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Molecular Characterization of Antibacterial Resistant *Salmonella* Isolated from Food Sources in Palestine

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Dedication

This thesis is dedicated to

My Great Parents (Ahmad and Samera)

Who have provided me with love, strength and confidence, deep thanks for always being there for me

My flowers of my life, Brothers and Sister

Who have all the time supported me to continue and to reach my goal

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Last but not least I would like to express my thanks and gratitude to my beloved family and friends for my encouragement and giving me all the love.

الاقرار

أنا الموقعة أدناه موقعة الرسالة التي تحمل العنوان:

Molecular Characterization of Antibacterial Resistant *Salmonella* Isolated from Food Sources in Palestine

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researchers own work, and has not been submitted elsewhere for any other degree or qualification.

Students Name: **إسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

ECDC	European center for disease prevention and control
MDR	Multi drug resistant
SGI1	Salmonella genomic island 1
PCR	Polymerase chain reaction
XLD	Xylose lysine desoxycholate
NB	Nutrient Broth
NA	Nutrient Agar
MHA	Mueller -Hinton agar
TSI	Triple sugar iron
LI	Lysine Iron
H_2S	Hydrogen sulfide
$BaCl_2 \cdot 2H_2O$	Barium chloride dehydrate
CFU	Colony forming units
H_2SO4	Sulfuric acid
CLSI	Clinical and Laboratory Standard Institute
AMP	Ampicillin
TE	Tetracycline
SXT	Trimethoprim/ Sulfamethoxazole
S	Streptomycin
C	Chloramphenicol
AK	Amikacin
CAZ	Ceftazidime
CIP	Ciprofloxacin
CN	Gentamicin
CTX	Cefotaxime
ETP	Ertapenem
FEP	Cefepime
FOX	Cefoxitin
N	Neomycin
NOR	Norfloxacin
CPD	Cefpedoxime
RPM	Revolutions Per Minute
CH_3COONa	Sodium Acetate
CS	Conserved Segment
Mg^{++}	Magnesium ion
Ca^{++}	Calcium ion
MOH	Ministry of Health
APCC center	Analysis, Poison Control and Calibration center

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Abstract

Introduction: Antibiotics resistance is an escalating health concern all over the world. The mis- and over-use of the antibiotics is not only noticed in human but also through the food chain. As one of the main food-borne pathogens; *Salmonella* is inclined for transferring the antibiotic resistance determinants. Currently, Salmonella genomic island 1 (SGI 1) and integrons are the most commonly studied determinants.

Materials and Methods: A total of 128 *Salmonella* isolates from different sources, mainly food samples, in the Palestinian market were collected and provided by Ministry of Health central lab and the Analysis, Poison Control and Calibration Center. Antibiotic susceptibility of the isolates was determined by disc diffusion method. Polymerase chain reaction (PCR) was used to detect class 1 and 2 integrons, as well as SGI 1. Sequencing was used to confirm the SGI1 and integrons positive isolates. Antibiotic resistance profile varied among the isolates that showed multi-drug resistant (MDR) isolates.

Rustles: Molecular characterization revealed that most of the isolates harbored class 1 integron with varied gene cassettes. Class 2 integron and

SGI1 were detected in few isolates. Interestingly, antibiotic resistance profile is highly variable at molecular level in correlation to its phenotypic characterization.

Conclusion: Antibiotic resistance determinants in *Salmonella* isolated from the different food sources arises the alarm for their direct role as a food-borne pathogen. Moreover, the possibility to transfer the antibiotics resistance determents horizontally that may be inclined indirectly in the super bug theory. The surveillance and monitor of the prevalence of antimicrobial resistance genes from other bacterial isolates and the antibiotics usage need strict protocols to control the emergence of the MDR bacterial isolates.

Chapter One
Introduction and Literature review

1.1. General background

The *Salmonella* subspecies are gram negative, facultative anaerobic, with peritrichous flagella, non- spore forming rods belonging to the family *Enterobacteriaceae*. The *Salmonella* genus consists of only two species, *S. enterica* and *S. bongori* and 6 subspecies. In addition, they contain more than 2500 different serotypes that are distinguished by their O, H, and Vi antigens (Grimont and Weill, 2007; Issenhuth-Jeanjean *et al.*, 2014).

Salmonella spp. generally range from 0.7 to 15 μm in width and 2 to 5 μm in length. They are chemoorganotrophic, catabolize glucose and other carbohydrate with production of acid and gas, but unable to metabolize lactose and sucrose. Generally, most *Salmonella* spp. can produce hydrogen sulfide, this can be confirmed by a triple sugar iron test that contains ferrous sulfate. Moreover, *Salmonella* will grow over a wider range of temperature from 2 °C to 54 °C. They grow well at room temperature, but their optimum temperature is about 37 °C. In addition, *Salmonella* grow at pH values ranging from 4.5 to 9.5, but optimal pH for growth is 6.5 to 7.5 (Doyle *et al.,* 1997). To be host specific, *Salmonella* serotypes involves a number of interconnected factors to survive in different host environments such as pH, temperature, site of attachment, host immune system and the pathogen itself (Foley *et al.*, 2011).

Many *Salmonella* serotypes, such as *S.* Typhimurium, *S.* Newport, *S.* Enteritidis and *S.* Heidelberg have the ability to infect broad host range (Foley *et al.,* 2008). Contrariwise, other serotype like *S.* Dublin, *S.* Typhi, *S.* Paratyphi, *S.* Gallinarum and *S.* Choleraesuis have restricted host ranges, which are associated primarily with one or a few hosts (Uzzau *et al.,* 2000).

Salmonella serotypes clearly seem to vary in their pathogenic potential for humans. Serotype distributions often vary vastly between human and animal populations as well as among different animal populations in the same geographic area. However, serotype-specific differences in virulence have been characterized in some cases. For example, in competition experiments with *Salmonella enterica* subspp., reptile-associated *S.* Arizona and *S.* Diarizonae showed a significantly reduced ability to colonize and persist in the intestine of BALB/c mice, clearly suggesting virulence differences (Kingsley *et al.,* 2000). Higher human infection rates caused by these broad host range over 50% of human cases of *S.* Typhi, *S.* Dublin, *S.* Paratyphi A, and *S.* Choleraesuis infections. Conversely, Lower percentages of infections by the broad-host-range serotypes, such as *S.* Typhimurium, *S.* Heidelberg and *S.* Enteritidis (Vugia *et al.*, 2004).

Salmonella strains that causes enteric fever could be typhoid fever or paratyphoid fever are related to *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B, and Paratyphi C, which are collectively known as typhoidal *Salmonella* strains, whereas other serovars are referred to collectively as non-typhoidal *Salmonella* (Voetsch *et al.,* 2004; Hendriksen *et al.,* 2004). In 2010, typhoid and paratyphoid fever were included in the Global Burden

of Disease. In 2000, typhoid fever was estimated to cause about 21.7 million illnesses and 216,000 deaths and paratyphoid fever 5.4 million illnesses (Crump *et al.,* 2015). Salmonellosis (food poisoning) is usually considered as an asymptomatic or self-limiting illness, but it can also become invasive and fatal, especially for patients who are immunocompromised. *Salmonella enterica* causes around 94 million cases, resulting in155000 deaths each year (World Health Organization, 2014). For instance, in Europe, approximately 29 % of the reported human salmonellosis cases are attributed to *Salmonella enterica* subspp. (ECDC, 2015). Another study in the United States of America alone, *Salmonella* is estimated to cause 1.4 million cases of salmonellosis, resulting in 17,000 hospitalizations and about 600 deaths each year (Voetsch *et al.,* 2004; Hendriksen *et al.,* 2004).

Salmonella infections can be chronic or invasive, these require antimicrobial treatment to prevent further morbidity or motility (Alcaine *et al.*, 2007). The first line antibiotic treatment is typically a fluoroquinolone, like ciprofloxacin or a third-generation cephalosporin β-lactam such as ceftriaxone (Mandal, 1990; Guerrant *et al.,* 2001; Habib, 2004). If resistance happens to the first line treatment of *Salmonella* infections, then alternative second line antimicrobials may be used, such as aminoglycosides, or folic acid pathway inhibitors like sulfisoxazole or sulfamethoxazole with or without trimethoprim (Guerrant *et al.,* 2001). The last line of treatment in multi-drug resistant (MDR) cases are usually

the aminoglycoside, amikacin or the carbapenems, imipenem or meropenem (Mundy *et al.*, 2000; Alanis, 2005; Foley and Lynne, 2008).

Pathogenicity factors of *Salmonella* spp. include type III secretion system (T3SS) that help in invading both phagocytic and non- phagocytic cells, cell surface lipopolysaccharids (LPSs), toxins, vacuole, flagella, fimbria and non-fimbrial adhesion on the surface. *Salmonella* spp. have gene clusters that give the ability to colonize the host, such as *Salmonella* Pathogenicity Islands (SPIs) (Ibarra and Steele-Mortimer, 2009).

1.2. Literature review

1.2.1. Food- borne infection

The transfer of antibiotic resistant bacterial strains to humans from food sources is the challenging issue as the control of usage of antibiotics in treating the food sources has limited control. The Animal Health Institute in the USA has estimated that more than 8000 tons of antimicrobials are used in food production, corresponding to 4–25 g antibiotics/ ton of feed (Carattoli, 2008).

Control of food-borne illness is complicated by several factors. The first is underreporting although foodborne illnesses can be severe or even fatal, milder cases are often not detected through routine surveillance. Second, many pathogens transmitted through food are also spread through person to person or from water, thus ambiguity of the role of foodborne transmission. Finally, some proportion of foodborne illness is caused by pathogens or

agents that have not yet been identified and thus cannot be diagnosed (Mead *et al.*, 1999).

Food-borne infections and diseases caused by pathogens that contaminate food, the major food borne pathogens include Gram-negative bacteria such as *E. coli* and *Salmonella* spp. (Adwan *et al.,* 2015), which are responsible for 14 million illnesses, 60,000 hospitalizations and 1800 deaths annually (Wang *et al*., 2012). For instance, the outbreak of food-borne disease in Taiwan increased rapidly from 121 in 1995 to 177 in 1996 and since then the frequency of disease keeps increasing (Chiou, 2000). In addition, about a quarter of the population is at higher risk for food-borne diseases nowadays (Oliver *et al.,* 2005).

1.2.2. Food is the main source of *Salmonella* infections

Salmonella is one of the most prevalent food-borne zoonotic pathogen (Threlfall, 2000; Chousalkar *et al.,* 2010). Among the different bacteria responsible for food-borne diseases, *Salmonella enterica* represents one of the leading causes of such infections with *S.* Typhimurium and *S.* Enteritidis being the most common serovars in the USA and Europe (Weill *et al.,* 2006), However, while in Italy it is the serovar *S.* Typhimurium, which is prevalent, while in Europe *S.* Enteritidis still remains the predominant serovar (Graziani *et al.*, 2008).

