J., & Nagy, B. (2007). Emergence of multidrug-resistant clones of Salmonella Infantis in broiler chickens and humans in Hungary. *Journal of Antimicrobial Chemotherapy*, *60*(3), 645–648. https://doi.org/10.1093/jac/dkm249

Pansegrau, W., Lanka, E., Barth, P. T., Figurski, D. H., Guiney, D. G., Haas, D., … Thomas, C. M. (1994). Complete nucleotide sequence of birmingham IncPα plasmids. Compilation and comparative analysis. *Journal of Molecular Biology*, *239*(5), 623–663. https://doi.org/10.1006/jmbi.1994.1404

Parker, C. T., Kloser, A. W., Schnaitman, C. A., Stein, M. A., Gottesman, S., & Gibson, B. W. (1992). Role of the rfaG and rfaP genes in determining the lipopolysaccharide core structure and cell surface properties of Escherichia coli K-12. *Journal of Bacteriology*, *174*(8), 2525–2538. https://doi.org/10.1128/jb.174.8.2525-2538.1992

Penders, J., Stobberingh, E. E., Savelkoul, P. H. M., & Wolffs, P. F. G. (2013). The human microbiome as a reservoir of antimicrobial resistance. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2013.00087

Pérez-Pérez, F. J., & Hanson, N. D. (2002). Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *Journal of Clinical Microbiology*, *40*(6), 2153–2162. https://doi.org/10.1128/JCM.40.6.2153

Pessoa-Silva, C. L., Toscano, C. M., Moreira, B. M., Santos, A. L., Frota, A. C. C., Solari, C. A., … Jarvis, W. R. (2002). Infection due to extended-spectrum beta-lactamase-producing Salmonella enterica subsp. enterica serotype infantis in a neonatal unit. *The Journal of Pediatrics*, *141*(3), 381–387.

Peterson, G., Kumar, A., Gart, E., & Narayanan, S. (2011). Catecholamines increase conjugative gene transfer between enteric bacteria. *Microbial Pathogenesis*, *51*(1–2), 1–8. https://doi.org/10.1016/j.micpath.2011.03.002

Ploy, MC., Chainier, D., Tran Thi, N. H., Poilane, I., Cruaud, P., Denis, F., Collignon, A., Lambert, T. (2003). Integron-associated antibiotic resistance in *Salmonella enterica* serovar typhi from Asia. Antimicrob Agents Chemother 47:1427-1429

Poppe, C., Smart, N., Khakhria, R., Johnson, W., Spika, J., & Prescott, J. (1998). Salmonella typhimurium DT104: A virulent and drug-resistant pathogen. *Canadian Veterinary Journal*.

Poutanen, S. M., & Low, D. E. (2003). Is it time to change fluoroquinolone MIC breakpoints for Salmonella spp.? *Clinical Microbiology Newsletter*, *25*(13), 97–102. https://doi.org/10.1016/S0196-4399(03)80027-2

Pradel, E., Parker, C. T., & Schnaitman, C. A. (1992). Structures of the rfaB, rfaI, rfaJ, and rfaS genes of Escherichia coli K- 12 and their roles in assembly of the lipopolysaccharide core. *Journal of Bacteriology*, *174*(14), 4736–4745. https://doi.org/10.1128/jb.174.14.4736-4745.1992

Randall, L. P., Cooles, S. W., Osborn, M. K., Piddock, L. J. V, & Woodward, M. J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy*, *53*(2), 208–216. https://doi.org/10.1093/jac/dkh070

Rhen, M. (2007). *Salmonella: molecular biology and pathogenesis*. Norfolk: Horizon bioscience.

Roberts, M. C. (2002). Resistance to Tetracycline,Macrolide-Lincosamide-Streptogramin, Trimethoprim,and Sulfonamide Drug Classes. *Molecular Biotechnology*, *20*(3), 261–284. https://doi.org/10.1385/MB:20:3:261

Roberts, M. C. (2005). Update on acquired tetracycline resistance genes. *FEMS Microbiology Letters*. https://doi.org/10.1016/j.femsle.2005.02.034

Robicsek, A., Jacoby, G. A., & Hooper, D. C. (2006). The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(06)70599-0

Roncero, C., & Casadaban, M. J. (1992). Genetic analysis of the genes involved in synthesis of the lipopolysaccharide core in Escherichia coli K-12: Three operons in the rfa locus. *Journal of Bacteriology*, *174*(10), 3250–3260. https://doi.org/10.1128/jb.174.10.3250-3260.1992

Rubinstein, E., Lagacé-Wiens, P., (2017). 144-Quinolones. In Infectious Diseases (Fourth Edition). 1239-1248.e2. https://doi.org/10.1016/B978-0-7020-6285-8.00144-1.

