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Role of Incompatibility Group 1 (IncI1) Plasmid-encoded Factors on Salmonella enterica Antimicrobial Resistance and Virulence

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Role of Incompatibility Group 1 (IncI1) Plasmid-encoded Factors on *Salmonella enterica* Antimicrobial Resistance and Virulence

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by

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Abstract

Foodborne illnesses are a leading cause of infectious diseases in the world. Among enteric organisms *Salmonella* is a key pathogen. It's high prevalence in poultry and other food-animal sources make it imperative to study. *Salmonella* has the ability to modify its genetic content with help of mobile genetic elements such as plasmids. Incompatibiltiy group 1 (IncI1) plasmids are commonly reported in *Salmonella*. This study evaluates role on IncI1 plasmids in antimicrobial resistance and virulence in *Salmonella*. Genetic determinants of resistance and virulence are noted among our IncI1-containing *Salmonella* isolates. These genetic elements are also transferable and reported to carry respective phenotypic traits with them. Whole genomes of selected strains were sequenced using Illumina MiSeq platform. This gave more comprehensive understanding of IncI1 plasmids and their host strains. Further studies using advanced sequencing methods and functional assays under various stress factors such as different pH, in presence of probiotic-like compounds, would give more accurate and comprehensive understanding of IncI1 plasmids and their role in foodborne illnesses. Overall, this study increases our understanding of foodborne illnesses and provides us efficient tools to manage them.

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Dedication

I dedicate my dissertation to my wife Mrs. Sharmila Kaldhone, parents, rest of my family members, and friends whose understanding, guidance and help in making this journey possible.

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Chapter 3. **Kaldhone PR**, Han J, Deck J, Khajanchi B, Nayak R, Foley SL, and Ricke SC. 2017. Evaluation of the genetics and functionality of Incompatibility 1 (IncI1) plasmids from *Salmonella enterica.* Foodborne Pathog Dis. Accepted. DOI:10.1089/fpd.2017.2332.

Chapter 4. **Kaldhone PR**, Khajanchi BK, Han J, Nayak R, Ricke SC, and Foley SL. 2017. Draft genome sequences of *Salmonella enterica* isolates containing the incompatibility group I1 plasmids form swine, poultry and human sources. Genome Announc. 5: e01056-17.

I. Introduction

This dissertation focuses on the impact of incompatibility group (Inc) I1 plasmids on *Salmonella* antimicrobial resistance, virulence and colonization. Each of the following chapters focus on different aspects of *Salmonella* and IncI1 plasmids. The common theme throughout these five chapters is as follows: 1) Foodborne pathogens are important contributors to infectious diseases. *Salmonella enterica* is one of the most predominant foodborne pathogens and has been responsible for several foodborne disease outbreaks in the U.S. (CDC, 2011). The first chapter focuses on one of the leading serotype *Salmonella* Heidelberg in layer hens and egg production. 2) Mobile genetic elements like plasmids allow *Salmonella* to adapt to external stress factors (Johnson et al, 2010). Plasmids are often defined by their incompatibility (Inc) grouping and plasmids representing multiple Inc groups have been found to play important roles in the dissemination of antimicrobial resistance among the members of Enterobacteriaceae family (Caratolli, 2011). Some plasmids such as IncI1, IncA/C and IncFII carry genes responsible for resistance to extended spectrum cephalosporins and other clinically important antimicrobial agents (Folster et al, 2016). The second chapter provides insight into IncI1 plasmids with regards to antimicrobial resistance, virulence and transfer potential. 3) IncI1 plasmids have been shown to encode multidrug resistance (MDR) phenotypes through the carriage of multiple antimicrobial resistance genes, which could serve as a reservoir to disseminate resistance genes to other bacteria (Wong et al, 2016). The third chapter evaluates genetics and functionality of IncI1 plasmids in *Salmonella enterica*. 4) The fourth chapter evaluates IncI1 plasmid-carrying strains. The epidemiological information of sequenced strains and summary of sequence analysis is included in this chapter. 5) Host- pathogen interactions are critical for *Salmonella* to survive and persist in their host (Patterson et al, 2016). The fifth chapter evaluates virulence

associated with IncI1 plasmids among *Salmonella enterica* isolates. This project provides critical phenotypical characteristics such as resistance profile, colicin inhibition assay and virulence pertaining to Caco-2 cell line for *Salmonella enterica* isolates containing IncI1 plasmids. Information about resistance, virulence- associated genes and replicon types of respective strains is also provided

The overall objective of this project was to evaluate role of IncI1 plasmids in antimicrobial resistance and virulence among *Salmonella enterica.* To achieve this objective, we have utilized the approaches outlined in next few lines. To assess the genetic diversity of IncI1 plasmids and the strains that carry them experiments such as polymerase chain reaction (PCR) and sequencing reactions were conducted. The PCR experiments were performed to detect antimicrobial resistance, conjugative-transfer and putative virulence genes. Whole genome sequencing was carried out on selected strains to get insight into their genetic composition. Bacterial inhibition associated with colicin toxin production was studied among our isolates using colicin inhibition assays. Conjugation experiments were subsequently conducted to know transfer dynamics of plasmids. The virulence potential of our isolates was tested using the Caco-2 human intestinal epithelial cells. Invasion and persistence assays in Caco-2 cells were conducted to reveal virulence ability of our isolates. Transconjugants obtained through conjugation experiment were put through genotypic screening, including the detection of resistance-related genes, replicon typing for plasmid detection and phenotypic characterizations such as colicin inhibition and Caco-2 virulence assays. The background, methods and results of experiments related to these approaches are discussed in details in the chapters of this dissertation.

References

Carattoli A. 2011. Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol. 301:654-658.

Center for Disease Control and Prevention. 2011. *Salmonella* surveillance. Annual Summary, 2009. Atlanta, GA.

Folster JP, Grass JE, Bicknese A, Taylor J, Friedman C, and Whichard JM. 2016. Characterization of resistance genes and plasmids from outbreaks and illness clusters caused by *Salmonella* resistant to ceftriaxone in the United States, 2011–2012. Microbial Drug Res. 23:188-193.

Johnson TJ, Thorsness JL, Anderson CP, Lynne AM, and Foley SL. 2010. Horizontal gene transfer of a ColV plasmid has resulted in a dominant avian clonal type of *Salmonella enterica* serovar Kentucky. PLoS ONE. 5: e15524.

Patterson SK, Kim HB, Borewicz K, and Isaacson RE. 2016. Towards an understanding of *Salmonella enterica* servar Typhimurium persistence in swine. Anim Health Res Rev. 17:159-168.

Wong MH, Kan B, Chan EW, Yan M, and Chen, S. 2016. IncI1 plasmids carrying various *bla*CTX-M genes contribute to ceftriaxone resistance in *Salmonella enterica* serovar Enteritidis in China. Antimicrob Agents Chemother. 60:982-989.

II. Chapter 1

Salmonella Heidelberg in Layer Hens and Egg Production: Incidence and Potential Issues

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Abstract

Foodborne *Salmonella* continues to be a public health issue both from an illness standpoint as well as economically. Poultry and eggs represent a primary source of foodborne *Salmonella* with several serovars usually being the primary isolates specifically identified as originating from these food product sources. *Salmonella* Heidelberg has emerged as a serovar of increasing prominence, particularly in some of the more recent egg-associated outbreaks. In this chapter the incidence of *S.* Heidelberg in poultry and eggs as well as characteristics and ability to cause disease as a foodborne pathogen are discussed. Particular issues such as virulence capabilities and antimicrobial resistance are also described. Finally, the adaptability of *S.* Heidelberg and implications are summarized.

Introduction

Pathogenic bacteria cause an estimated 3.6 million cases of foodborne illnesses per year in the U.S.; approximately one third (1.04 million cases) of which are caused by the non-typhoid *Salmonella* (Scallan et al, 2011). Foodborne illnesses caused by organisms such as *Salmonella* associated with food animals have continued to remain a prominent concern over the past several decades. Several factors have contributed to this continued prevalence of foodborne illnesses. For example, centralization of food production, food processing, and distribution system increases the possibility for larger outbreaks (Rose et al, 2002, Bhatt and Zhang, 2013). In addition, increased and divergent sources of foods and food ingredients such as eggs add to the uncertainty (Ricke et al, 2013a). Likewise, changes in consumer dining habits, food preferences, and increased consumption of raw foods such as vegetables are likely contributors as well (Hanning et al, 2009).