Another study that investigated the source of *Salmonella* infections in humans shows that approximately 55% (range 32-88%) of human *Salmonella* cases are food-borne infection, 14% (range 3-26%) are travel-

related, 13% (range 0-29%) are acquired through environmental sources, 9% (range 0-19%) are attributable to direct animal contact and 9% (range 0-19%) occur due to direct human-to-human transmission (Aarestrup and Nørrung, 2008; Galindo and Cooke, 2007).

1.2.3. Food contamination by *Salmonella*

Food borne pathogens have been commonly identified from contamination of various food samples such as milk, chicken, veal, beef and lamb meat, as well as in food production animals such as cattle, chickens, pigs and cows (Xu *et al*., 2012). For instance, *Salmonella* was detected in Gaza from food samples (7.3%) in fresh chicken, fresh minced meat, frozen minced meat, fresh meat and frozen meat with prevalence 13.3%, 10%, 6.7%, 3.3% and 3.3% respectively (El Nakhala, 2013). In Australia, the egg industry leads to food poisoning by *Salmonella* specially in uncooked or partially cooked food containing raw eggs accounted for 14% of food-borne outbreaks in 2006, 13% in 2007, and 28% in the first quarter of 2008 (OzFoodNet Working Group, 2010). Food-borne outbreaks also can arise from human or animal faeces contaminating the surface of fruits and vegetables (World Health Organization, 2014). In addition, food borne diseases are often associated with the consumption of raw or undercooked foods such as seafood and poultry (Omurtag *et al.,* 2013; Oliver *et al.,* 2005).

Antibiotic resistant bacteria, which are isolated from the food arise in several ways, the first way is the presence of antibiotic resistant bacteria on food selected by the use of antibiotic agricultural production and the second

way is the presence of resistant genes in bacteria that are added during the processing of food (Verraes *et al*., 2013).

1.2.4. Appearance of MDR *Salmonella* strains

Over the past years, the number of human infections caused by antimicrobial resistant bacteria increased, which makes treatment more difficult (Alanis, 2005). Gram-negative bacteria, in particular the *Enterobacteriaceae*, are adapted to exchange of genetic information and antibiotic resistance by acquisition of genes from a shared pool (Iredell & Partridge, 2010). The escalation of antimicrobial resistant *Salmonella* strains resulted in the need for careful *Salmonella* infection treatment protocols to target the drug of choice (Carattoil, 2003). During the late 1990s and early 2000s, MDR *Salmonella* emerged, and since then they have expanded worldwide, for example, in *Salmonella enterica* serotype Typhimurium phage type DT104, is the second most prevalent *Salmonella* serotype isolated in England and Wales (Threlfal *et al.* 1997; Threlfall *et al.*, 1996). Furthermore, MDR *Salmonella* increases the prevalence in the United States (Glynn et al., 1998; Hosek *et al.*, 1997) and Canada (Ng et al., 1997). According to the Centers for Disease Control and Prevention, in the United States 800,000 to 4 million *Salmonella* infections occur annually, of which approximately 1 to 5% are confirmed due to *S. enterica* serotype Typhimurium (Glynn et al., 1998).

Outbreaks of MDR *S.* Typhimurium DT104 have also been reported in animal's populations such as poultry, cow, and swine in numerous

countries (Cody *et al.,* 1999; Davies *et al.,* 1996; Mølbak *et al.,* 1999). The genomic element that carries resistance to antimicrobial drugs such as ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline play a major role on the emergence of the MDR *Salmonella*, which have been associated with a higher risk of invasive infection, higher frequency and duration of hospitalization, longer illness, and increased risk of death as compared to infections caused by susceptible strains (World Health Organization, 2014). Furthermore, the increasing incidence of trimethoprim and nalidixic acid resistance together with reduced susceptibility to ciprofloxacin (fluoroquinone) is of particular concern (Cormican *et al.,* 1998; Heurtin-Le Corre *et al.,* 1999).

1.2.5. Change of susceptibility of *Salmonella* strains with time

Susceptibility *Salmonella* strains to drugs can change with time. For instance, in Spain, antibiotic resistance of different *Salmonella* servars such as *S.* Enteritidis and *S.* Typhimurium (the main types responsible for human salmonellosis) isolated in 1993 from chicken increased from 3.98 antibiotics to 5 antibiotics for each strain in 2006 (Álvarez-Fernández *et al.,* 2012). In Great Britain between 1994 and 2010, *S.* Typimurium was the main *Salmonella* serovar isolated from pigs and its resistance increased from 27.2% in 1994 to 58.3% in 2010 (Mueller-Doblies *et al.,* 2013).

Prior to the mid- 1970 *S.* Typhi serovar were known to have resistance to chloramphenicol, streptomycin, sulfonamide and tetracycline (Rowe *et al.,* 1997). The development of chloramphenicol resistance leads to increased

use of other antibiotics such as, ampicillin and trimethoprim-sulfamethoxazole. However, trimethoprim- sulfamethoxazole until 1975 remained effective in France (Zaki and Karande, 2011).

The resistance to certain antibiotics can lead to resistance to other antibiotics that are chemically related. Multidrug resistance in bacterial strains results from environmental factors including pH, anaerobic atmosphere, cations such as $Mg^{++}$and Ca^{++}. Moreover, Other mechanisms can exhibit resistance to antibiotics such as production of enzymes that destroy the active antibiotic, change their permeability barrier and altered structural target for the antibiotic (Reddy, 2009).

1.2.6. Principal biological pathways used in the development of resistance in bacterial strains

There are two principal biological pathways involved in the evolution and development of resistance in bacterial strains. First, intrinsic or innate resistance (vertical transmission), which is natural. During the evolutionary process, bacterial cells accumulate genetic errors in existing genes and transfer the resistant genes to progeny cells via vertical gene transfer (VGT) (Reddy, 2009; Holmes *et al*. 2016; Apata, 2009). Figure 1.1a. shows the vertical transmission **(**Dantas, Sommer, 2014).

The second, acquired resistance (horizontal transmission) involves genetic exchanges within and between bacterial species that lead to resistance to some antibiotics acquired by mutation or genetic exchange such as conjugation, transposition, transformation, transduction and lysogeny

(Reddy, 2009; Holmes *et al.* 2016; Apata, 2009). Figure 1.1b. shows the horizontal transmission **(**Dantas, Sommer, 2014**)**.

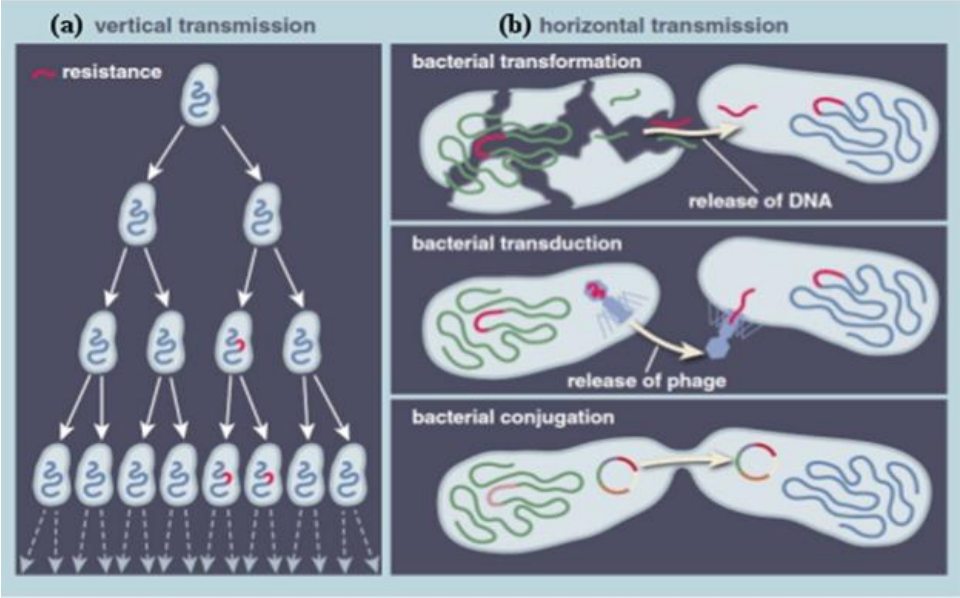


Figure 1.1: Principal biological pathways involved in the evolution and development of antibiotic resistance. (A) Vertical transmission (B) Horizontal transmission (Dantas, Sommer, 2014).

Genetic origin of antibiotic resistance may be chromosomal or extrachromosomal. In chromosomal origin, mainly due to spontaneous mutation in a gene locus that determine susceptibility to antibiotics. Whereas extrachromosomal genetic elements (plasmids) may carry genes for resistance to different antibiotics and heavy metals. Plasmids are responsible for the spread of multiple drug resistant (MDR). Moreover, plasmids may code for enzymes that destroy or inactivate an antibiotic. For instance, plasmids carrying genes for β-lactamases confer resistance to penicillin or plasmid genes for the production of acetyl transferase confer resistance to chloramphenicol or carry genes for enzymes that determine the active transport of tetracycline's across the cell membranes (Reddy, 2009).

1.2.7. Emergence of multi -drug resistance in *Salmonella* strains

Over the last decade, high increase in antibiotic resistance in *Salmonella* strains was found. Special multidrug- resistant strains have led to public health problems around the world (Salehi *et al.*, 2005). The overuse of antibiotics in different fields has led to increase in the prevalence of MDR *Salmonella* strains from poultry to humans through the food chain or by physical contact (Guerra *et al.*, 2000). In addition, infections caused by antibiotic resistant bacteria have also been shown to result in increased morbidity and mortality in humans and animals (Mundy *et al.*, 2000; Alanis, 2005; Foley and Lynne, 2008).

In the late 1980s, multiple drug resistance (MDR), defined as resistance to trimethoprim- sulfamethoxazole, ampicillin and chloramphenicol, was reported from various countries (Wang *et al.*, 1989; Mirza *et al.*, 1996). In Vietnam in 1993- 1996, 80% of *Salmonella* Serovar Typhi isolates were reported to be MDR (Wain *et al.*, 1999). In Europe, between 2002 to 2003, a 22% prevalence of MDR phenotype in *Salmonella* Serovar Typhi isolates was documented (Cooke *et al.*, 2007). Another study Nigeria and Kenya in 2004- 2006, where the *Salmonella* serovar Typhi was prevalence of was MDR 61% and 70%, respectively (Akinyemi *et al.*, 2005; Mengo *et al.*, 2010). In India, it was found that MDR *Salmonella* Serovar Typhi increased from 34% in 1999 to 66% in 2005 (Kumar *et al.*, 2008).

An increasing prevalence of antibiotic resistance among non-typhoidal *Salmonella* strains over recent decades have also been reported. The most

important development of MDR has been observed in *Salmonella* Serovar Typhimurium, which began to appear in the early 1980s in the United Kingdom (Threlfall, 2002). Figure 1.2. shows the resistant genes in *Salmonella* Typhimurium DT104 (Briggs and Fratamico, 1999).