Rychlik, I., Gregorova, D., & Hradecka, H. (2006). Distribution and function of plasmids in Salmonella enterica. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2005.10.030

Samadi, N., Pakzad, I., Sefidan, A. M., Hosainzadegan, H., & Tanomand, A. (2015). Study of aminoglycoside resistance genes in enterococcus and salmonella strains isolated from ilam and milad hospitals, Iran. *Jundishapur Journal of Microbiology*, *8*(4). https://doi.org/10.5812/jjm.18102

Schnaitman, C. a, & Klena, J. D. (1993). Genetics of lipopolysaccharide biosynthesis in enteric bacteria. *Microbiological Reviews*, *57*(3), 655–682.

Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews*. https://doi.org/10.1016/j.femsre.2004.04.001

Seth, R., & Seth, S. D. (2009). Chapter74/ Chloromphenicol. In *Textbook of Pharmacology*, *3/e*(3rd ed.). New Delhi, India:Elsevier.

Shanahan, P. M., Jesudason, M. V, Thomson, C. J., & Amyes, S. G. (1998). Molecular analysis of and identification of antibiotic resistance genes in clinical isolates of Salmonella typhi from India. *Journal of Clinical Microbiology*, *36*(6), 1595–1600.

Shaw, K. J., Rather, P. N., Hare, R. S., & Miller, G. H. (1993). Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiological Reviews*, *57*(1), 138–163. https://doi.org/10.1016/j.abb.2004.09.003

Shibata, N., Doi, Y., Yamane, K., Yagi, T., Kurokawa, H., Shibayama, K., … Arakawa, Y. (2003). PCR Typing of Genetic Determinants for Metallo-β-Lactamases and Integrases Carried by Gram-Negative Bacteria Isolated in Japan, with Focus on the Class 3 Integron. *Journal of Clinical Microbiology*, *41*(12), 5407–5413. https://doi.org/10.1128/JCM.41.12.5407-5413.2003

Singh, O. V. (2017). *Foodborne pathogens and antibiotic resistance*. Hoboken, NJ: John Wiley&Sons, Inc.

Sköld, O. (2000). Sulfonamide resistance: Mechanisms and trends. *Drug Resistance Updates*. https://doi.org/10.1054/drup.2000.0146

Sommer, M. O. A., Dantas, G., & Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science*, *325*(5944), 1128–1131. https://doi.org/10.1126/science.1176950

Spratt, B. (1994). Resistance to antibiotics mediated by target alterations. *Science*, *264*(5157), 388–393. https://doi.org/10.1126/science.8153626

Spratt, B. G., & Cromie, K. D. (1988). Penicillin-binding proteins of gram-negative bacteria. *Rev Infect Dis*, *10*(4), 699–711. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3055170

Stokes, H. W., & Gillings, M. R. (2011). Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. *FEMS Microbiology Reviews*. https://doi.org/10.1111/j.1574-6976.2011.00273.x

Thomas, C. M. and Smith, C. A. (1987). Incompatibility group P plasmids: genetics, evolution and use in genetic manipulation. Annu. Rev. Microbiol. 41, 77^101.

Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro1234

Threlfall, E., Frost, J., Ward, L., Rowe, B. (1994). Epidemic in cattle and humans of Salmonella typhimurium DT 10 with chromosomally integrated multiple drug resistance *Veterinary Record* 134, 577.

Threlfall, E. J., Hampton, M. D., Schofield, S. L., Ward, L. R., Frost, J. A., & Rowe, B. (1996). Epidemiological application of differentiating multiresistant Salmonella typhimurium DT104 by plasmid profile. *Communicable Disease Report. CDR Review*, *6*(11), R155-9.