Among the bacterial foodborne pathogens *Salmonella*-related infections annually account for 35% of hospitalizations and 65% of deaths in the U.S. (Scallan et al, 2011). Human salmonellosis is dominated by the broad host-range serovars (Foley et al, 2011, 2013, Ricke et al, 2013b). Similarly, several of the most commonly detected serovars in the U.S. poultry industry are able to colonize multiple host species (Foley et al, 2011, 2013). Not all serovars behave the same way in their respective host and some serovars exhibit a broader host range than others. Invasive diseases associated with these serovars can arise when gastrointestinal organisms are able to undergo extra-intestinal spreading leading to bacteremia and focal (localized to one organ or system) infection causing systemic manifestations (Jones et al, 2008, Suez et al, 2013). For example, invasive non-typhoidal *Salmonella* have surfaced as a leading cause of bloodstream infections in Sub-Saharan Africa in juveniles and adults (Feasy et al, 2012).

Poultry continues to be one of the primary reservoirs of *Salmonella* among animals (Edwards, 1958, Foley et al, 2008, 2011, 2013, Finstad et al, 2012, Howard et al, 2012). It has been estimated that 90% of cases of salmonellosis in the U.S. originated from chickens, eggs or egg-products (Chittick et al, 2006, Foley et al, 2008). In the U.S., 3.9% of the whole chicken, 18% of the ground chicken, 1.6% of the ground beef, 15% of the ground turkey, and 2.3% of the turkey samples tested positive for *Salmonella* during 2013 (CDC, 2013). Among the known human disease causing *Salmonella* serovars, a limited number are considered to be significant causes of foodborne infection. *Salmonella enterica* serovar Heidelberg, *S.* Kentucky, *S.* Senftenberg, *S.* Enteritidis, *S.* Typhimurium and *S.* Hadar have all been identified as prominent serovars in samples isolated from chickens over the past twenty years (Foley et al, 2008, 2011, 2013). In more recent times, *Salmonella enterica* serovar Heidelberg has emerged as a leading foodborne disease causing serovar. Among the documented outbreaks, *S.* Heidelberg has been associated with those originating from eggs and egg production suggesting that it can occupy this specific niche. Given this concern, the overall objective of this review is to discuss what is currently known about *S.* Heidelberg as a foodborne pathogen in general and future perspectives for understanding its incidence in poultry and eggs and potential issues associated with antibiotic resistance.

Emergence of *Salmonella* Heidelberg as a Foodborne Pathogen

Salmonella Heidelberg was initially discovered in 1933 in Heidelberg, Germany (Habbs, 1933). In 1954, it was isolated for the first time in the U.S. (Smyser et al, 1965). More recently, the serovar has consistently remained among the top ten most common etiologic agents for non-typhoid *Salmonella* infection (Harris et al, 1990, Stanley et al, 1992, Threlfall et al, 1992, Foley

and Lynne, 2008, Donado-Godoy et al, 2015). Specifically in the U.S., *S.* Heidelberg is typically among the top five most common serovars causing human salmonellosis (CDC, 2011) and has been responsible for several outbreaks in the U.S. and Canada (Dutil et al, 2010, Hoffman et al, 2013). For example, a multistate outbreak that occurred in 2013 and 2014 was traced back to contaminated chicken that managed to sicken 634 people in 29 different states (CDC, 2014).

According to USDA's National Veterinary Service Laboratory data from 1968 to 2010, 71% of the *S.* Heidelberg isolates collected over that time period originated from poultry-related sources (CDC, 2013). Among isolates at slaughter, poultry accounted for 86% of the food animal isolates, which is noteworthy, given the high percentage of the isolates that originates from one class of hosts. Thus, the serovar likely has evolved to survive in the chicken gastrointestinal environment. Genetic adaptation to environmental conditions associated with the host could be one reason for this predominance as has been indicated for other serovars (Johnson et al, 2010). The environments where birds are raised can influence the patterns of predominant organisms in their gut ecosystem (Nordentoft et al, 2011). The avian gut provides a diverse polymicrobial environment that could potentially provide selective pressure to alter the genetic composition of *S.* Heidelberg in such a manner to better adapt to the poultry environment (Han et al, 2012). In addition, antimicrobial exposure may impact the populations of organisms present in an environment, especially since several *S.* Heidelberg strains have been reported to be multidrug resistant (Lynne et al, 2009). If these strains outcompete other bacteria under selective pressure, it may help explain the relative prominence of this pathogen along the food production continuum, where *S.* Heidelberg isolates displaying antimicrobial resistance have been recovered at many different steps (Lynne et al, 2009). A detailed examination of the incidence and

distribution in poultry and egg production is warranted to develop a more in-depth understanding regarding potential explanations for the prevalence of this serovar.

Epidemiology of Egg Associated-*Salmonella* Outbreaks

A substantial proportion of the increase in poultry associated salmonellosis is no doubt due to increase in poultry and egg market sales. Since 1910, per capita consumption of poultry products in the U. S. has increased 6.5-fold (Buzby and Farah, 2006). Egg consumption in the U.S. has also recently reached a thirty year high, with the per person consumption increasing by twelve eggs over the past four years (Clarke, 2015). To meet this demand, the USDA reported that 242 million cases of shell eggs (15 dozen eggs per case) were produced in the U.S. during 2014 by 305 million shell egg laying hens (Egg Facts, 2015). In one month alone, 5.814 millions of cases of eggshells were broken for egg products in the U.S (Ibarburu, 2015). Global egg production also grew from 35.2 million tons to 62.6 million tons in the last few years (Windhorst, 2009). Regional imbalances in egg production and demand have led to substantial growth in egg transportation globally. In North America, the U.S. is a major exporter and Canada and Mexico are importers (Windhorst, 2009). South and Southeast Asian countries such as Malaysia and Thailand are now exporters, while the U.A.E. and Kazakhstan are leading importers in Western and Central Asian countries (Windhorst, 2009). In the EU, the ban on cage-rearing for birds has led to an increase in the import of eggs from neighboring non-EU countries such as Belarus (Windhorst, 2009). These increased levels of imports have increased the odds of foodborne diseases caused by eggs, because the corresponding effects of transit time adds to the likelihood of a break in the cold chain and opportunities for cross-contamination (Carrasco et al, 2012).

Egg consumption has been identified as a risk factor for *S*. Heidelberg infection (Hennessy et al, 2004). Hennessy et al, (2004) estimated that approximately 37% of *S*. Heidelberg population-attributable infections originate from consuming eggs prepared outside the home. In addition, eggs can be consumed in many different forms, including either eggs alone or through products containing eggs that may be raw or lightly cooked, such as Caesar salad dressing, homemade ice cream, hollandaise sauce, and fresh pasta dishes (Ricke et al, 2013a). This diverse use of eggs in a wide range of food products makes it a critical potential vehicle for *S*. Heidelberg transmission, and also a challenge to pinpoint specific sources of infections.

In 2010, the nation's largest egg recall was due to *Salmonella enterica* serovar Enteritidis contamination on egg layer farms in Iowa (Flynn, 2012). When one of the farms associated with the 2010 outbreak was inspected in 2012, investigators isolated *S.* Heidelberg from the poultry houses (Hoffman et al, 2013). They were confirmed initially using pulsed field gel electrophoresis (PFGE) followed by whole genome sequencing (WGS) to differentiate isolates with the same PFGE pattern (Hoffman et al, 2013). The four isolates related to this outbreak were linked to the JF6X01.0022 XbaI and JF6A26.0001 BlnI patterns.