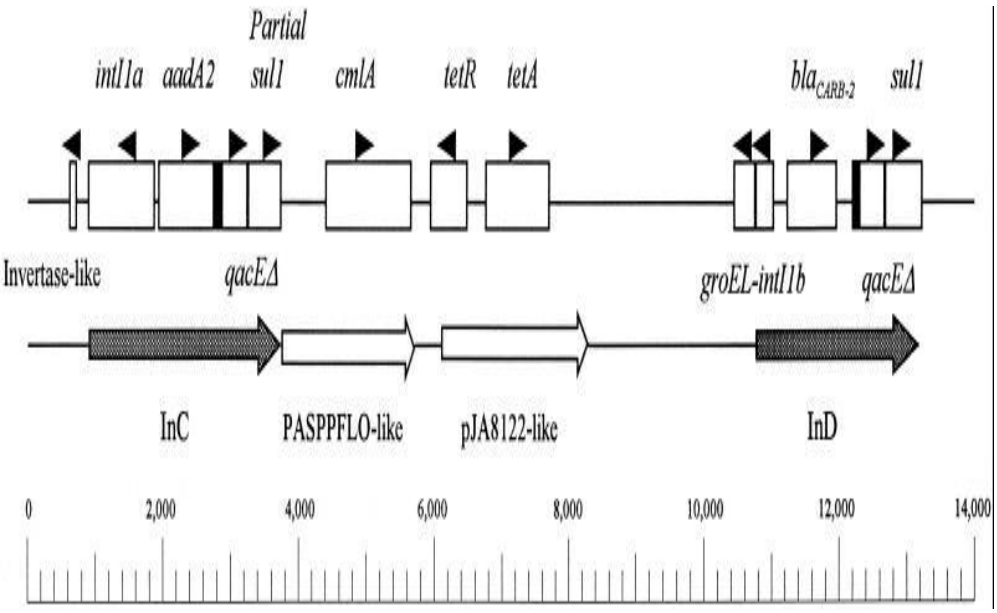


Figure 1.2: Arrangement of antibiotic resistance genes of *S.* Typhimurium DT104 isolate (Briggs and Fratamico, 1999).

In 1990, studies about *Salmonella* Serovar Typhimurium conducted in European countries as well as the United States, Canada, Turkey and Japan reported resistance to five antibiotics, Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracycline. This resistance phenotype is called ACSSaT (Threlfall, 2000; Parry, 2003).

1.2.8. Integrons

1.2.8.1. Integrons role in the spread of resistance genes in *Salmonella* strains

Antimicrobial resistance genes can be spread via mobile genetic elements such as plasmids, transposons and integrons (Hsu *et al.*, 2006). Plasmids and transposons are known to have ability to transfer resistance genes from one strain to another. In addition, they can be interacting with each other in different ways for enhancing their collective ability to transfer resistance genes. Furthermore, some of the plasmids that carry multiple resistance genes have transfer systems, which are aid in the transfer of DNA between unrelated species (Hall *et al.*, 1996).

In general, integrons were characterized in the late 1980s in Gram-negative bacteria (Stokes and Hall, 1989). integrons play a key role in the dissemination and spread of resistance genes for a wide range of distinct antibiotics among diverse bacterial strains (Nemergut *et al.*, 2008). Moreover, integrons play a major role in the development of MDR *Salmonella* strains. Thus, integrons are genetic elements that have the ability to the acquisition, rearrangement and expression of genes usually contained in antimicrobial resistance gene cassettes and associated with transposons and mobilized when located on plasmids (Hsu *et al.*, 2006). Each integron has at least five cassettes, which leads to multi-resistance. All the time, more than one integron is present within the same bacterial

cell. Integrons are normally reported for *Enterobacteriaceae* and other gram-negative bacteria (Fluit and Schmitz, 1999).

1.2.8.2. Integron classes

The integrons have four distinct classes, each encoding a distinct integrase gene (Martinez- Freijo *et al.* 1998). Class 1 integrons are the most common type present in clinical isolates and are strongly associated with the multi-resistance in hospital cases. Integron 1 at 5'CS downstream, have about 60 different gene cassettes that confer resistance to aminoglycosides, chloramphenicol, erythromycin, trimethoprim, cephalosporins, carbapenems, penicillins, rifampin, and quaternary ammonium compounds (Fluit and Schmitz, 1999). Often, class 1 integrons also contain an additional resistance gene in the 3'CS, downstream from the gene cassettes, namely, *sulI*. This gene confers resistance to sulfamethoxazole (Hall and Collis, 1998). Class 1 integron has been studied in various microorganisms, especially in gram negative bacteria such as *Salmonella*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Providencia*, *Pseudomonas*, *Serratia*, *Shigella*, *Stenotrophomonas*, *Burkholderia*, *Campylobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella, and Vibrio,* which their prevalence commonly reported to be ranging from 22 to 59 % (Xu *et al.,* 2011; Partridge *et al.,* 2009; Pérez-Valdespino *et al.,* 2009; Deng *et al.,* 2015). The highest prevalence for class 1 integrons was recorded from Gram-negative bacteria in Guangzhou, southern China during 2001–2006 (Xu *et al.,* 2011; Deng *et al.,* 2015). In another study in Eastern China in 2009, the prevalence rate of class I integron was found among all

Salmonella isolates to be 64.9% in the 310 *Salmonella* strains that were isolated from chicken (Lu *et al.*, 2014), where as in another study class 1 integron was found to be in *Salmonella* strains 59%–75% (Fluit and Schmitz, 1999).

Organization of class 2 integrons have similarity to class 1 integrons, are commonly associated with Tn7 transposon family, carrying both of its recombination site *attI2* and promoter Pc found within such transposons (Xu *et al.*, 2009). Its 3′ conserved segment (3′-CS) contains 5 *tns* genes (*tnsA*, *tnsB*, *tnsC*, *tnsD* and *tnsE*) functioning in the movement of transposon (Senda *et al.*, 1996). However, class 2 integrons share identical gene cassettes with class 1 integrons. The gene cassettes, including dihydrofolate reductase (*dfrA1*), which confer resistance to trimethoprim, streptothricin acetyltransferase (*sat1*), which confer resistance to streptothricin and aminoglycoside adenyltransferase (*aadA1*), which confer resistance to streptomycin/spectinomycin (Xu *et al.*, 2009; Hansson *et al.*, 2002). In some species of Gram-negative organisms such as *Acinetobacter*, *Psuedomonas*, *Enterobacteriaceae and Salmonella*, class 2 integrons have been commonly reported with a low occurrence and prevalence compared with class 1 integron (Ramírez *et al.*, 2005; Crowley *et al.*, 2008). Class 3 and 4 integrons have low prevalence compared with class 1 and 2 integrons (Deng *et al.*, 2015).

1.2.9. Salmonella genomic island 1

The Salmonella genomic island 1(SGI1) consisting of 43Kb integrative mobilization element (Boyd *et al*., 2000), which has been inserted in the last 18bp into 3` end of the *thdF* gene (specific *attB* site) in the chromosome of *Salmonella* serovars (Boyd *et al*., 2008). This SGI1 contains five antibiotic resistances genes: *aadA2, sul1, floR* (*cmlA*-like), *Tet (G)* and *blaP1* (also named *blaPSE -1* or *blaCARB-2*) (Threlfall, 2000). The genomic island SGL1 classification into five types SGI1-A, SGI1- B, SGI1-C, SGI1-D and SGI1- E according to antibiotic resistance phenotypes were ApCmFfSmSpSuTcTm, ApSu, SmSpSu, SmSpSuTm, and ApSmSpSuTc, respectively (Boyd, *et al.*, 2002). In addition, the researcher discovered new types of SGI1 variant MDR regions were accordingly classified as SGI1-A to SGI1-O (Boyd *et al.*, 2008; Cloeckaert *et al.*, 2006; Mulvey *et al.*, 2006).

Identification of the genomic island SGI1 in *S. enterica* serovar Typhimurium DT104, variant SGI1 MDR regions have been described for a wide variety of *S. enterica* serovars such as serovars Agona, Meleagridis, Newport, Kentucky, Kiambu, Albany, Cerro, Derby, Dusseldorf, Emek, Infantis, Kingston, and Paratyphi B (Boyd *et al.*, 2001; Khemtong and Chuanchuen, 2008). In 2005, reported that SGI1 may be transferred by conjugation from *S. enterica* donor strains to non-SGI1 recipient strains such as *S. enterica* and *Escherichia coli*, where it integrated into the recipient chromosome in a site-specific manner (Doublet *et al.*, 2005). Lately, SGI1 and variants of it have been identified in *Proteus mirabilis*

clinical and food isolates (Ahmed *et al.,* 2006; Boyd *et al.,* 2008; Doublet *et al.,* 2007). The identification of SGI1 in other than *Salmonella* isolates is of great concern as the spread of the SGI1 MDR phenotype may have significant clinical implication (Boyd *et al.,* 2008; Cloeckaert *et al.,* 2006).

The SGI1 contains multi-drug resistance region (MDR), which is located at 3` end of SGI1. The MDR in SGI1 have complex structure of class1 integron called In104 (Mulvey *et al*., 2006). The In104 complex integron possesses two cassette attachment sites (*attI1*). At the first *attI1* site of this complex integron, the cassette carries the *aadA2* gene, which confers resistance to streptomycin and spectinomycin (Str^R and Spt^R), and downstream, a 3′ conserved segment (3′-CS) with a truncated *sul1* gene (*sul1*Δ) is found. At the second *attI1* site, the cassette contains the β-lactamase gene bla_{PSE-}, conferring resistance to ampicillin (Amp^R), and downstream, the 3′-CS comprises a complete *sul1* gene conferring resistance to sulfonamides. Flanked by the two cassettes are the *floR* gene, which confers cross-resistance to chloramphenicol and florfenicol (Chm^R/Flo^R), and the tetracycline resistance (Tet^R) genes *tetR* and *tet*(G). Figure 1.3 shows the maps of SGI1 of *Salmonella* Typhimurium DT104 (Boyd *et al*., 2001; Partridge and Hall, 2003).

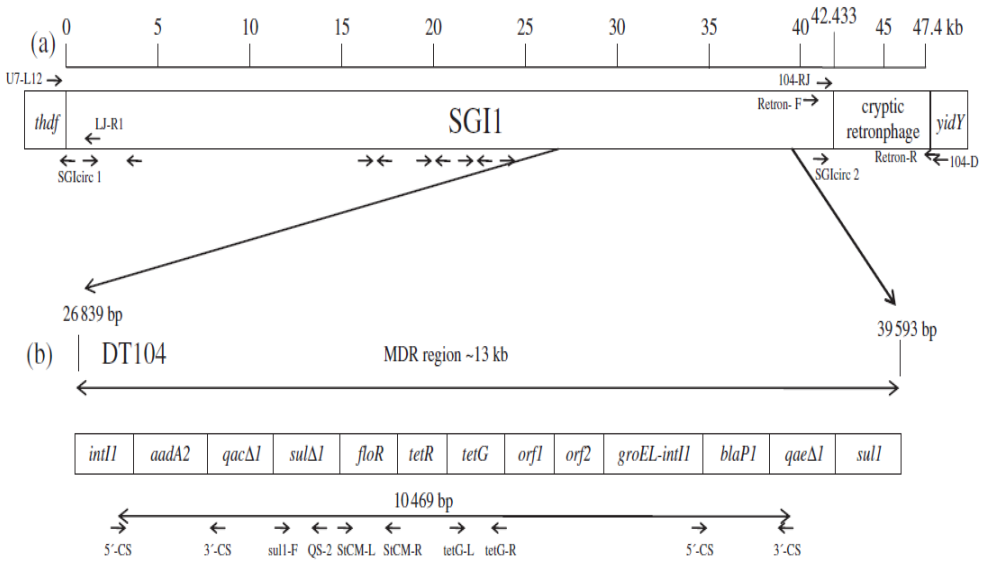


Figure1.3. (a) Map of SGI1 of *Salmonella* Typhimurium DT104. (b) The MDR region (13 kb) of SGI of *Salmonella* Typhimurium DT104 (Boyd *et al.*, 2001).