Threlfall, E. J., Rowe, B., Ferguson, J. L., & Ward, L. R. (1986). Characterization of plasmids conferring resistance to gentamicin and apramycin in strains of Salmonella typhimurium phage type 204c isolated in Britain. *Journal of Hygiene*, *97*(3), 419–426. https://doi.org/10.1017/S0022172400063609

Threlfall, E. J., Ward, L.R., Skinner, J.A., and Rowe, B. (1997). Increase in multiple antibiotic resistance in nontyphodial salmonella from humans in England and Wales: a comparison of data for 1994 and 1996. Microb. Drug. Resist. 3: 263-266.

Tolmasky, M. E. (2013). Plasmids. In Brenner's Encylopedia of Genetics (Second Edition). 362-366. https://doi.org/10.1016/B978-0-12-374984-0.01174-8.

Tosini, F., Visca, P., Luzzi, I., Dionisi, A. M., Pezzella, C., Petrucca, A., & Carattoli, A. (1998). Class 1 integron-borne multiple-antibiotic resistance carried by IncFI and IncL/M plasmids in Salmonella enterica serotype typhimurium. *Antimicrobial Agents and Chemotherapy*, *42*(12), 3053–3058.

Van Hoek, A. H. A. M., Mevius, D., Guerra, B., Mullany, P., Roberts, A. P., & Aarts, H. J. M. (2011). Acquired antibiotic resistance genes: An overview. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2011.00203

Van Meervenne, E., Van Coillie, E., Kerckhof, F. M., Devlieghere, F., Herman, L., De Gelder, L. S. P., … Boon, N. (2012). Strain-specific transfer of antibiotic resistance from an environmental plasmid to foodborne pathogens. *Journal of Biomedicine and Biotechnology, 2012*. https://doi.org/10.1155/2012/834598

Villa, L., Mammina, C., Miriagou, V., Tzouvelekis, L. S., Tassios, P. T., Nastasi, A., & Carattoli, A. (2002). Multidrug and broad-spectrum cephalosporin resistance among Salmonella enterica serotype Enteritidis clinical isolates in southern Italy. *Journal of Clinical Microbiology*, *40*(7), 2662–2665. https://doi.org/10.1128/JCM.40.7.2662-2665.2002

von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., … Wolffs, P. F. G. (2016). Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Frontiers in Microbiology*, *7*, 173. https://doi.org/10.3389/fmicb.2016.00173

Walsh, C. (2003). "Antibiotics, Action, Origins, Resistance". pp. 3-49. ASM Press, Washington DC.

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature*. https://doi.org/10.1038/35021219

Walsh, C. (2003). Where will new antibiotics come from? *Nature Reviews Microbiology*, *1*(1), 65–70. https://doi.org/10.1038/nrmicro727

Wang, P., Xiong, Y., Lan, R., Ye, C., Wang, H., Ren, J., … Xu, J. (2011). PO157-Sal, a novel conjugative plasmid detected in outbreak isolates of Escherichia coli O157:H7. *Journal of Clinical Microbiology*, *49*(4), 1594–1597. https://doi.org/10.1128/JCM.02530-10

Watanabe, T., Arai, T., & Hattori, T. (1970). Effects of cell wall polysaccharide on the mating ability of salmonella typhimurium. *Nature*, *225*(5227), 70–71. https://doi.org/10.1038/225070a0

Waters, V. L. (1999). Conjugative transfer in the dissemination of beta-lactam and aminoglycoside resistance. *Frontiers in Bioscience : A Journal and Virtual Library*, *4*, D433-56. https://doi.org/10.2741/Waters

Watkins, R. R., Bonomo, R. A., (2017). 140- β-Lactam Antibiotics. In Infectious Diseases (Fourth Edition). 1203-1216.e2. https://doi.org/10.1016/B978-0-7020-6285-8.00140-4.