In another instance, outbreak-strains isolated from clinical patients exhibited a specific PFGE pattern (MMWR, 2013) that matched the PFGE pattern of a strain isolated from suspected slaughterhouse examined during the traceback investigation with similar PFGE patterns being subsequently differentiated by WGS (Evans et al, 2014). Two isolates from this outbreak exhibited a multi-drug resistant phenotype. The patients, from whom these two isolates were collected, were under one year of age. The variable antimicrobial resistance profile of these isolates and patients' immature immune status would have made outpatient treatment difficult,

thus the infants required hospitalization for their illness. It is probable that these clinical isolates could have acquired their antimicrobial resistance via the poultry house environment (MMWR, 2013). Due to the occurrence of *S*. Heidelberg in poultry houses, poultry could be considered as a primary source of the pathogen, thus making poultry associated *S*. Heidelberg a potential public health concern (FDA, 2012).

Ecology of *Salmonella* Heidelberg Colonization and Invasion in Poultry

In general, *S*. Heidelberg possesses potential extensive colonization capacity for poultry associated with its ability to attach and invade the host intestinal epithelial cells. Some *S.* Heidelberg strains have been identified that contain mobile genetic elements such as the incompatibility group (Inc) FIB plasmids. These plasmids often contain genes for iron acquisition, toxin (colicin) production, serum survival, and antimicrobial resistance (Han et al, 2012). The presence of these plasmids and their aerobactin operon and *sit* iron transport systems, likely play a role in allowing *S*. Heidelberg to successfully colonize the epithelial lining of poultry.

Chicken macrophages are an important component in the defense against bacterial invasion (He et al, 2012). Newly hatched chickens possess a naïve immune system and rely on transferred maternal immunity for their defense against infection. The macrophage response in these young birds is attenuated (He et al, 2012). Therefore, young birds are particularly susceptible to *S*. Heidelberg infection due to the pathogen's ability to weaken the macrophage response coupled with the weaker host immune system. These factors make *S.* Heidelberg an important player in terms of invasive infection in newly hatched chicks.

Macrophage survival is a key for boosting virulence leading to invasive *Salmonella* infections. Gokulan et al. (2013) examined the infection of J774 mouse macrophages by *S.*

Heidelberg and concluded that *S.* Heidelberg was able to enter and survive in this macrophage cell line with differing abilities (Gokulan et al, 2013, Agnihothram et al, 2015). Those strains that survived the best contained a plasmid-encoded type 4 secretion system (T4SS), which likely diminished host immune response that resulted in increased uptake and survival of *S.* Heidelberg in the macrophages after 24 hrs. of incubation (Gokulan et al, 2013). He et al, (2012) conducted a study examining the interaction of *S.* Heidelberg and HD-11 chicken macrophage cells, which are MC29 virus-transformed chicken macrophage cells (Beug et al, 1979). An effective, robust oxidative burst was considered an indicator of effective defense functions of the cell line, however the study demonstrated that HD-11's phorbol myristate acetate-stimulated oxidative burst decreased after infection by *S.* Heidelberg (He et al, 2012). Thus, this ability of *S.* Heidelberg to limit macrophage function could be critical to its extra-intestinal survival.

If *S.* Heidelberg infects the reproductive tract of egg laying hens, eggs produced have the potential to be contaminated as occurs with other serovars particularly *S.* Enteritidis. *Salmonella* Enteritidis can contaminate eggs in two distinct ways, either by external penetration of the eggshell or internally via transovarian infection (Gantois et al, 2009; Howard et al, 2012; Martelli and Davies, 2012). The external eggshell penetration route includes transmission from the feces of colonized birds to the egg surface followed by penetration to the interior of eggs and growth during the storage (Cockburn and Vernon, 1956). *Salmonella* Heidelberg would appear to be a candidate for external egg contamination as it has been isolated from layer feces in commercial layer houses (Li et al, 2007). Gast et al, (2007b) studied *in vitro* egg contamination by *S.* Heidelberg and *S.* Enteritidis under ambient temperature. They observed *S.* Enteritidis exhibits a remarkably greater rate of eggshell penetration and yolk multiplication as compared to

S. Heidelberg. They also reported a significantly lower rate of penetration and multiplication at incubation temperatures between 20°C than 30°C for *S.* Heidelberg.

While *S.* Heidelberg appears to be less capable of penetration, prevention measures focused on temperature control at the poultry farm and during processing and transportation may still be important for controlling *S.* Heidelberg. Although little direct evidence has been established that *S.* Heidelberg possesses characteristics that allow it to be prevalent in eggs, it has been reported to grow in Brain Heart Infusion broth at 19°C and 37°C, with only slight variation when compared to other *Salmonella* serovars including *S.* Enteritidis (Juneja et al, 2003). When McConnell and Schaffner, (2014) incubated *S.* Heidelberg as part of a cocktail of *Salmonella* serovars in raw ground beef they validated recommended U.S. FDA guidelines for the length of time that food can be kept out of temperature control if the food product starts at 5°C and does not exceed 21°C (McConnell and Schaffner, 2014). While it remains to be determined if similar criteria would be applicable for S. Heidelberg in eggs, there may be potential for a relatively high prevalence of *S.* Heidelberg in eggs if the opportunity for initial contamination arises and sufficient temperature abuse occurs to allow substantial growth. Certainly, improper transport and a break in the cold chain could enhance growth of *S.* Heidelberg in contaminated eggs (Schoeni et al, 1995). While pasteurizing egg whites appears to cause a greater than 8 log reduction of *S.* Heidelberg (Muriana, 1997), cooking may not be able to always eliminate the organism as several *Salmonella* serovars are capable of surviving simulated domestic conditions for various forms of cooking eggs (Humphrey et al, 1989).

Among *Salmonella* serovars, *S.* Heidelberg, *S.* Enteritidis, and *S.* Typhimurium are able to colonize the reproductive tract of layer hens with *S.* Enteritidis exhibiting tissue tropism for the reproductive tract (Gast et al, 2004, 2005, 2007a, 2011, Gantois et al, 2008). In a more recent

study, Gast et al. (2011) observed the same rate of isolation for *S*. Heidelberg and *S*. Enteritidis in ovaries and oviducts of chicken. This indicates both serovars might possess the similar capabilities to colonize the reproductive tract and also implies that factors other than colonization of the bird's main reproductive tract plays an important role in the contamination of egg. *Salmonella* Enteritidis has been shown to produce high molecular weight lipopolysaccharides and be able to grow to high cell densities (Guard-Petter, 1998; Parker et al, 2001, Gast et al, 2011). These characterstics could have a role in colonization of bacteria to the epithelium of the gastrointestinal tract and could be the reason for the greater ability of *S*. Enteritidis to colonize and invade gastrointestinal tract than other serovars such as *S*. Heidelberg (Gast et al, 2011). *Salmonella* Enteritidis uses diverse types of fimbriae such as SEF 14, SEF 17, and SEF 21 to attach to the host luminal lining. It also possesses long polar and plasmid-mediated fimbriae (Foley et al, 2008), while *S*. Heidelberg expresses fimbriae such as FliA, FliB, and FliC. *Salmonella* Heidelberg has been isolated from ovaries of naturally infected chickens (Barnhart et al, 1991), which may provide an opportunity for transovarian contamination of eggs. The egg-contamination ability could be attributed to expression of potential virulence factors such as the outer membrane proteins, fimbriae and flagella. Environmental factors including temperature and pH might affect the expression of these virulence factors as well (McDermid et al, 1996, Morales et al, 2007; Gast et al, 2011) and impact the ability of *Salmonella* to infect eggs. Clearly, more research needs to be done to elucidate whether *S*. Heidelberg possesses specific mechanisms associated with colonization of the layer hen reproductive tract.

Virulence and Pathogenesis of *S*. Heidelberg

While the *S*. Heidelberg association with laying hens and eggs remains to be fully explored, its pathogenesis in humans is assumed to be fairly typical of other foodborne disease causing *Salmonella* serovars. *Salmonella* infections in humans can lead to gastrointestinal illness which is characterized by nausea, vomiting, abdominal pain, and diarrhea that begins 12 to 36 hours following consumption of the contaminated food. The severity of the symptoms depends on various factors including the level of virulence gene expression of the organism and the host immune status (Robertson et al, 2003).