1.3. Aims of the study

This study was conducted to address part of the deficient information in molecular antibiotic resistance characterization of *Salmonella* isolates from food sources in Palestine.

Specifically, the aims of this study were:

1- To determine the levels and patterns of antibiotic resistance of *Salmonella* isolates from food sources in Palestine.

2- To assess the prevalence and molecular epidemiology of class 1 and 2 integrons in *Salmonella* isolates.

3- To determine the prevalence and molecular epidemiology of SGI1in *Salmonella* isolates by using molecular techniques (PCR and sequencing).

Chapter Two
Materials and Methods

2.1. Collection and identification *Salmonella* isolates

A total of 128 *Salmonella* isolates collected from food sources were provided by the Ministry of Health (MOH) central laboratory in Ramallah-Palestine and Analysis, Poison Control and Calibration (APCC) Center at An-Najah National University- Nablus, Palestine. *Salmonella* isolates identification was as provided from the central laboratory and the APCC Microbiology laboratories at An-Najah National University - Nablus, Palestine.

2.2. Media preparation

2.2.1 Nutrient Broth (NB)

Nutrient broth (Oxoid, England) preparation as labeled on the bottle following manufacturer's instructions. In a 1 L Erlenmeyer flask, 4 g of NB were added to 500 ml deionized water then mixed, boiled and distributed to 5-10 ml tubes. The tubes were sealed with cotton plug before autoclaving at 121 °C for 15 minutes. Cooled NB tubes stored at 4°C.

2.2.2 Nutrient Agar (NA)

Nutrient agar (ACUMEDIA, USA) preparation as labeled on the bottle following manufacturer's instructions. In a 1 L Erlenmeyer flask, 11.5 g of NA were added to 500 ml deionized water then mixed and boiled. The flask was sealed with cotton plug before autoclaving at 121 °C for 15

minutes. After that it tempered to about 55°C. Approximately 20 ml of agar poured in sterile Petri dishes that left at room temperature overnight. During next day, the Petri dishes kept at 4°C.

2.2.3. Mueller -Hinton (MHA) agar

As labeled on the bottle following manufacturer's instructions, Mueller-Hinton agar (Oxoid, England) was prepared. A weight of 19 g of MHA was mixed and heated with 500 ml of deionized water in the flask. The mixture was heated and stirred until the agar was dissolved. The flask was sealed with cotton plug before autoclaving at 121 °C for 15 minutes. After that it tempered to about 50°C. Approximately 20 ml of agar poured in sterile Petri dishes that left at room temperature overnight. During next day, the Petri dishes kept at 4°C.

2.2.4. Triple sugar iron (TSI) agar

TSI agar (Acumedia, USA) prepared as directed by the manufacturer’s instructions. The powder of TSI agar (30 g) was mixed with 500 ml deionized water and heated to dissolve. Using a pipette, 10 ml of TSI medium dispensed into tubes, then autoclaved for 15 minutes at 121°C. Slant agar made by letting the media solidify in the tubes at room temperature and then stored at 4°C.

2.2.5. Urea agar

As directed by the manufacturer, Urea agar (Oxoid, England) was prepared according to label on the bottle. In a 250 ml bottle, 95 ml distilled water

was mixed and boiled with 2.4 g of Urea agar to dissolve. This was followed by autoclaving for 15 minutes at 121°C. The agar then was tempered to 50°C and aseptically one ampoule of sterile urea solution (SR20) was added. The media was then distributed into sterilized tubes to have approximately 10 ml in each. The tubes were plugged with sterile cotton. The medium was prepared as slant agar in the tube by leaving the tubes to solidify in slanted position and then stored at 4°C.

2.2.6. Xylose lysine desoxycholate (XLD) agar

XLD agar (Oxoid, England) preparation as labeled on the bottle following manufacturer's instructions. In a 0.5 L bottle flask, 250 ml deionized water was mixed and boiled with 13.25 g XLD agar. After that it tempered to about 55°C. Approximately 20 ml of agar poured in sterile Petri dishes that left at room temperature overnight. During next day, the Petri dishes kept at 4°C.

2.2.7. Lysine iron (LI) agar

LI agar (Oxoid, England) preparation as labeled on the bottle following manufacturer's instructions. A bottle flask containing 500 ml deionized water and 17 g of LI agar was mixed thoroughly, dissolved by heating. Using a pipette, 10 ml of LI agar medium dispensed into tubes, then autoclaved for 15 minutes at 121°C. Slant agar made by letting the media solidify in the tubes at room temperature and then stored at 4°C.

2.3. Sample identification

2.3.1 XLD test

A test was carried out by subculturing 1 or 2 colonies from a pure culture on the XLD agar plate followed by streaking the inoculum on the medium. This was followed by incubation of plates for 24 hours at 37°C. In positive result, a pink colony with or without black centers develop. Few *Salmonella* species produce yellow colonies with or without black centers (US Food and Drug Administration, 2009).

2.3.2 TSI test

TSI agar tube inoculated with a pure culture of Salmonella by streaking the slant and stabbing the butt, then the tube was incubated at 37°C for 24 hours. Culture of Salmonella produced yellow butt, the slant red with or without blackening as indication for H_2S production in TSI agar (US Food and Drug Administration, 2009).

2.3.3 LI test

A pure *Salmonella* culture inoculated into LI tube by streaking the slant and stabbing the butt, and then the tube was incubated at 37°C for 48hours. In this test, Salmonella isolate produced purple butt and most Salmonella cultures produce H_2S (blackening) in LI agar (US Food and Drug Administration, 2009).

2.3.4 Urease test

Urea agar was inoculated with a pure culture of *Salmonella* by streaking the slant and stabbing the butt, tested *Salmonella* incubated at 37°C for 24 hours. Urease positive result is indicated by turn of urea agar to purple- red color (US Food and Drug Administration, 2009).

2.3.5. Serological test (*Salmonella* Latex Test)

One or two colonies from *Salmonella* isolates were mixed with a drop of latex reagent (Oxoid, England). Followed by mixing and spreading the drop to cover the reaction area using the loop for 10–15 seconds. For up to 2 minutes, gently moving the card in a circular motion to observe the positive agglutination reaction.

2.4. Antibiotic susceptibility test

2.4.1. Preparation of 0.5 McFarland turbidity standard.

A total of 10 ml of the McFarland 0.5 turbidity standard was prepared by adding 0.05 ml of 1.175% (wt/Vol) barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution to 9.95 ml of 1%(Vol/Vol) sulfuric acid (H_2SO4) solution (Andrews, 2009). Then the tube was closed with parafilm to avoid evaporation. The absorbance was measured using a S-22 UV/VIS spectrophotometer (BOECO, Germany) at a wavelength of 625 nm. The absorbance must be 0.08 to 0.13(Andrews, 2001).

The absorbance of 0.5 McFarland standards is equivalent to that of a bacterial suspension with a concentration of 1.5 x 10^8 colony forming units/ml (CFU/ml).

2.4.2. Antibiotic susceptibility test

Antimicrobial susceptibility of *Salmonella* isolates using disk diffusion method was determined according to Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2012). Antibiotic disks (Oxoid, England) used in this research were Ampicillin (AMP,10µg), Tetracycline (TE,30µg), Trimethoprim/ Sulfamethoxazole (SXT, 25µg), Streptomycin (S, 25µg), Chloramphenicol (C, 30 µg), Amikacin (AK, 10µg), Ceftazidime (CAZ, 30µg), Ciprofloxacin (CIP, 5 µg), Gentamicin (CN, 5 µg), Cefotaxime (CTX, 30µg), Ertapenem (ETP, 10µg), Cefepime (FEP, 30µg), Cefoxitin (FOX, 30µg), Neomycin (N, 30µg), Norfloxacin (NOR, 10µg) and Cefpedoxime (CPD, 10µg). The standard size (9 cm) inoculated plates contains 5-6 discs were incubated overnight at 37°C. The diameter of the zone of inhibition was measured in millimeters. CLSI guidelines were used to classify *Salmonella* isolates as resistant, intermediate, and sensitive according to the recommended criteria (CLSI, 2012).

2.5. PCR amplification

2.5.1. DNA isolation

DNA of the *Salmonella* isolates was extracted by boiling method (Ahmed *et al.*, 2006). Briefly, an overnight nutrient agar culture plate was scraped

off with a sterile loop and re-suspended in 2 ml of sterile distilled H_2O, centrifuged at 11,500 X *g* for 5 min and supernatant discarded. 2ml sterile distilled H_2O were added then suspended and boiled for 10min. The tube incubated on ice for 10 min, pelleted by centrifugation at 11,500 X *g* for 5 min. The supernatant solution was collected as the DNA template. DNA templates were stored at -20ºC till used.

2.5.2. Detection of class 1 integrons, class 2 integron and the whole SGI1 in *Salmonella*

Detection of genes encoding the *intI1, intI2 and* SGI1 were performed by PCR (Hussein *et al*., 2009). Primers and the expected amplicons size are described in Table 2.1. PCR reaction reagents are as described in Table 2.2. PCR, run were on Prime Thermal Cycler (Prime, UK). For class 1 integron was performed using the following conditions: initial denaturation at 95ºC for 2min; followed by 35 cycles; denaturation at 95ºC for 25 secs, annealing at 51 ºC for 35 sec and extension at 72ºC for 65 sec; with a final extension at 72 ºC for 5 min. PCR products amplified using the above conditions were detected using 1.4% agarose gel electrophoresis stained with ethidium bromide. Class 2 integron and whole SGI1 PCR were performed with the same thermal cycler as in class 1 integron with different annealing temperatures. Using 60 ºC annealing temperature for class 2 integron and 50.5 ºC annealing temperature for SGI1. Pure water was used as a negative control. Class 1 integron from *Klebsiella* isolates (From Dr. Ghaleb Edwan / Biology department) were used to check the protocol as

positive controls. This study isolates gave the positive results for class 1, 2 integron and SGI1 that were confirmed by sequencing.

Table 2.1: *Salmonella* target gene for PCR amplification, amplicon size and primer sequencing.

Primer	Primer sequence (5` → 3`)	Target	Amplicon size (bp)	Reference
5`-CS	GGCATCCAAGCAGCAAGC	Integron 1	variable	Hussein *et al*., 2009
3`-CS	AAGCAGACTTGACCTGAT			
hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Integron 2	500-491	Ahmed et al., 2007
hep51	GATGCCATCGCAAGTACGAG			
U7-L12	ACACCTTGAGCAGGGCAAAG	Left junction SGI1	500-461	Hussein *et al*., 2009
LJ-R1	AGTTCTAAAGGTTCGTAGTCG			

Table 2.2: PCR reaction reagent used in this study.