White, D. G., Goldman, J. D., Demple, B., & Levy, S. B. (1997). Role of the acrAB locus in organic solvent tolerance mediated by expression of marA, soxS, or robA in Escherichia coli. *Journal of Bacteriology*, *179*(19), 6122–6126. https://doi.org/10.1128/jb.179.19.6122-6126.1997

Yan, J. J., Ko, W. C., Chiu, C. H., Tsai, S. H., Wu, H. M., & Wu, J. J. (2003). Emergence of ceftriaxone-resistant Salmonella isolates and rapid spread of plasmid-encoded CMY-2-like cephalosporinase, Taiwan. *Emerging Infectious Diseases*, *9*(3), 323–328. https://doi.org/10.3201/eid0903.010410

YONEYAMA, H., & KATSUMATA, R. (2006). Antibiotic Resistance in Bacteria and Its Future for Novel Antibiotic Development. *Bioscience, Biotechnology, and Biochemistry*, *70*(5), 1060–1075. https://doi.org/10.1271/bbb.70.1060

Zatyka, M., & Thomas, C. M. (1998). Control of genes for conjugative transfer of plasmids and other mobile elements. *FEMS Microbiology Reviews*. https://doi.org/10.1016/S0168-6445(97)00061-2

ZINDER, N. D., & LEDERBERG, J. (1952). Genetic exchange in Salmonella. *Journal of Bacteriology*, *64*(5), 679–699.

Ziegelin, G., Furste, J. P., & Lanka, E. (1989). TraJ protein of plasmid RP4 binds to a 19-base pair invert sequence repetition within the transfer origin. *Journal of Biological Chemistry*, *264*(20), 11989–11994.

APPENDIX A

CHEMICALS USED IN THE STUDY

Table 19 List of the chemicals.

10X PCR Buffer (Genoks)
Agar Bacteriological (Agar No.1) (Oxoid)
Agarose (AppliChem)
Brain Heart Infusion (BHI) Broth (Oxoid)
Buffered Peptone Water (Oxoid)
dNTPs (10 mM) (Genoks)
Ethanol (Sigma-Aldrich)
Ethidium Bromide (10 mg/µl)(Sigma Aldrich)
Ethylenediaminetetraacetic acid (EDTA) Stock Solution [0.5 M] (Bioshop)
Isopropanol (Sigma-Aldrich)
Ladders (Scientific Laboratory Supplies)
Loading Buffer (6X) (Genoks)
Luria-Bertani (LB) Broth (Sigma-Aldrich)
$MgCI_2$ (25mM) (Genoks)
Mueller-Hinton Agar (Oxoid)
Mueller-Hinton Broth (Oxoid)
MyTaq DNA Polymerase (Bioline)
MyTaq Red Reaction Buffer (Bioline)
Primers (Sentegen)
Qiagen Plasmid Mini Kit (Qiagen)
Sodium Hydroxide, NaOH (Merck)
Tetracycline (Sigma-Aldrich)
Tris (Merck)
Tsg DNA Polymerase (Lamda Biotech)
Xylose Lysine Deoxycholate (XLD) Agar (Oxoid)

APPENDIX B

PREPARATION OF BUFFERS AND SOLUTIONS

1. Preparation of Tetracycline Stock Solution

120 mg of tetracycline was dissolved in 4 ml of sterile sterile H_2O and 4 ml of ethanol under fume hood. The test tube was wrapped in foil.

2. Preparation of Buffered Peptone Water

10 g of buffered peptone water mixture was dissolved in 500 ml of ddH_2O. Buffered peptone water solution was sterilized in autoclave at 121 °C for 15 minutes.

3. Preparation of Xylose Lysine Deoxycholate (XLD) Agar

26.5 g of XLD agar was dissolved in 500 ml of ddH_2O. After the solution was boiled without overheating, it was transferred to water bath at 50°C for 25 minutes. It was poured into the petri dishes nearby Bunsen burner.

4. Preparation of Xylose Lysine Deoxycholate (XLD) Agar Containing 10 µg/ml Tetracycline

26.5 g of XLD agar was dissolved in 500 ml of ddH_2O. After the solution was boiled without overheating, it was transferred to water bath at 50°C for 25 minutes. 0.333 ml of stock solution was added directly to 500 ml of cooling XLD agar. The solution was mixed for 15 minutes. It was poured into the petri dishes nearby Bunsen burner.

5. Preparation of Brain Heart Infusion (BHI) Agar

37 g of BHI broth and 15 g of bacteriological agar were dissolved in 1 liter of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes. After cooling in the water bath at 50°C for 25 minutes, it was poured into the petri dishes nearby Bunsen burner.