The type of diarrhea caused by *S*. Heidelberg and other pathogenic serovars is inflammatory diarrhea, which is the result of the interaction of bacterial enterotoxin and host epithelium (Foley et al, 2011, 2013). Following ingestion, *Salmonella* adheres to the intestinal epithelium with the help of flagella and fimbriae (Van Asten and Van Dijk, 2005, Foley et al, 2013). Conserved and host-specific factors expressed by *Salmonella* helps the organism to colonize the host gastrointestinal epithelium (Stevens et al, 2009, Foley et al, 2013). The pathogen crosses the intestinal epithelial barrier with the aid of the *Salmonella* pathogenicity island (SPI) 1-encoded type 3 secretion system (T3SS), which is a molecular transporter that facilitates the transfer of toxins and effector proteins such as InvJ, SpaO, PrgI/J, SipA/B/C/D, SptP, AvrA, SopA/B/D/E/E2, SlrP, and SspH1 from the cytoplasm of the bacteria into the host cells (Galán and Wolf-Watz, 2006, Schlumberger and Hardt, 2006, Foley et al, 2013). Thus, the T3SS promotes cellular uptake and invasion. Some of the effector proteins such as SopA/B/D/E2 and SipA activate the host-signal transduction cascade (Hopkins and Threlfall, 2004, Foley et al, 2013), which leads to induction of membrane ruffling at the contact site where *Salmonella*

interacts with the host cell (Al-Mousawi et al, 2010). Membrane ruffling leads to engulfment of the bacterium and the formation of *Salmonella* containing vacuoles within the host cells.

The virulence phenotype displayed by the pathogen is largely determined by the virulence factors that the organism carries. In addition to the SPI-1 coded T3SS, there is a second T3SS coded by SPI-2 that plays an important role in the virulence of serovars such as *S.* Enteritidis (Hensel et al, 1998; Rosselin et al, 2011, Foley et al, 2013, Ricke et al, 2013b). The SPI-2 coded T3SS is involved in post-invasion changes in the intra-cellular environment (Malik-Kale et al, 2011). Each of the T3SSs forms complex systems that deliver at least forty distinct virulence effectors into the host cells to facilitate invasion, survival, and replication within host cells (Malik-Kale et al, 2011). These virulence effectors are responsible for various functions including decreasing the activating and trafficking of free oxygen radicals, and inhibiting phagocyte maturation. Free oxygen radicals, such as nitrous oxide, are one of the macrophages' primary defense tools against microbial pathogens (Rosselin et al, 2011). Defective macrophages can be responsible for intracellular survival and proliferation of bacterial pathogens (Withanage et al, 2005).

Salmonella Heidelberg, along with other invasive non-typhoidal serovars (iNTS), possess additional genetic elements that can facilitate invasive infections. In addition to SPI-1 and 2, *Salmonella* can carry several additional SPIs, including 3-6, 9, 13, and 14 that are important for *Salmonella* virulence (Suez et al, 2013). For example, SPI-6 encodes genes such as *invasin*, *pagN*, *CS54*, and *sinH*, which contribute to *Salmonella*'s invasive phenotype. Fimbriae gene clusters, such as *bcf*, *csg*, *stb*, *sth*, and *sti* also aid infection of the host by forming a filamentous structure on the cell surface that assists colonization in chicken gastrointestinal epithelium (Foley et al, 2013). *Salmonella* Heidelberg, like other *Salmonella* serovars is able to penetrate the

intestinal epithelium, spread from one epithelial cell to another and eventually enter into the macrophages and dendritic cells (Wallis et al, 1986, Richter-Dahlfors et al, 1987, Jones et al, 1994, Rescigno et al, 2001, Salcedo et al, 2001, Meyerholz et al, 2002, Geddes et al, 2007, Malik-Kale et al, 2011).

Antimicrobial Resistance in *Salmonella* Heidelberg

The other phenotype characteristic that *S*. Heidelberg shares with several *Salmonella* serovars is antimicrobial resistance. Several strains of *S*. Heidelberg have been shown to cause invasive disease, which often requires antimicrobial therapy for treatment (Suez et al, 2013). Consequently, antimicrobial resistance is a major health concern due to potential clinical treatment failure. Analysis of resistance trends has shown that *S*. Heidelberg isolates collected in recent years are more likely to be to clinically important antimicrobial agents than they were historically (Folster et al, 2012). These findings may be due, at least in part, to selective pressure from continued use of antimicrobial agents in animal feeds, veterinary and human medicine (Crump et al, 2011). For example, the numbers of cephalosporin- resistant *S*. Heidelberg infections occurring in chickens and humans exhibited a distinctive trend in Quebec, Canada during the past few years. From 2004 to 2007 the number of resistant infections was decreasing, however, from 2007 to 2011 the trend reversed and subsequently the numbers of resistant infections increased. This trend has been suggested to correlate with the reintroduction of the use of ceftiofur in hatcheries in Quebec that began in late 2006 after a period of disuse (Otto et al, 2014).

Overall, antimicrobial resistance increases the cost of illness by increasing the number of cases, severity, and duration of illness (Rabsch et al, 2001, Foley et al, 2008). Resistance also leads to clinical treatment failure if the administered therapy is ineffective and the healthcare

provider is forced to apply the next line of therapy. This regimen lengthens the time of recovery and heightens the odds of the patient developing bacteremia, septicemia, and organ system failure. Once organ system failure ensues, it can cause irreversible damage to the body, potentially leading to death. In this way, increased resistance can lead to chronic sequelae and increased mortality (Barza and Travers, 2002).

Human antimicrobial use is also a risk factor for salmonellosis. Antimicrobial treatment can disrupt the normal microbiome of the host. The microbiome serves an important function by occupying the epithelial surface and preventing colonization by new organisms (Rashid et al, 2015). This colonization resistance can be hampered by antimicrobial therapy (Barza and Travers, 2002, Molbak 2005). As noted above, antimicrobial therapy can disrupt colonization resistance in the host. If there are *Salmonella* present or subsequent ingestion of organisms that are resistant to the drug used for previous therapy, it increases the likelihood of infection and proliferation increasing the severity of the foodborne illness (Koningstein et al, 2010).

As in Canada, *Salmonella* strains isolated in the U.S. have shown extended spectrum cephalosporin (ESC) resistance (Taylor et al, 2015). This phenomenon is due in large part to the ability of *Salmonella* to synthesize AmpC-like β-lactamase (Phillippon et al, 2002). This enzyme is coded by the bla_{CMY} genes, which are often located on plasmids, including those of incompatibility groups (Inc) A/C and IncI1. Both IncI1 and IncA/C plasmids have been identified in *S*. Heidelberg isolated from poultry (Han et al, 2012). The IncI1 plasmids carrying bla_{CMY} gene have been observed to acquire kanamycin resistance along with cephalosporins (Folster et al, 2011). IncI1 plasmids carrying bla_{CMY} genes, typically belongs to sequence type 12 (ST12) of the plasmid multi-locus sequencing typing (pMLST) analysis scheme (Jolley and Maiden, 2010). This sequence-based technique relies on genes contained on the plasmid. If genes

carrying antimicrobial resistance are present on the plasmid then there are enhanced odds that resistance could have been acquired through horizontal genetic transfer (Kaldhone et al, 2008, Krauland et al, 2010, Cain and Hall, 2012). Similar findings from pMLST have been reported for *S*. Kentucky isolates originating from poultry (Fricke et al, 2009) and *Salmonella* and *E. coli* from environmental, animal, and human sources in Canada (Mataseje et al, 2010). The increased dissemination of ESC resistance in the North American continent is likely due to the transmission of the plasmid-encoded bla_{CMY} genes. The fact that ESC strains display different PFGE patterns indicates the ability of the plasmids to incorporate into a variety of genetic backgrounds across multiple serovars and even species (Folster et al, 2012).

Ceftiofur resistance has been reported to be frequent among *S*. Heidelberg isolates from chicken (9%) and human sources (33%) in Quebec, Canada (CIPARS, 2011). A rotational administration of antibiotics was implemented to mitigate increasing antimicrobial resistance. It was subsequently observed that ceftiofur resistance decreased from 70 cases per 100,000 in 2004 to 29 cases per 100,000 in 2007, (CIPARS, 2011). Changes in antimicrobial agent use in veterinarian and agricultural practices might act as a risk reduction strategy to decrease antimicrobial resistance while treating foodborne illnesses.