PCR Reaction Reagent	Volume for each sample
2X ReddyMix PCR Master Mix (Thermal Scientific)	12.5µl
water (nuclease- free)	10.75 µl
primermix (forward primer +reverse primer)	1.25µl
DNA	0.5µl
Total volume for each sample	25µl

2.6. DNA extraction from agarose gel

Salmonella PCR product was prepared for sequencing as described previously (Sun *et al.,* 2012). Briefly, a cut of DNA band from the gel was frozen at -80 ºC for 5 min. The frozen DNA band from the gel were centrifuged at 14000 RPM for 5 min at 4 ºC filtrated through an Eppendorf tube to separate the agarose gel. The filtrate was added to the same quantity of phenol/chloroform/alcohol (25:24:1), centrifuged at 14000 RPM and the upper layer transferred to new tubes. After measuring the volume of the upper layer (1/10) CH_3COONa and 2.5 fold of 100% ethanol volumes to the solution at room temperature. The last solution shacked then were

added centrifuged for 40 min at 4 ºC. The supernatant discarded and pellet washed by adding 70% ethanol. The sample was dried overnight.

2.7. Sequencing

Sequencing services was purchased from Bethlehem University using dideoxy chain termination method using ABI PRISM sequencer, model 3130 (Hitachi Ltd, Tokyo, Japan). DNA Sequences similarity were searched using the BLAST program services available from the NCBI GenBank homepage (https://blast.ncbi.nlm.nih.gov). An example for the comparison of the sequences for class 1 integron, class 2 integron and SGI1 were shown in Appendix B.

Chapter Three
Results

3.1. Identification of *Salmonella* isolates

A total of 128 of *Salmonella* isolates were collected and identified in the central laboratory of MHO and the APCC center microbiology laboratory, An-Najah National University-Nablus, Palestine. Each sample was subcultured at XLD agar were pink colored colonies with or without black center are confirmed by the biochemical test using TSI agar (red slant and yellow/black butt), LI agar (purple butt with blackening) and Urea agar with no color change that confirmed by the Serological test.

3.2. Antibiotic sensitivity test

In the present study, the *Salmonella* bacterial isolates were tested for their susceptibility against 16 antimicrobial agents (Table 3.1) were 93 isolates (72.7%) showed multidrug resistance for two or more antibiotics. The data are shown in Appendix A. However, the antimicrobial resistance pattern showed that most *Salmonella* isolates have high resistance to Tetracycline (79.7%) then to Trimethoprim/ Sulfamethoxazole (48.4%) and Ampicillin (17.1%), while *Salmonella* isolates showed resistance to Streptomycin (6.25%), the rest of the antibiotics with lower resistance ranged from 0.78 – 6.25 % as shown in Table 3.1. In addition, the results showed that all *Salmonella* isolates have a susceptibility to Amikacin and Cefepime.

Table 3.1: Antibiotic resistance of *Salmonella* isolates.

Antibiotic	Resistant isolates	
	%	No. of Isolates
Ampicillin (10 µg)	17.1	22
Cefoxitin (30 µg)	0.78	1
Cefotaxime (30 µg)	1.56	2
Ceftazidime (30 µg)	1.56	2
Cefpodoxime (10 µg)	0.78	1
Cefepime (30 µg)	0	0
Neomycin (30 µg)	0.78	1
Amikacin (10 µg)	0	0
Streptomycin (25 µg)	6.25	8
Gentamicin (5 µg)	3.1	4
Norfloxacin (10 µg)	1.56	2
Ciprofloxacin (5 µg)	2.34	3
Chloramphenicol (30 µg)	2.34	
Tetracycline (30 µg	79.9	102
Trimethoprim/ Sulfamethoxazole (25 µg)	48.4	62
Ertapenem (10 µg)	0.78	1

3.3. Detection of class 1 and 2 integrons and characterization of the gene cassettes

Class1 and 2 integrons were screened in the 128 *Salmonella* bacteria isolated from food by molecular techniques (PCR and Sequencing).

Salmonella isolates that carried class 1 integron with varied gene cassettes were detected in 108 (84.4%) isolates. The detected gene cassettes are aminoglycoside adenyltransferase (*aadA*) gene, which confer resistance to Spectinomycin and Streptomycin; aminoglycoside adenyltransferase type B (*aadB*) gene, which confer resistance to Tobramycin, Gentamicin and Kanamycin; dihydrofolate reductase gene cassettes (*dfrA*), which confer Trimethoprim resistance and β-lactamase gene (*blaPSE-1*), which confers Ampicillin resistance were as reported in other studies (Beutlich *et al.*,

2011; Yu *et al.*, 2014; Firoozeh *et al.*, 2012). Class 1 integron detected in the isolates were sequenced and compared to the GenBank data that showed similarity more than 96%. Results are presented in Figure 3.1 and Table 3.2. An example for the sequence of class 1 integron is shown in Appendix B.

The detected Class 2 integrons, in 2 (1.6%) isolates using hep74/hep51 primers were as reported in other study (Araújo *et al.*, 2017) carried *dfrA1, sat1 and aadA1* cassette confers resistance to Trimethoprim, Streptothricin and Streptomycin/Spectinomycin, respectively. As the detected Class 2 integron in isolates were sequenced and compared to GenBank database, revealed the similarity to GenBank accession number KX579880 from *Escherichia coli* strain. Results are presented in Figure 3.2 and Table 3.2. An example for the sequence of class 2 integron is shown in Appendix B.

The detection of class 1 and 2 integrons showed the strong relationships between the presence of the integrons and the multi drug resistance in the isolates. Class 1 integrons were present in 80(74.7%) of the multi-resistance isolates, which possessed resistance to two or more antibiotics drugs. Two isolates detected with the class 2 integron are multi-resistance strains. The data are shown in Appendix A.

Table 3.2. Integron classes and gene cassettes characterization.

Integron classes	No. of isolates	Amplicon size (bp)	Gene cassettes
Class 1 integron	1	1600	*dfrA-aadA* gene
	4	1500	*dfrA-aadA* gene
	10	1200	*blaPSE1* gene
	86	1000	*aadA* gene
	2	800	*aadB* gene
	5	750	*dfrA* gene
Class 2 integron	2	500	*dfrA-sat-aadA*

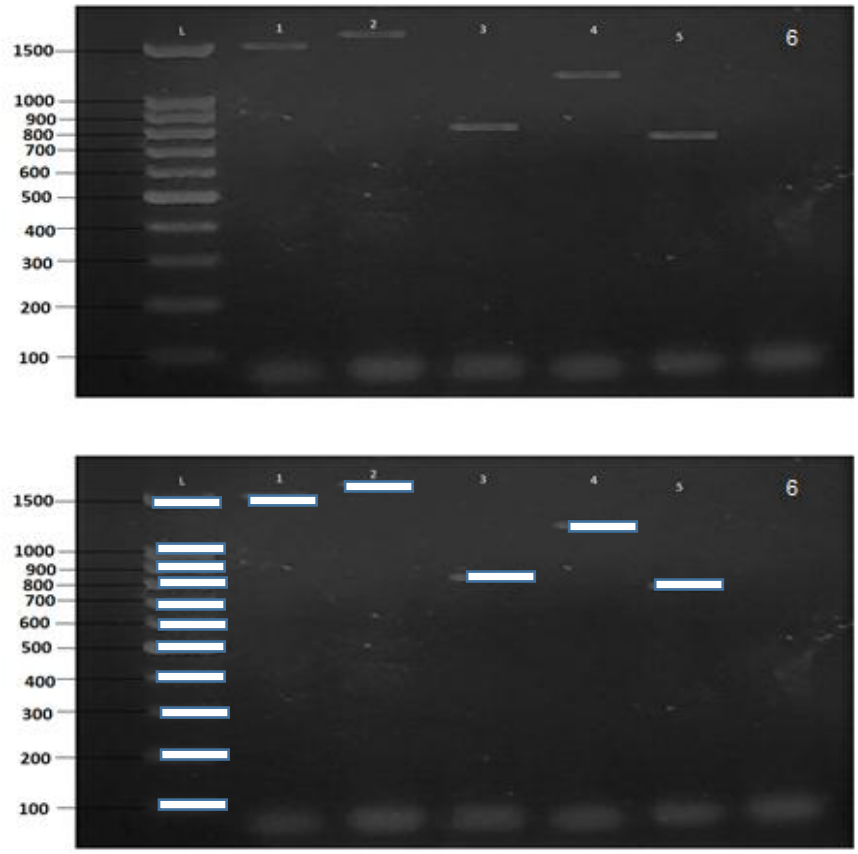


Figure 3.1. Representative PCR profile for class 1 integron detected in *Salmonella* isolates from food sources. (L: Ladder. Lane 1, 2, 3, 4 and 5 are isolates number 111443, 1625443, 1107023, 1210679 and 1303450 respectively as indicated in appendix A. Lane 6: Negative control).

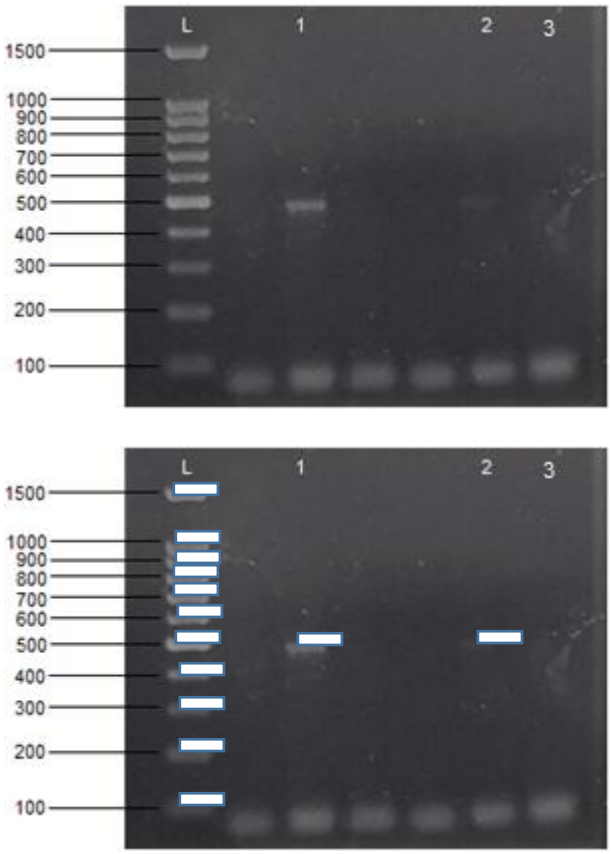


Figure 3.2. Representative PCR profile for class 2 integron detected in *Salmonella* isolates from food sources. (L: Ladder. Lane 1 and 2 are isolates number 1303450 and 1617130 respectively as in appendix A. Lane 3: Negative control).