6. Preparation of Brain Heart Infusion (BHI) Broth

37 g of BHI broth was dissolved in 1 liter of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes.

7.Preparation of Luria-Bertani (LB) Broth

25 g of LB broth was dissolved in 1 liter of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes.

8. **Preparation of Luria-Bertani (LB) BrothContaining 10 µg/ml Tetracycline**

12.5 g of LB broth was dissolved in 500 ml of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes. After cooling in the water bath at 50°C for 25 minutes, 0.333 ml of stock solution was added directly to the LB broth.

9. Preparation of pH 8.0 Tris-EDTA Buffer

Firstly, to prepare 1M Tris solution, 60.57 g of Tris (MWT 121.4 g/mol) was dissolved in 500 ml ddH_2O by a magnetic stirrer. The pH of the solution was done 8.0 using HCI. To prepare 0.5M EDTA, 18.6 g of Diaminoethane tetraacetic acid was mixed with 100 ml ddH_2O by a magnetic stirrer with a moderate heat. The pH of the solution was brought to 8.0 by using NaOH. 5 ml of 1M Tris pH 8.0, 1 ml of 0.5M EDTA pH 8.0, and 494 ml of ddH_2O were mixed and sterilized in autoclave at 121 °C for 15 minutes.

10. Preparation of Agarose Gel

1.7 g and 0.7 g of agarose were dissolved in 100 ml of 0.5 X TBE solution individually. Until the solution became clear, it was brought to boiling temperature on a magnetic stirrer. After cooling at room temperature for 15 minutes, it was poured into the gel tank.

11. Preparation of Mueller-Hinton Agar

19 g of Mueller-Hinton agar was dissolved in 500 ml of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes. After cooling in the water bath at 50°C for 25 minutes, it was poured into the petri dishes nearby Bunsen burner.

12. Preparation of Mueller-HintonBroth

10.5 g of Mueller-Hinton broth was dissolved in 500 ml of ddH_2O. The solution was sterilized in autoclave at 121 °C for 15 minutes.

APPENDIX C

PHENOTYPIC CONJUGATION RESULTS OF *ESCHERICHIA COLI* ISOLATES

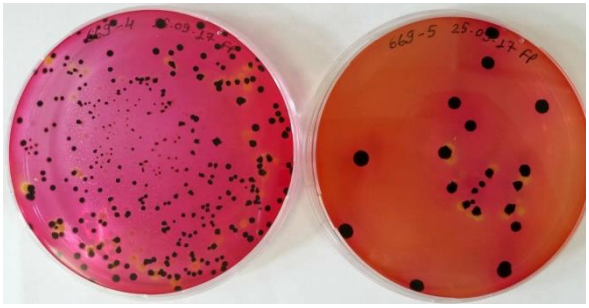

Figure 21 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-669+MET-A1-018).

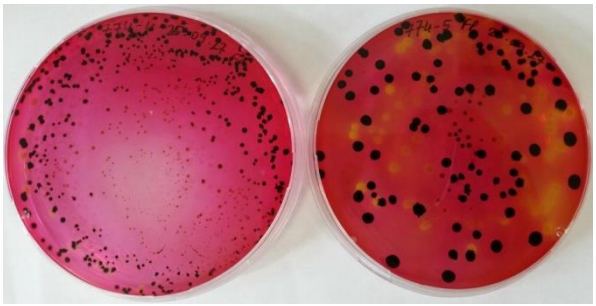

Figure 22 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-774+MET-A1-018).

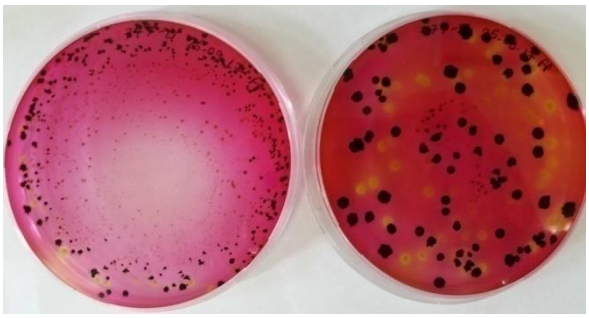

Figure 23 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-777+MET-A1-018).