Isolation, Identification and Detection

There are numerous methods for the isolation, identification and detection of *Salmonella* (Ricke et al, 2013b, Park et al, 2014). Several conventional isolation and identification methods are described by the U.S. FDA, European Committee for Standardization (CEN) and International Organization of Standardization (ISO) and are culture-based. These classical techniques follow a standard sequence beginning with non-selective preenrichments, followed by selective enrichments, isolation on selective agar media and finally biochemical screening with

triple sugar and lysine iron agars. Serological testing using poly-O and poly-H antisera is used as a step to identify the specific serotype of the isolate based on the Kaufmann-White scheme. In the case of *S.* Heidelberg, Maurischat et al, (2015) listed the serological profile (serotype) as 4,[5],12:r:1,2 when using it as one of the serovars for a real-time multiplex PCR assay developed to differentiate *S.* Enteritidis and *S.* Typhimurium. Numerous broths and plating media have been employed for *Salmonella* growth and some of them have experienced significant modifications during the course of time to increase their efficacy. The media used may have an impact on the efficacy of isolating different *Salmonella* serovars. This difference could be a reason for the variability in isolation rates and prevalence of certain *Salmonella* serovars in different locales (Richardson, 2011).

Polymerase chain reaction (PCR) approaches represent a more recent molecular based methodology used to detect and identify foodborne pathogens including *Salmonella*. The technique uses enzymatic amplification of specific DNA sequences in an isolate (Gharieb et al, 2015). Over time several different variations of PCR have been used for *Salmonella* identification, these include multiplex PCR, SYBR Green based real time (RT) PCR (Roche Diagnostics, Indianapolis, IN), and the BAX System (DuPont, Wilmington, DE) (Park et al, 2014, Gunel et al, 2015). These molecular methods are more rapid and reproducible, yet a positive result is only considered presumptive and needs to be confirmed by a standard method that leads to isolating an organism.

While molecular methods are more rapid than culture identification, their speed is reduced by the fact that many of these rapid methods require that the samples undergo a culture-enrichment step that can take several hours before analysis. Issues with including an enrichment step arise when considering that certain enrichment protocols may favor the odds of detection of

certain serotypes over others (Gorski, 2012). For example, *S.* Heidelberg spent media has been shown to limit the growth of certain *S.* Typhimurium isolates (Rivera Calo et al, 2015). While the mechanism is not clear specific metabolites produced by *S.* Heidelberg could play a role in this inhibition. In addition, several different techniques have been used across laboratories (Singer, 2009). In a study comparing common culturing methods for *Salmonella* it was found that each of the five methods compared resulted in a different *Salmonella* prevalence from swine fecal samples (Love and Rostagno, 2008). However, only the combination of results from two or more methods agreed most closely with the known level of positives (Love and Rostagno, 2008).

Advanced molecular typing techniques can be used to identify the effects of horizontal gene transfer among bacterial strains and thus used for traceback identification of pathogen sources. The techniques often employed include PFGE, clustered regulatory interspaced short palindromic repeats (CRISPR) - multiple variable locus sequence typing (MVLST) analysis (Young et al, 2012) and more recently WGS. The CRISPR are unique genetic elements that are made up of short sequences called spacers and conserved direct repeats (Haft et al, 2005). Analysis of CRISPR loci has been used to differentiate clinical isolates of *Salmonella* (Fabre et al, 2012). *Salmonella*-associated MVLST is a sequencing-based typing method that relies on the comparison of sequences of two virulence genes, *fimH1* and *sseL* (Liu et al, 2011). Molecular subtyping methods can be used in concert, for example, the combined CRISPR-MVLST and PFGE analysis has been shown to possess more discriminatory power than individual methods for *S.* Heidelberg isolates (Shariat et al, 2013). This means that if isolates have similar PFGE profiles, then they can be differentiated from each other using CRISPR-MVLST. Among these techniques, PFGE has proven to have utility for identifying horizontal gene transfer among *S.* Heidelberg strains from poultry-associated sources (Kaldhone et al, 2008).

Additional typing methods include multiple loci variable number tandem repeat analysis (MLVA), which is a PCR-based method that relies on differences in the number of tandem repeats that are observed at multiple loci known to have strings of repetitive sequence in the bacterial genome (Broschat et al, 2010). Multiple amplification of prophage locus typing (MAPLT) represents another sequence-based technique that depends on loci located in integrated prophage sequences (Ross and Heuzenroeder, 2005). When MLVA and MAPLT were combined they were found to be better able to distinguish among *S.* Heidelberg isolates of phage type (PT) 1, compared with the respective individual methods (Young et al, 2012). These approaches have been used to distinguish between human and non-human associated isolates among PT1 isolates (Demczuk et al, 2003).

With increasing ease and availability of sequencing, WGS has been promoted as the ultimate tool for the investigation of foodborne pathogens such as *S*. Heidelberg (Hoffmann et al., 2013, 2014). In a retrospective study of a recent *Salmonella* outbreak, Hoffmann et al. (2014), used WGS to examine the genetic relatedness of *S*. Heidelberg isolates associated with the 2011 multistate outbreak. The sequencing confirmed the presence of multiple antimicrobial resistance genes and likely enhanced virulence genes associated with T4SS. Single-nucleotide polymorphism (SNP) analysis based on WGS data has proven to be helpful for in depth differentiation of isolates; in one study 284 significant SNPs were found in 44 *S*. Heidelberg isolates that exhibited nearly identical PFGE patterns (Hoffmann et al, 2014). Similarly, Bekal et al. (2015) used a high-quality core genome single-nucleotide variant (hqSNV) to discriminate among the more prevalent and highly clonal *S*. Heidelberg isolates. More than 59 hqSNVs were measured among 46 *S*. Heidelberg isolates from three different outbreaks in Quebec that possessed the same PFGE and PT patterns. The ability to use SNP analyses to discriminate

highly clonal isolates demonstrates that the WGS-based approach could be a superior typing tool while working with events where the isolates were previously considered identical with conventional subtyping methods.

Future Issues-Evolution of *Salmonella* Heidelberg

Salmonella serotypes vary in their host specificities, for example *S.* Gallinarum and *S.* Dublin are very host-specific serovars; while *S.* Typhimurium, *S.* Enteritidis and *S.* Heidelberg represent examples of more broad host-range serovars (Baümler et al, 1998). Host range is dictated by several factors including genome plasticity and interaction with the host and its immune system (Foley and Lynne, 2008). The acquisition of new genes that allow for the attachment or colonization of new host environments can facilitate expansion of a respective microorganism's host range (Methner et al, 2011). These new genes can be obtained through horizontal gene transfer by a variety of vehicles including phages, plasmids, and transposons (Foley et al, 2013). Particularly troublesome is the continued isolation of strains resistant to multiple antimicrobial agents that makes them more problematic for treatment (Hennessy et al, 2004, Foley et al, 2008). Mutations in virulence related genes and presence of pseudogenes indicate that alterations of its virulence profile may also be occurring (Chiu et al, 2005). *Salmonella* Heidelberg appears to be continually evolving, likely in response to exposure to different external pressures (Beltran et al, 1988, Suez et al, 2013). These changing phenotypic characteristics of *S.* Heidelberg are brought about by alterations in genomic composition of strains through acquisition of new genes or mutation of existing gene contents (Foley et al, 2013) allowing bacteria to adapt to external stress and to alter their genetic content (Onchman and Moran, 2001, Maurelli, 2007).

In general, there are approximately 10^{-10} mutations per base pair in bacteria (Bars et al,, 2012). Some of the bacteria express mutations higher than this frequency and are referred to as "mutators" (Bars et al,, 2012). *Salmonella* Heidelberg strain, SHB182, is one example of such a mutator. The SHB182 strain has been associated with the bovine intestinal microbiome (Le Gall et al,, 2009). Mutations that this strain has accumulated are believed to facilitate increased adaption to the changing bovine intestinal lining. To study this phenomenon, Bars et al. (2012) created a twelve base pair deletion in a methyl mismatch repair (MMR) system for SHB182. This led to enhanced adherence to epithelial cells through increased expression of *fliC* and decreased expression of *fliA* and *fliB*. Allelic differences in *fliC* gene among *S*. Heidelberg, *S*. Typhimurium, and *S*. Muenchen are the result of recombination (Milkman and Stoltzfus, 1988, Smith et al, 1990). These findings from studies with SHB182 indicate that at least some strains of *S*. Heidelberg are able to undergo genetic changes in response to environmental stress factors that are reflected in altered pathogenic phenotypes.