3.4. Detection of Salmonella genomic island 1(SGI1)

To assay Salmonella genomic island 1 presence in integron 1 positive strain, PCR was performed by using specific primers that are mentioned in Table 2.1.

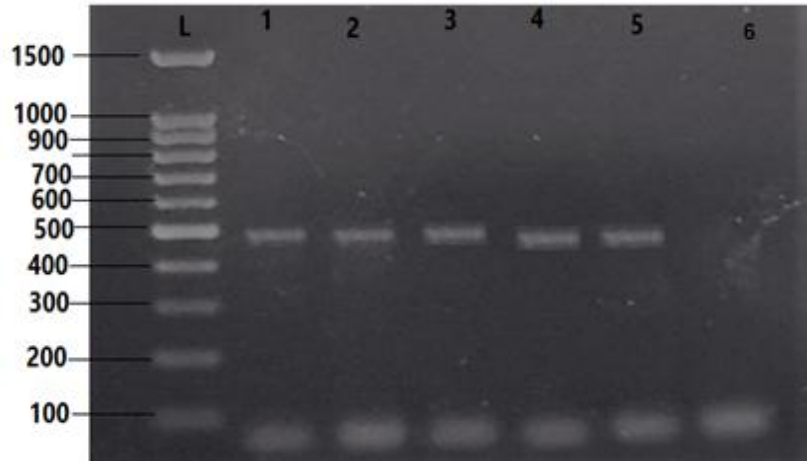
Salmonella genomic island 1 were detected in five isolates, which represent 4.63% of the isolates with various antimicrobial resistance profiles and variable gene cassettes. All SGI1 positive isolates with the expected sizes

of approximately 500 bp. The results are presented in Table 3.3 and Figure 3.3.

Moreover, SGI1 five isolates PCR products were sequenced showed similarity greater than 98% to other *Salmonella* isolates in GenBank. An example SGI1 sequence shown in Appendix B.

Table 3.3. Characterization of SGI1 of *Salmonella* isolates.

Gene cassettes	**Integron class**	**Antibiogram**	**Isolate source**	**Isolate No.**
*aadA*gene	Class 1 integron *	AMP TE CN S	Chicken	1616461
*dfrA-aadA*gene	Class 1 integron**	AMP TE CIP N	Schnitzel	1625443
*aadA*gene	Class 1 integron *	AMP TE CIP CN	Kabab	1625441
*aadA*gene	Class 1 integron *	TE S	Stool	1312916
*dfrA-aadA*gene	Class 1 integron**	Sensitive for all antibiotics	Parsley /Salad	1502734
*: Class 1 integron that represent with 1000bp **: Class 1 integron that represent with 1500bp				



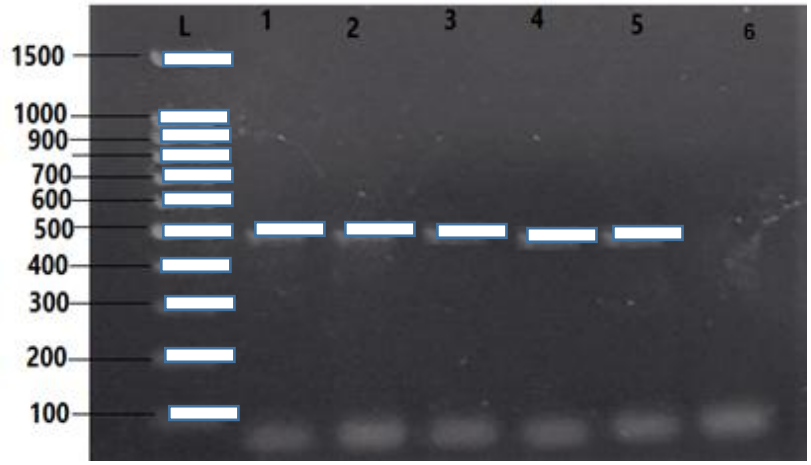


Figure 3.3. Representative PCR profile for SGI1 genes detected in *Salmonella* isolates from food sources. (L: Ladder. Lane: 1, 2, 3, 4 and 5 are isolates number 1616461, 1625443, 1625441, 1312916 and 1502734 respectively (Appendix A). Lane 6: Negative control).

Chapter four
Discussion, Conclusion and Recommendations

4.1. Discussion

Antimicrobial resistance in *Salmonella* is an extra burden to the notion that this bacterium is considered one of the most important foodborne diseases all over the world that needs special consideration to reduce its impact on human health. Lately, the usage of antimicrobial agents on big scale, not only as a therapeutic and preventive measure, but as growth promoter factors, could force the bacteria to adapt and emerge as MDR bacteria affecting humans. Human infections with MDR *Salmonella* could be caused by eating contaminated food (Gomez *et al.,* 1997; Fey *et al.,* 2000). The mis-use of antimicrobials can lead to emergence of resistance in *Salmonella* (Threlfall, 2002), which makes it more difficult to choose the suitable antimicrobial (Gebreyes *et al.,* 2000). Molecular characterization of antimicrobial resistance gives the knowledge, insight and benefit about the spread of resistance genes among *Salmonella* isolates (Swaminathan *et al.,* 2001). Therefore, the phenotypic and genotypic resistance monitoring is necessary to control antibiotic resistance in *Salmonella* species from food sources (Antunes *et al.,* 2006).

In total, the frequency of multi-drug resistance in *Salmonella* isolates was 72.7%, which was similar to results in Romania (Colobatiu *et al.,* 2015), but higher than MDR *Salmonella* strains isolated from raw chicken in Pennsylvania (31.0%) (Mikanatha *et al.,* 2010). Also, less MDR

Salmonella isolates were recovered from the poultry environment in the USA (56.0%) (Zou *et al.*, 2009). In contrast, other studies in Ethiopia showed higher isolation rate of MDR *Salmonella* isolate (85.1%) than defined in the present study (Eguale *et al.*, 2014).

Interestingly, only one isolate isolated from food gave ACSSuT phenotype that correlates with the classical SGI-1 genomic characterization. In the current study, higher rates of antibiotic resistance were against Tetracycline (79.7%) and trimethoprim/ Sulfamethoxazole (48.4%), similar to reports of *Salmonella* isolates from Brazil in which samples exhibited resistance to Tetracycline (83.0%) and trimethoprim/ Sulfamethoxazole (50.0%) (Zishiri *et al.*, 2016). This is not surprising as these antibiotics are affordable, cheap and commonly used in food processing as growth promoters, which forces bacteria such as *Salmonella* to adapt and acquire resistance and then transfer this resistance to other isolates that can infect humans through the food chain leading to MDR bacterial isolates emergence that represent a serious public health risk that may affect drug efficacy.

Low resistance to Streptomycin (6.25%) and Chloramphenicol (2.34%), similar to reports of *Salmonella* isolates from Brazil, Streptomycin (12.5%) and Chloramphenicol (4.2%) (Zishiri *et al.*, 2016). Moreover, the resistance of *Salmonella* to Ampicillin (17.1%) and Ciprofloxacin (2.34%) was lower than that mentioned in other reports. In China, the percentage of resistance of *Salmonella* to Ampicillin was 75.0% and Ciprofloxacin was 80.4%, but they reported similar susceptibility of isolated strains to Amikacin. (Zhao *et al.*, 2017). In another report in Ethiopia showed a high resistance rate of

Salmonella to Ampicillin (31.0%), Ciprofloxacin (28.7%) and neomycin (41.4%) than the present (Eguale *et al.*, 2014). Resistance to Chloramphenicol, Ceftazidime, Cefotaxime, Ciprofloxacin and Gentamicin, were similar to the values mentioned in a report in Tunisia (Soufi *et al.*, 2012). In our research the *Salmonella* strains showed variable resistance rates with lower resistance to Cephalosporins such as Ceftazidime, Cefepime, Cefpodoxime, Cefotaxime and Cefoxitin and Carbapenem such as Ertapenem. These results indicate that cephalosporins as well as carbapenem are still appropriate drugs that can be used against invasive and systemic infection caused by *Salmonella* especially if they inclined as a foodborne disease. The shown variable resistance to the different kinds of antibiotics reflects the variable usage of the antibiotics in the Palestinian market. Notably, the low rate of resistance to Chloramphenicol in the studied *Salmonella* isolates can be correlated to its forbidden use in Palestinian market in animal production as its health potential risk. However, the Chloramphenicol resistance in certain isolates in this study could raises a question about the source of this resistance that needs further investigation.

Integrons play an important role in the spread of antibiotic resistance genes among Gram-negative bacterial strains. Integron gene cassettes have been identified as a main source of resistance, which serve as antimicrobial resistance generator within bacteria (Ochman *et al.*, 2000; Lucey *et al.*, 2000). Of the many identified classes of integrons, classes 1 and 2 are the most frequent in Gram-negative bacteria (White *et al.*, 2001). The present

study showed a high frequency of MDR among integron- positive *Salmonella* strains isolated from food sources. There is good indication for the association between MDR in *Salmonella* bacterial isolates and the presence of class 1 integrons (Rayamajhi *et al.*, 2008). Class 1 integron positive strains totaled 84.4% (108/128), that is comparable to other reports of 86.5 % (Krauland *et al.*, 2009), 59.5% in Iran (Firoozeh *et al.*, 2012), 36.2% in Japan (Ahmed *et al.*, 2009), 30% in Tunisia (Soufi *et al.*, 2012), 16.9% in China (Zhao *et al.*, 2017) and was very low in USA (2.8%) (Van *et al.*, 2013). In the present study, we detected one gene cassette in isolate 103 integron 1 positive strains where they were diverse in size (*aadA*, *dfrA*, *blaPSE1*and *aadB*) and only five strains have two gene cassettes (*aadA* and *dfrA*).

The studied isolates generally harbored the *aadA* gene, which encodes streptomycin and spectinomycin resistance. A reasonable explanation for the high prevalence of *aadA* gene as cassettes could be due to overuse of streptomycin and spectinomycin in food animal production and agriculture for many proposals as well as use in human medicine (Marshall and Levy, 2001). After *aadA* gene cassette commonly detected among class1 integrons of *Salmonella* isolates from the food samples we found *blaPSE1 gene* which confers resistance to ampicillin. However, the gene cassette *dfrA*, which confer resistance to trimethoprim, was detected alone in five isolates. Moreover, more than one isolate contained two gene cassettes (*dfrA-aadA*), Which is an important point that indicates that class 1 integrons support and facilitate acquisition of resistance to a broad

spectrum of antibiotic agents among *Salmonella* isolates. The predominant expression of the *aadA* gene in class 1 integrons has been reported in bacteria from other species, suggesting that this gene can be either the first cassette to be acquired by an integron or to be inserted into integrons of other gene cassettes (Rayamajhi *et al.*, 2008). The present survey showed high expression of resistance genes to aminoglycosides among *Salmonella* isolates. These results are similar to those from the reports that revealed that cassettes for aminoglycosides are common in class1 integrons (Firoozeh *et al.*, 2012; Soufi *et al.*, 2012). Class 2 integron positive isolates totaled 1.6% (2/128) while other reports revealed the prevalence of class 2 integrons in *Salmonella* isolates 8.3% (Corrêa *et al.*,2014), 20.3% in Iran (Naghoni, *et al.*, 2010), 4.3% in Japan (Ahmed *et al.*, 2009). Conversely, in Hong Kong and Tunisia class 2 integrons were not detected (Jin and Ling, 2009; Soufi *et al.*, 2012).