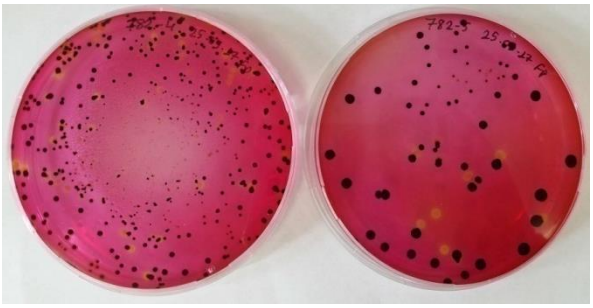

Figure 24 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-782+MET-A1-018).

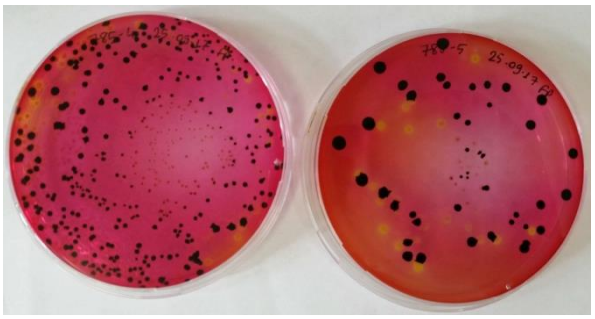

Figure 25 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-785+MET-A1-018).

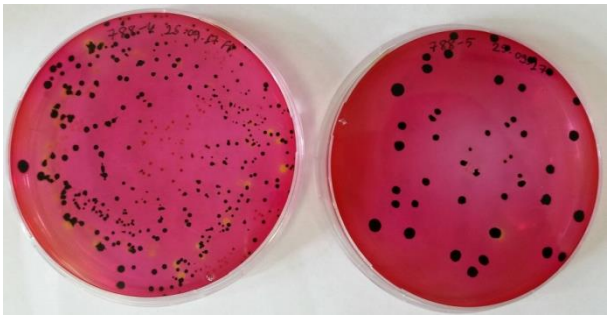

Figure 26 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-788+MET-A1-018).

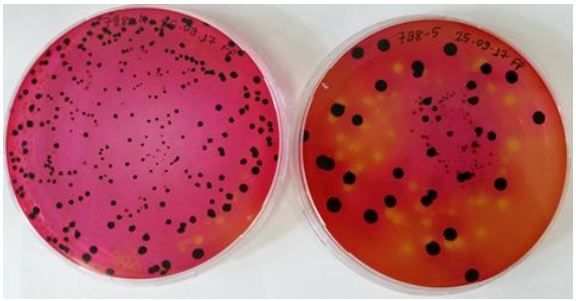

Figure 27 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-798+MET-A1-018).

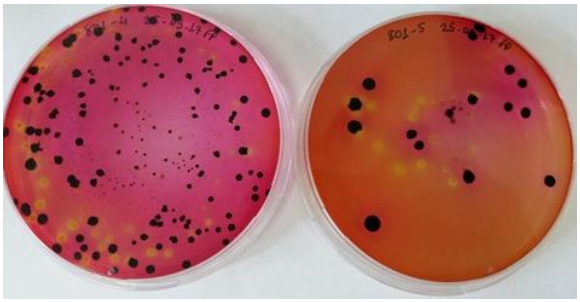

Figure 28 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-801+MET-A1-018).

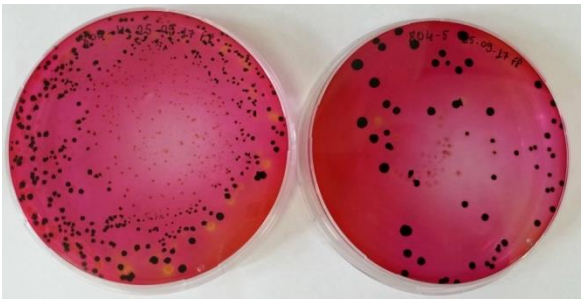

Figure 29 Conjugated *E. coli* colonies on XLD agars containing 10^{-4} and 10^{-5} diluted mating solutions from left to right respectively (MET-S1-804+MET-A1-018).