Thus, a historical analysis of *S*. Heidelberg strains may explain the genetic adaptations that members of the serovar have undergone to survive in their respective environments (Kivisaar, 2003). For example, in a study exploring *Escherichia coli*, a mutation phenotype enabled organisms to adapt rapidly to the mouse gut environment (Giraud et al, 2001). *Escherichia coli* can acquire and accumulate mutations to adapt rapidly to its environment. Genomic plasticity enables organisms to alter their genomic content (Liu et al, 2007), and in *S*. Heidelberg's case to potentially increase its host range to infect a broad range of species. Therefore, a high resolution genomic map will be useful for identifying the correlation between parent strains and newer strains. Genomic rearrangements could be either in the form of an insertion or a deletion. Addition or deletion of genes helps that organism to divert their resources

towards more critical functions such as survival in a stressful environment. Consequently, *S.* Heidelberg with its ability of genomic plasticity is able to survive across a diverse host range.

Conclusions

Eggs have been an important source of protein in human diets and as such there has been increasing demand for egg production on a global scale, which makes identifying ways to improve egg safety imperative. *Salmonella* Heidelberg has become one of the more common organisms isolated along the poultry and egg production, processing and consuming continuum (Foley et al, 2011). The continued isolation of strains of *S.* Heidelberg that are resistant to multiple antimicrobial agents adds to the importance of studying ways to mitigate the risk of this organism in egg production.

In order to survive in the chicken intestinal tract, *S.* Heidelberg likely has displayed genomic plasticity to adapt to the host environment, either through the acquisition of required genes and deletion of unnecessary genes. Mobile genetic elements such as plasmids make this genetic information exchange possible. For example the acquisition of IncFIB plasmids carrying iron transport and toxin production genes is an example of genetic adaptability of some *S.* Heidelberg strains (Han et al, 2012). Other factors such as the expression of certain fimbriae may enhance transovarian spread and perhaps indicate a phenotypic adaptation of *S.* Heidelberg to gain an ecological advantage in the avian environment. Increased virulence coupled with antimicrobial resistance makes *S.* Heidelberg a challenge for egg safety. Several different antimicrobial resistance genes have been identified on different plasmids detected in *S.* Heidelberg (Folster et al, 2011), which can facilitate the horizontal spread of antimicrobial resistance among bacteria and makes resistance difficult to manage in *Salmonella*.

Technical advances dictate the methods for *Salmonella* detection. Conventionally used culture methods are still considered as the gold standard, however variability in outcome based on the media used and a longer duration to obtain results are drawbacks of culture methods (Richardson, 2011). PCR-based techniques are rapid and replicable, but often require culture confirmation to verify the results (Gunel et al, 2015). Advanced molecular techniques such as PFGE, MVLST and CRISPR analyses, especially in concert with one another, are important to understanding the molecular epidemiology of disease transmission (Liu et al, 2011, Young et al, 2012, Shariat et al, 2013). The rise of WGS-based methods provide valuable tools to gain detailed information on the genetics and natural history of *S.* Heidelberg strains (Bekal et al, 2015), which may provide useful data for identifying tactics to intervene and decrease bacterial contamination. *Salmonella* Heidelberg has become an important foodborne pathogen in eggs as well other food products, so there is critical need to understand the genetic mechanisms this organism uses to adapt to the avian environment and cause human disease, in order to provide better strategies to intervene and improve food safety.

Disclaimer

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References

Agnihothram SS, Basco MDS, Mullis L, Foley SL, Hart ME, Sung K, and Azevedo MP. 2015. Infection of murine macrophages by *Salmonella enterica* serovar Heidelberg blocks murine norovirus infectivity and virus-induced apoptosis. PLoS ONE 10: e0144911. doi:10.1371/journal.pone.0144911

Al-Mousawi A, Eissa A, Abu-Zant F, Drobiova H, Al-Saif I, and Al-Saleh E. 2010. Correlation between cluster analyses of *Salmonella* strains isolated from diarrhetic patients in Kuwait and biofilm formation. WIT Transactions on Ecology and the Environment. Environmental Toxicology. 132: 67-74.

Barnhart HM, Dreesen DW, Bastein R, and Pancorbo OC. 1991. Prevalence of *Salmonella* Enteritidis and other serovars in ovaries of layer hens at time of slaughter. J Food Prot 54: 488-491.

Bars HE, Gall-David SL, Renoux VM, Bonnaure-Mallet M, Jolivet-Gougeon A, and Bousarghin L. 2012. Impact of a mutator phenotype on motility and cell adherence in *Salmonella* Heidelberg. Vet Microbiol 159: 99-106.

Barza M, and Travers K. 2002. Excess infections due to antimicrobial resistance: The "attributable fraction". Clin Infect Dis 34: S126-30.

Baümler AJ, Tsolis RM, Ficht TA, and Adams LG. 1998. Evolution of host adaptation in *Salmonella enterica*. Infect Immun. 66: 4579-4587.

Bekal S, Berry C, Reimer AR, Domselaar GV, Beaudry G, Fournier E, Doualla-Bell F, Levac E, Gaulin C, Ramsay D, Huot C, Walker M, Sieffert C, and Tremblay C. 2015. Usefulness of hqSNV analysis for subtyping the highly clonal and the most prevalent *Salmonella* Heidelberg clone in the context of outbreak investigations. J Clin Microbiol. doi:10.1128/JCM.02200-15.

Beltran P, Musser JM, Helmuth R, Farmer III JJ, Frerichs WM, Wachsmuth IK, Ferris K, McWhorter AC, Wells JG, Cravioto A, and Selander RK. 1988. Toward a population genetic analysis of *Salmonella*: Genetic diversity and relationships among strains of serotypes *S. choleraesuis*, *S. derby*, *S. dublin*, *S. enteritidis*, *S. heidelberg*, *S. infantis*, *S. newport*, and *S. typhimurium*. Proc Natl Acade Sci U S A. 85:7753-7757.

Beug H, Von- Kirchbach A, Doderlein G, Conscience JF, and Graf T. 1979. Chicken hematopoietic cells transformed by seven strains of detective avian leukemia viruses display three distinct phenotypes of differentiation. Cell. 18:375-390.

Bhatt T and Zhang J. 2013. Food product tracing technology capabilities and interoperability. J Food Sci. 78(Suppl.2): B28-33.

Broschat SL, Call DR, Davis MA, Meng D, Lockwood S, Ahmed R, and Besser T E. 2010. Improved identification of epidemiologically related strains of *Salmonella enterica* by use of a fusion algorithm based on pulsed- field gel electrophoresis and multiple-locus variable-number tandem-repeat analysis. J Clin. Microbiol. 48: 4072-4082.

Buzby JC, and Farah HA. 2006. Chicken consumption continues longrun rise. Amber Waves. 4: 5.

Cain AK, and Hall RM. 2012. Evolution of a multiple antibiotic resistance region in IncHI 1 plasmids: Reshaping resistance regions in situ. J Antimicrob Chemother. 67: 2848-2853.

Canadian integrated program for antimicrobial resistance surveillance (CIPARS). 2011. Antimicrobial resistance short report. Public Health Agency of Canada, Guelph, Ontario, http://www.phac-aspc.gc.ca/cipars-picra/pubs-eng.php accessed on March 26, 2015.

Carrasco E, Morales-Rueda A, and Garcia-Gimeno RM. 2012. Cross-contamination and recontamination by *Salmonella* in foods: A review. Food Res Int. 45: 545-556.

Center for Disease Control and Prevention. 2011. *Salmonella* surveillance. Annual Summary, 2009. Atlanta, GA.

Center for Disease Control and Prevention. 2013. An atlas of *Salmonella* in the United States, 1968- 2011.