The studied isolates showed that integron- negative isolates as well as integron- positive isolates showed resistance to tetracycline. These results indicate that the class1 integrons among our *Salmonella* isolates do not support the total resistance phenotypes, this can occur due to the presence of the other integrons, in addition to class1 integrons or chromosomal mutation or other genetic elements like plasmids and transposons. The present study showed low resistance to some antimicrobial drug even being integron- positive such as Ciprofloxacin and Gentamycin such results may be due to a low level of resistance gene expression.

SGI1 identified for the first time in the *S. enterica* in *S.* Typhimurium phage type DT104, which is the first described genomic island conifers antibiotic resistance, in which giving ability to cause diseases in humans as well as animals. After that SGI1 was identified in other *S. enterica* serovars. For instance, serovar Agona strains isolated in Belgium from poultry and Paratyphi B serovar strain isolated in Singapore from fish (Boyd *et al.*, 2001; Cloeckaent *et al.*, 2000; Meunier *et al.*, 2002). Moreover, SGI1 has been reported in various serovars, including Kentucky, Infantis, and so on (Hall, 2010). This wide distribution provides an indication that the SGI1 is a significant determinant of MDR strains of *Salmonella* and is clearly able to move from one strain to another. SGI1 is non-self-transmissible, but it can be mobilized by the IncA/C plasmid conjugative machinery (Hall, 2010), as a result, it is an integrative mobilizable element (Doublet *et al.*, 2005).

The studied isolates showed low prevalence rate of SGI1 (4.6%), which similar to prevalence rate in the Dutch study (7.9%) (Vo *et al.*, 2006). In contrast, other studies estimated higher rates. For instance, a French study detected high prevalence rate for SGI1 (57%) (Bugarel *et al.*, 2011). Another study detected 19% of examined isolates to be SGI1- positive isolates (Krauland *et al.*, 2009), which was nearly similar to the prevalence rate in Australian study, they detected 20.5% of SGI1- positive isolates (Levings *et al.*, 2005), while was 12% in another report (de Curraize *et al.*, 2017). Our results demonstrated that the Penta- resistance profile (ACSSuT) was not a sensitive and specific marker for SGI1 since the

ACSSuT did not appear in any SGI1- positive strain defined in the present study.

4.2. Conclusions and recommendations

To the best of our knowledge, this is the first report in Palestine concerned with molecular characterization of antibiotics resistance from food sources that illustrates a potential public health risk of *Salmonella* in Palestine. Moreover, conducting molecular characterization of multi-drug resistance from food sources increases the alarm that the reservoir could be in such food sources for MDR isolates. Our results about antibiotic resistance in *Salmonella* isolates is of great concern for health issues. *Salmonella* has been isolated from various food items, particularly in cooked meat products, Taheeniah and raw meats as collected locally by MOH central laboratory and Analysis, Poison Control and Calibration center.

The findings of this study enabled researcher to set the following recommendation:

1- Implementation of programs to reduce the incidence of MDR *Salmonella* Isolates to reduce the foodborne diseases such as following the Good Manufacturing Practice (GMP), Hazard Analysis and Critical Control Points (HACCP)

2- Hygiene control for adequate consumer protection.

3- Control the misuse of antibiotics in both veterinary and human medicine.

4- Antimicrobial resistance monitoring among foodborne *Salmonella* is important as transferable elements of resistance genes spread from food products to humans.

5- Further studies to identify *Salmonella* serotypes prevalent in Palestine and to be connected with MDR *Salmonella* results.

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Appendix A
Distribution of resistance genes and phenotype profile in *Salmonella* isolates

solate code	Isolate No.	Sample Source	Antibiotic resistance pattern*	Integron amplicon size(bp)**	Class 1 Integron Gene Cassette **
S1	1618649	Chicken	TE, SXT, (S)	1000	*aadA* gene
S2	1622996	Chicken	TE, SXT, S	1000	*aadA* gene
S3	1623992	Chicken	AMP, TE, SXT, S, C	1200	*blaPSE1* gene
S4	1625440	Kabab	AMP, TE, C	800	*aadB* gene
S5	1616461	Chicken	AMP, TE, CN, (S)	1500	*dfrA-aadA* gene
S6	1617206	Chicken	-	1000	*aadA* gene
S7	1622163	Chicken	TE, SXT, (S)	1000	*aadA* gene
S8	1623330	Meat	-	1000	*aadA* gene
S9	1625438	Schnitzel	TE	1000	*aadA* gene
S10	1623272	Chicken	TE, SXT, (S)	1000	*aadA* gene
S11	1626464	Chicken	TE, SXT, S	750	*dfrA* gene
S12	1622472	Chicken	TE	1000	*aadA* gene
S13	1625003	Chicken	(AMP)	1000	*aadA* gene
S14	1625444	Beef burger	(AMP), TE, SXT, (S)	1000	*aadA* gene
S15	1617204	Chicken	CN	1000	*aadA* gene
S16	1625443	Schnitzel	AMP, TE, CIP, N	1500	*dfrA-aadA* gene
S17	1622473	Chicken	TE, SXT, (S)	1000	*aadA* gene
S18	1623993	Chicken	TE, SXT, (S)	1000	*aadA* gene
S19	1623369	Chicken	AMP, (S)	1000	*aadA* gene
S20	1617130	Chicken	AMP, TE, SXT	750	*dfrA* gene
S21	1624159	Humous	TE, (S)	1000	*aadA* gene
S22	1625441	Kabab	AMP, TE, CIP, CN	1000	*aadA* gene
S23	1622165	Chicken	TE, SXT, (S)	-	-
S24	1617205	Meat	-	-	-
S25	1622997	Chicken	TE, (S)	-	-
S26	1502337	Chicken	TE, SXT, (S)	-	-
S27	1504919	Chicken	TE, (S)	-	-
S28	1529723	Chicken	TE, SXT, (S)	-	-
S29	150947	Chicken	TE, SXT, (S)	1000	*aadA* gene
S30	1514653	Beef meat	AMP, TE	1000	*aadA* gene
S32	1527253	Human	TE, SXT	1000	*aadA* gene
S33	1510548	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S34	1512211	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S35	1502734	Parsley/salad	-	1000	*aadA* gene
S36	1506221	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S37	1522567	Chicken	TE, SXT, (S)	1000	*aadA* gene
S38	1500346	Meat	-	1000	*aadA* gene
S39	1530767	Chicken	-	1000	*aadA* gene
S40	1525987	Chicken	TE, SXT, (S)	1000	*aadA* gene

S41	1507083	Chicken	TE, SXT, (S)	1000	*aadA* gene
S42	1528778	Taheeniah	-	1000	*aadA* gene
S43	1504915	Chicken	TE, (S)	1000	*aadA* gene
S44	1425011	Chicken	TE, SXT, (S)	1200	*blaPSE1* gene
S45	1511410	Human	TE, SXT, (S)	1000	*aadA* gene
S46	1510549	Chicken	TE, SXT, (S)	1000	*aadA* gene
S47	1525866	Humous	TE, SXT	1000	*aadA* gene
S48	1524591	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S49	1525867	Salad	TE, SXT	1000	*aadA* gene
S50	1526234	Human	TE, SXT, (S)	1200	*blaPSE1* gene
S51	1501420	Chicken	TE, SXT, (S)	1000	*aadA* gene
S52	1322617	Taheeniah	TE	1000	*aadA* gene
S53	1325841	Chicken	TE, SXT, (S)	1000	*aadA* gene
S54	1322628	Taheeniah	TE, (S)	1000	*aadA* gene
S55	1314839	Taheeniah	TE, (S)	1000	*aadA* gene
S56	1325891	Chicken	TE, SXT, (S)	1000	*aadA* gene
S57	1322051	Halawa	TE	1000	*aadA* gene
S59	1318938	Chicken	TE, SXT, S	1200	*blaPSE1* gene
S60	1318937	Chicken	TE, SXT, (S)	1200	*blaPSE1* gene
S62	1329892	Beef burger	AMP, TE	1000	*aadA* gene
S63	1326253	Kabab	TE, S	1000	*aadA* gene
S64	1311107	Chicken	TE, SXT, (S)	1000	*aadA* gene
S65	1312823	Human	TE, S	1000	*aadA* gene
S66	1305495	Beef burger	TE, SXT, S	1200	*blaPSE1* gene
S67	1310917	Meat	TE, SXT	1000	*aadA* gene
S68	1309466	Chicken	TE, SXT, (S)	1000	*aadA* gene
S69	1318956	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S70	1316604	Beef burger	TE, SXT, (S)	1000	*aadA* gene
S71	1313666	Taheeniah	TE, SXT	1000	*aadA* gene
S72	1314840	Taheeniah	TE	1000	*aadA* gene
S73	1304624	Chicken	TE, SXT	1000	*aadA* gene
S75	1303450	Chicken	AMP, TE, SXT	750	*dfrA* gene
S76	1302793	Beef burger	TE	1000	*aadA* gene
S77	1225992	Beef meat	TE, SXT, (S)	1000	*aadA* gene
S78	1227585	Beef burger	TE, SXT, (S), CAZ	1000	*aadA* gene
S79	1228215	Cheese	TE	1000	*aadA* gene
S80	1226840	Beef burger	TE, (S), SXT	1000	*aadA* gene
S81	1228101	Chicken	TE, (S), SXT	1000	*aadA* gene
S82	1220694	Salad	TE	1000	*aadA* gene
S83	1226844	Chicken	TE, SXT, (S)	1000	*aadA* gene
S84	1226072	Beef meat	AMP, TE	1000	*aadA* gene
S85	1225991	Beef meat	AMP, TE	-	-
S86	1225151	Chicken	TE, SXT, (S)	-	-
S87	1221149	Chicken	TE, SXT, (S)	-	-
S88	1222904	Chicken	TE, SXT, (S)	1000	*aadA* gene
S89	1203318	Fish	TE	1000	*aadA* gene
S90	1209224	Beef burger	TE, SXT, (S)	1200	*blaPSE1* gene
S91	1111326	Chicken	TE, (S)	1000	*aadA* gene