Center for Disease Control and Prevention. 2014. http://www.cdc.gov/*Salmonella*/heidelberg-10-13access/ accessed on July 12, 2015.

Chittick P, Sulka A, Tauxe R, and Fry A. 2006. A summary of national reports if foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: Clues for disease prevention. J Food Prot. 69: 1150-1153.

Chiu CH, Tang P, Chu C, Hu S, Bao Q, Yu J, Chou Y Y, and Wang HS. 2005. The genome sequence of *Salmonella enterica* serovar Choleraesuis, a highly invasive and resistant zoonotic pathogen. Nucleic Acids Res. 33:1690–1698.

Clarke P. 2015. Bacon helped US lead the way in boosting egg sales. Poultry World. 170: 18.

Cockburn W, and Vemon E. 1956. Food poisoning in England and Wales, 1956. Public Health Laboratory Service Report. Section II, 233-241.

Crump J A, Medalla FM, Joyce KW, Krueger AL, Hoekstra RM, Whichard JM, and Barzilay EJ. 2011. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Emerging Infections Program NARMS Working Group. Antimicrob Agents Chemother. 55: 1148–1154.

Demczuk W, Soule G, Clark C, Ackermann H-W, Easy R, Khakria R, Rodgers F, and Ahmed R. 2003. Phage-based typing scheme for *Salmonella enterica* serovar Heidelberg, a causative agent of food poisoning in Canada. J Clin Microbiol. 41: 4279- 4284.

Donado-Godoy P, Byrne BA, Hume M, Leon N, Perez-Gutierrez E, Flores MJV, Clavijio V, Holguin A, Romer-Zunniga JJ, Castellanos R, Tafur M, and Smith WA. 2015. Molecular characterization of *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg from poultry and retail chicken meat in Colombia by pulsed-field gel electrophoresis. J Food Prot. 78: 802-807.

Dutil L, Irwin R, Finley R, Ng LK, and Avery B. 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis. 16: 48-54. doi : 10.3201/ eid 1601.090729.

Edwards P. 1958. Salmonellosis: Observation on incidence and control. Annals New York Acad Sci. 598-613.

Egg facts. 2015. The egg business. http://www.aeb.org/farmers-and-marketers/industry-overview accessed November 23, 2015.

Evans PS, Luo Y, Muruvanda T, Ayers S, Hiatt B, Hoffman M, Zhao S, Allard MW, and Brown E. 2014. Complete genome sequences of *Salmonella enterica* serovar Heidelberg strains associated with a multistate food-borne illness investigation. Genome Announc 2: e01154-13.

Fabre L, Zhang J, Guigon G, LeHello S, Guibert V, Accou-Demartin M, De Romans S, Lim C, Roux C, Passet V, Diancourt L, Guibourdenche M, Issenhuth-Jeanjean S, Achtman M, Brisse S, Sola C, and Weill F-X. 2012. CRISPR typing and subtyping for improved laboratory surveillance of *Salmonella* infections. PLoS ONE. 7:e36995.

Feasey NA, Dougan G, Kingsley RA, Heyderman RS, and Gordan MA. 2012. Invasive non-typhoidal *Salmonella* disease: An emerging and neglected tropical disease in Africa. Lancet. 379: 2489-2499.

Finstad S, O'Bryan CA, Marcy JA, Crandall PG, and Ricke SC. 2012. *Salmonella* and broiler processing in the United States: Relationship to foodborne salmonellosis. Food Res Int. 45:789-794.

Flynn D. 2012. *Salmonella* Heidelberg found in Iowa poultry houses. Food Safety News. http://www.foodsafetynews.com/2012/09/*Salmonella*-heidelberg-found-in-iowa-poultry-houses/#.VZQIpKbcG50 accessed June 28, 2015.

Foley SL, Lynne AM, and Nayak R. 2008. *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. J Anim Sci. 86: E149-E162.

Foley SL, and Lynne AM. 2008. Food-animal associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance. J Anim Sci. 86: E173-E187.

Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, and Ricke SC. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Appl Environ Microbiol. 77: 4273-4279.

Foley SL, Johnson TJ, Ricke SC, Nayak R, Danzeisen J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev. 77:582-607.

Folster JP, Pecic G, McCullough A, Rickert R, and Whichard JM. 2011. Characterization of bla (CMY)-encoding plasmids among *Salmonella* isolates in the United States in 2007. Foodborne Pathog Dise 8: 1289-1294.

Folster J P, Pecic G, Singh A, Duval B, Rickert R, Ayers S, Abbott J, McGlinchey B, Bauer-Turpin J, Haro J, Hise K, Zhao S, Fedorka-Cray P J, Whichard J, and McDermott P F. 2012. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat, and humans in the United States 2009. Foodborne Pathog Dis. 9: 638-645.

Food and Drug Administration. 2012. Inspections, compliance, enforcement and criminal investigations. Warning letters http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2012/ucm315877.htm accessed on July 30, 2015.

Fricke WF, McDermott PF, Mammel MK, Zhao S, Johnson TJ, Rasko DA, Fedorka-Cray PJ, Pedroso A, Whichard J M, Leclerc JE, White DG, Cebula TA, and Ravel J. 2009. Antimicrobial resistance-conferring plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella enterica* serovar Kentucky isolates from poultry. Appl Environ Microbiol. 75: 5963-5971.

Galán J E, and Wolf-Watz H. 2006. Protein delivery into eukaryotic cells by type III secretion machines. Nature. 444: 567–573.

Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, and Van Immerseel F. 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Micobiol Rev. 33: 718-738.

Gantois I, Eeckhaut V, Pasmans F, Haesebrouck F, Ducatelle R, and Van Immerseel F. 2008. A comparative study on pathogenesis of egg contamination by different serotypes of *Salmonella*. Avian Pathol 37:399-406.

Gast RK, Guard-Bouldin J, and Holt PS. 2004. Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with *Salmonella heidelberg* and *Salmonella enteritidis*. Avian Dis. 48: 863-869.

Gast RK, Guard-Bouldin J, and Holt PS. 2005. The relationship between the duration of fecal shedding and the production of contaminated eggs by laying hens infected with strains of *Salmonella* Enteritidis and *Salmonella* Heidelberg. Avian Dis. 49: 382-386.

Gast RK, Guraya R, Guard-Bouldin J, Holt PS, and Moore RW. 2007a. Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs by hens infected with *Salmonella* Enteritidis or *Salmonella* Heidelberg. Avian Dis. 51: 40-44.

Gast RK, Guraya R, Guard-Bouldin J, and Holt PS. 2007b. In vitro penetration of egg yolks by *Salmonella* Enteritidis and *Salmonella* Heidelberg strains during thirty-six-hour ambient temperature storage. Poultry Sci. 86:1431-1435.

Gast RK, Guraya R, Guard J, and Holt PS. 2011. The relationship between the numbers of *Salmonella* Enteritidis, *Salmonella* Heidelberg, or *Salmonella* Hadar colonizing reproductive tissues of experimentally infected laying hens and deposition inside eggs. Avian Dis. 55:243-247.

Geddes K, Cruz F, and Heffron F. 2007. Analysis of cells targeted by *Salmonella* type III secretion *in vivo*. PLoS Pathology. 3: e196. doi:10.1371/journal.ppat.0030196.

Gharieb RM, Tartor YH, and Khedr MH. 2015. Non-typhoidal *Salmonella* in poultry meat and diarrhoeic patients: Prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug resistant strains. Gut Pathog. 7: doi:10.1186/s13099-015-0081-1.

Giraud A, Matic I, Tenaillon O, Clara A, Radman M, Fons M, and Taddei F. 2001. Costs and benefits of high mutation rates: Adaptive evolution of bacteria in the mouse gut. Science. 291: 2606- 2608.

Gokulan K, Khare S, Rooney AW, Han J, Lynne AM, and Foley SL. 2013. Impact of plasmids, including those encodingVirB4/D4 type IV secretion systems, on *Salmonella enterica* serovar Heidelberg virulence in macrophages and epithelial cells. PLoS ONE 8: e77866. doi:10.1371/journal.pone. 0077866

Gorski L. 2012. Selective enrichment media bias the types of *Salmonella enterica* strains isolated from mixed strain cultures and complex enrichment broths. PLoS ONE. 7: e34722. doi:10.1371/journal.pone.0034722.