S92	1106931	Beef burger	AMP, TE, (S)	1000	*aadA* gene
S93	16110447	Chicken	TE	1000	*aadA* gene
S95	17020065	Taheeniah	TE	1000	*aadA* gene
S96	17020066	Taheeniah	TE	1000	*aadA* gene
65	1222895	Meat	AMP, TE	1000	*aadA* gene
66	1301761	Beef burger	-	-	-
67	1228099	Chicken	TE, SXT, (S)	1000	*aadA* gene
69	1221924	Chicken	TE, (S)	-	-
72	1222168	Beef meat	-	-	-
74	1229413	Hamburger	TE, SXT, (S)	750	*dfrA* gene
75	1228656	Chicken	TE, SXT, (S)	1000	*aadA* gene
81	1303707	Chicken	AMP, TE, CIP, CN	1000	*aadA* gene
84	1223699	Chicken	TE, SXT, (S)	1000	*aadA* gene
85	1222895	Chicken	AMP, TE	1000	*aadA* gene
89	1221998	Beef meat	AMP, TE	1000	*aadA* gene
91	1221147	Chicken	-	-	*aadA* gene
98	1215533	Chicken	AMP, TE	1000	*aadA* gene
103	1208177	Chicken	AMP	750	*dfrA* gene
105	1210679	Chicken	TE, SXT, S	1200	*blaPSE1* gene
111	1206036	Milk	-	-	*aadA* gene
113	1115756	Beef burger	TE, (S)	1000	*aadA* gene
115	1107595	Chicken	TE, SXT, (S), CAZ, CTX	1000	*aadA* gene
118	1115028	Chicken	TE, SXT, (S)	1000	*aadA* gene
120	1110568	Chicken	TE, SXT, (S)	1000	*aadA* gene
124	1107182	Halawa	-	-	-
125	1116292	Beef burger	TE, (S)	-	-
127	1208177	Beef burger	AMP	-	-
129	1124360	Beef burger	-	-	-
130	1210679	Beef burger	TE, SXT, (S)	1000	*aadA* gene
132	1107023	Beef meat	TE, SXT, (S)	800	*aadB* gene
133	1114437	Beef meat	AMP, TE, SXT, (S), C	1600	*dfrA-aadA* gene
136	1105163	Beef meat	TE, (S)	1200	*blaPSE1* gene
138	1107869	Halawa	-	-	-
139	1201817	Chicken	-	-	-
140	1109413	Chicken	AMP, CTX, CPD, ETP, FOX, NOR	1500	*dfrA-aadA* gene
143	1111326	Beef burger	TE, (S)	1000	*aadA* gene
144	1201849	Egg	-	1200	*blaPSE1* gene
148	1107870	Halawa	-	1000	*aadA* gene
149	1202943	Beef burger	(SXT)	1000	*aadA* gene
199	1312916	Stool	TE, S	1000	*aadA* gene
350	1502734	Parsley/salad	-	1500	*dfrA-aadA* gene

*: Antibiotics between brackets represent intermediate resistant, - represent an isolate sensitive for all antibiotics

**: Class 1 Integron amplicon size and cassettes, - represent an isolate negative for Class 1 Integron.

Appendix B
Examples of Sequence Alignment results.

1- Class 1 Integron: Isolates number 1617130

Salmonella enterica subsp. enterica serovar Worthington strain S204 class 1 integron trimethoprim-resistant dihydrofolate reductase type I DfrA1 (dfrA1) and aminoglycoside adenylyltransferase AAD(3") (aadA1) genes, partial cds

Sequence ID: KJ848449.1 Length: 1588 Number of Matches: 1

Related Information

Range 1: 242 to 394 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
283 bits(153)	2e-72	153/153(100%)	0/153(0%)	Plus/Plus

```
Query  118  TGGCTGTTGGTTGGACGCAAGACTTTTGAATCAATGGGAGCATTACCCAACCGAAAGTAT  177
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Sbjct  242  TGGCTGTTGGTTGGACGCAAGACTTTTGAATCAATGGGAGCATTACCCAACCGAAAGTAT  301

Query  178  GCGGTCGTAACACGTTCAAGTTTTACATCTGACAATGAGAACGTAGTGATCTTTCCATCA  237
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302  GCGGTCGTAACACGTTCAAGTTTTACATCTGACAATGAGAACGTAGTGATCTTTCCATCA  361

Query  238  ATTAAAGATGCTTTAACCAACCTAAAGAAAATA  270
            |||||||||||||||||||||||||||||||||
Sbjct  362  ATTAAAGATGCTTTAACCAACCTAAAGAAAATA  394
```

2- Class 2 integron: Isolate number 1303450

Escherichia coli strain Y15W2.4 class 2 integron dihydrofolate reductase (dfrA1), streptothricin acetyltransferase (sat2), and aminoglycoside-3"-adenylyltransferase (aadA1) genes, complete cds

Sequence ID: KX579880.1 Length: 2055 Number of Matches: 1

Related Information

Range 1: 1714 to 2041 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
433 bits(234)	2e-117	302/334(90%)	11/334(3%)	Plus/Plus

```
Query  4     AACCTTAACGCTATGGAACTCGCCGCCCGACTGGCCTGGCCAGGAGCGAAATGGTGTGCT  63
             ||||||||||||||||||||||||||||||||||| ||||| | ||||||||||  |||||
Sbjct  1714  AACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCGAAATGTAGTGCT  1773

Query  64    TACGTTGT---GAAAATGGCACATACAGCGCAATGTTGA-TGC-AAATCRSGCCAAAGGA  118
             ||||||||   | |||    |||||||| | ||     || ||||||  ||| |||||
Sbjct  1774  TACGTTGTCCCGCATTTGG----TACAGCGC-A-GTAACCGGCAAAATCGCGCCGAAGGA  1827

Query  119   TGTCGCTGCCGACTGGCCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGA  178
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1828  TGTCGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGA  1887

Query  179   AGCTAGGCAGGCTTATCTTGGACAAGAAGATCGCTTGGCCTCGCGCGCAAATCAGTTGGA  238
             ||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
Sbjct  1888  AGCTAGGCAGGCTTATCTTGGACAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGA  1947

Query  239   AGAATTTGTTCACTACGTGAAAGGCGAGATAACCAAGGTAGTCGGCAAATAATGTCTAAC  298
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Sbjct  1948  AGAATTTGTTCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGTCTAAC  2007

Query  299   AATTCGTTCAAGCCGACGCCGCTTTGTGTCGCGG  332
             |||||||||||||||||||||||| | | |||||
Sbjct  2008  AATTCGTTCAAGCCGACGCCGCTTCGCGGCGCGG  2041
```

3- Salmonella genomic island 1: Isolate number 1616461

Salmonella enterica strain SRC4 TrmE (trmE) gene, complete cds; Salmonella Genomic Island 1 variant SGI1-I, complete sequence; YidY (yidY) genes, complete cds

Sequence ID: KU563154.2 Length: 44922 Number of Matches: 1

Related Information

Range 1: 814 to 1173 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
660 bits(357)	0.0	359/360(99%)	0/360(0%)	Plus/Plus

```
Query  1     AAGAGTTGCGCCTGGCGCAGCAAAGCTTAAGCGAGATTACCGGCGAGTTTACCTCCGACG  60
             ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  814   AAGAGTTGCGCCTTGCGCAGCAAAGCTTAAGCGAGATTACCGGCGAGTTTACCTCCGACG  873

Query  61    ACCTGCTGGGACGGATTTTCTCCAGCTTCTGTATTGGGAAGTAAATCTCCTAAATTAAAT  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  874   ACCTGCTGGGACGGATTTTCTCCAGCTTCTGTATTGGGAAGTAAATCTCCTAAATTAAAT  933

Query  121   TAAAAACGAAGTAAAACCTTTCAAAACATATCAAAACAACCAATTGTTATTTATGGCAAT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  934   TAAAAACGAAGTAAAACCTTTCAAAACATATCAAAACAACCAATTGTTATTTATGGCAAT  993

Query  181   TTTAGGAAGTTCTCTTATGGTTAGGTTTTACTTCCCTTCTCACGATTTTGCTCCGTTTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  994   TTTAGGAAGTTCTCTTATGGTTAGGTTTTACTTCCCTTCTCACGATTTTGCTCCGTTTTG  1053

Query  241   CTAATTATTGATCCACATTTGCTCCCGAGTTAAAATTTTGGAGTAACGGGAGCAAAGCGG  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1054  CTAATTATTGATCCACATTTGCTCCCGAGTTAAAATTTTGGAGTAACGGGAGCAAAGCGG  1113

Query  301   AGCAATTATGAAGGTATCAGTAAACAAGCGTAACCCTAACTCGAAGGGGCTCCAGCAACT  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1114  AGCAATTATGAAGGTATCAGTAAACAAGCGTAACCCTAACTCGAAGGGGCTCCAGCAACT  1173
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جامعة النجاح الوطنية

كلية الدراسات العليا

الوصف الجزيئي لمقاومة المضادات الحيوية في بكتيريا السالمونيلا المعزولة من مصادر غذائية في فلسطين

إعداد

مجد أحمد محمد جناجرة

إشراف

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د. معتصم يوسف المصري

قدمت هذه الأطروحة استكمالاً لمتطلبات نيل درجة الماجستير في برنامج العلوم الحياتية بكلية الدراسات العليا، في جامعة النجاح الوطنية، في نابلس- فلسطين.

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الملخص

مقاومة المضادات الحيوية هي مصدر قلق صحي متصاعد في جميع أنحاء العالم. لا يلاحظ سوء استخدام المضادات الحيوية والإفراط في استعمالها في الإنسان فحسب، بل أيضًا من خلال السلسلة الغذائية باعتبارها واحدة من مسببات الأمراض الرئيسية التي تنقلها الأغذية. يميل السالمونيلا إلى نقل محددات مقاومة المضادات الحيوية. حالياً، تعتبر(integron و SGI1) الاكثر شيوعا للدراسة.

تم جمع ما مجموعه 128 عزلة من السالمونيلا من مصادر مختلفة، معظمها عينات غذائية من الاسواق الفلسطينية، وتم توفيرها من قبل المعمل المركزي التابع لوزارة الصحة ومركز التحاليل والتحكم في السموم والمعايرة. تم تحديد الحساسية للمضادات الحيوية من العزلات من خلال طريقة انتشار القرص. واستخدام تفاعل البلمرة المتسلسل للكشف عن جينات محددات المقاومة وايضا استخدام طريقة التسلسل لتحديد العازلات الموجبة للجينات المقاومة. تفاوتت مقاومة مقاومة المضادات الحيوية بين العزلات التي أظهرت عزلات مقاومة متعددة الأدوية.

وقد أوضحت الخصائص الجزيئية أن معظم العزلات كانت تؤوي مجموعة متكاملة من الدرجة الأولى مع أشرطة جينات متنوعة .تم الكشف عن الفئة 2 integron و SGI1 في عدد قليل من العزلات. ومن المثير للاهتمام، أن مظهر مقاومة المضادات الحيوية متغير بدرجة كبيرة على المستوى الجزيئي في الارتباط بتوصيفه الظاهري.

إن محددات مقاومة المضادات الحيوية في السالمونيلا المعزولة من المصادر الغذائية المختلفة تثير ناقوس الخطر لدورها المباشر كمُمْرِض منقولة بالأغذية. علاوة على ذلك، فإن إمكانية نقل مضادات مقاومة المضادات أفقيا قد تكون مائلة بشكل غير مباشر في نظرية الأخطاء العظيمة. تتطلب مراقبة ومراقبة انتشار جينات المقاومة المضادة للميكروبات من عزلات بكتيرية أخرى واستخدام المضادات الحيوية بروتوكولات صارمة للتحكم في ظهور العزلات البكتيرية المقاومة للعديد من الادوية