Guard-Petter J. 1998. Variants of smooth Salmonella enterica srovar Enteritidis that grow to higher cell density than the wild type are more virulent. Appl Environ Microbiol. 64: 2166-2172.

Gunel E, Killic GP, Bulut E, Durul B, Acar S, Alpas H, and Soyer Y. 2015. *Salmonella* surveillance on fresh produce in retail in Turkey. Int J Food Microbiol. 199: 72-77.

Habbs VH. 1933. About a new type of bacteria form the paratyphoid enteritis group. J Bact. 130: 367-374.

Haft DH, Selengut J, Mongodin EF, and Nelson KE. 2005. A guild of 45 CRISPR associated (Cas) protein families and multiple CRISPR/Cas subtypes exists in prokaryotic genomes. PLoS Comput Biol. 1: e60.

Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, Kaldhone PK, Logue CM, and Foley SL. 2012. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PloS ONE. 7: e51160.

Hanning IB, Nutt JD, and Ricke SC. 2009. Salmonellosis outbreaks in the United States due to fresh produce: Source and potential intervention measures. Foodborne Pathog Dis. 6: 635-648.

Harris A, Cherubin C, Biek R, and Edwards LC. 1990. Frequency of *Salmonella* Typhimurium the year after a massive outbreak. Diagn Microbiol Infect Dis. 13, 25-30.

He H, Genovese KJ, Swaggerty CL, Nisbet DJ, and Kogut MH. 2012. A comparative study of invasion, survival, modulation of oxidative burst, and nitric oxide responses of macrophages (HD11), and systemic infection in chickens by prevalent poultry *Salmonella* serovars. Foodborne Pathog Dis. 9: 1104-1110.

Hennessy T, Cheng L, Kassenborg H, Ahuja S, Mohle-Boetani J, Marcus R, Shiferaw B, and Angulo F. 2004. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: A case-control study in FoodNet sites. Clin Infect Dis. 38: S237-243.

Hensel M, Shea JE, Waterman SR, Mundy R, Nikolaus T, Banks G, Vazquez-Torres A, Gleeson C, Fang FC, and Holden DW. 1998. Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Mol Microbiol 30: 163-174.

Hoffman M, Luo Y, Lafon PC, Timme R, and Allard MW. 2013. Genome sequences of *Salmonella enterica* serovar Heidelberg isolates isolated in the United States from a multistate outbreak of human *Salmonella* infections. Genome Announc 1 e00004-12. Pubmed: 23405335.

Hoffmann M, Zhao S, Pettengill J,Luo Y, Monday SR, Abbott J, Ayers SL, Cinar H N, Muruvanda T, Li C, Allard MW, Whichard J, Meng J, Brown EW, and McDermott PF. 2014. Comparative genomic analysis and virulence differences in closely related *Salmonella enterica* serotype Heidelberg isolates from humans, retail meats, and animals. Genome Biol Evol. 6: 1046-1068.

Hopkins KL, and Threlfall EJ. 2004. Frequency and polymorphism of *sopE* in isolates of *Salmonella enterica* belonging to the ten most prevalent serotypes in England and Wales. J Med Microbiol. 53: 539-543.

Howard ZR, O'Bryan CA, Crandall PG, and Ricke SC. 2012. *Salmonella* Enteritidis in shell eggs: Current issues and prospects for control. Food Res Int. 45: 755-764.

Humphrey TJ, Greenwood M, Gilbert RJ, Rowe B, and Chapman PA. 1989. The survival of salmonellas in shell eggs cooked under simulated domestic conditions. Epidem Infect. 102: 35-45.

Ibarburu M. 2015. U.S. flock trends and projections. The egg industry center market reports and industry analysis. American Egg Board. October 7, 2015.

Juneja VK, Marks HM, and Huang L. 2003. Growth and heat resistance kinetic variation among isolates of *Salmonella* and its application to risk assessment. Risk Analysis 23: 199-213.

Johnson TJ, Thorsness JL, Anderson CP, Lynne AM, and Foley SL. 2010. Horizontal gene transfer of a ColV plasmid has resulted in a dominant avian clonal type of *Salmonella enterica* serovar Kentucky. PLoS ONE. 5: e15524.

Jolley KA, and Maiden MC. 2010. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 11: 595.

Jones BD, Ghori N, and Falkow S. 1994. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's Patches. J Exp Med. 180: 15-23.

Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Dangelo MT, Hurs S, Medus C, Cronquirst A, and Angulo FJ. 2008. Salmonellosis outcomes differ substantially by serotype. J Infect Dis. 198: 109-114.

Kaldhone PR, Nayak R, Lynn AM, David DE, McDermott PF, Logue CM, and Foley SL. 2008. Characterization of *Salmonella enterica* serovar Heidelberg from turkey-associated sources. Appl Environ Microbiol. 74: 5038-5046.

Kivisaar M. 2003. Stationary phase mutagenesis: Mechanisms that accelerates adaptations of microbial populations under environmental stress. Environ Microbiol. 5: 814-827.

Koningstein M, Simonsen J, Helms M, and Molbak, K. 2010. The interaction between prior antimicrobial drug exposure and resistance in human *Salmonella* infections. J Antimicrob Chemother. 65:1819-1825.

Krauland M, Harrison L, Paterson D, and Marsh J. 2010. Novel integron gene cassette arrays identified in a global collection of multi-drug resistant non-typhoidal *Salmonella enterica*. Curr Microbiol. 60: 217-223.

Le Gall S, Desbordes L, Gracieux P, Saffroy S, Bousarghin L, Bonnaure-Mallet M, and Jolivet-Gougeon A. 2009. Distribution of mutation frequencies among *Salmonella enterica* isolates from animal and human sources and genetic characterization of *Salmonella* Heidelberg hypermutator. Vet Microbiol. 137: 306-312.

Li X, Payne JB, Santos FB, Levine JF, Anderson KE, and Sheldon BW. 2007.

Salmonella populations and prevalence in layer feces from commercial high-rise houses and characterization of the *Salmonella* isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult Sci. 86: 591-597.

Liu WQ, Liu GR, Li JQ, Xu GM, Danni Q, He XY, Juan D, Zhang HN, Randal JN, and Liu SL. 2007. Diverse genome structures of *Salmonella* Paratyphi C. BMC Genomics. 8: 1-10.

Liu F, Barrangou R, Gerner-Smidt P, Ribot EM, Knabel SJ, and Dudley EG. 2011. Novel virulence gene and clustered regularly interspaced short palindromic repeat (CRISPR) multilocus sequence typing of the major serovars of *Salmonella enterica* subsp. *enterica*. Appl Env Microbiol. 77: 1946-1956.

Love BC, and Rostagno MH. 2008. Comparison of five culture methods for *Salmonella* isolation from swine fecal samples of known infection status. J Vet Diagn Inves. 20: 620-624.

Lynne AM, Kaldhone P, David D, White DG, and Foley SL. 2009. Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. Foodborne Pathog Dis. 6: 206-215.

Malik-Kale P, Jolly CE, Lanthrop S, Winfree S, Luterbach C, and Steele-Mortimer O. 2011. *Salmonella*- at home in the host cell. Front Microbiol. 2: 125.

Martelli F, and Davies RH. 2012. *Salmonella* serovars isolated from table eggs: An overview. Food Res Int. 45: 745-754.

Mataseje LF, Baudry PJ, Zhanel GG, Morck DW, Read RR, Louie M, and Mulvey MR. 2010. Comparison of CMY-2 plasmids isolated from human, animal and environmental *Escherichia coli* and *Salmonella* spp. from Canada. Diagn Microbiol Infect Dis. 67: 387-391.

Maurelli AT. 2007. Black holes, antiviral genes, and gene inactivation in the evolution of bacterial pathogens. FEMS Microbiol Letters. 267: 1-8.

Maurischat S, Baumann B, Martin A, and Malorny B. 2015. Rapid detection and specific differentiation of *Salmonella enterica* subsp. *enterica* Enteritidis, Typhimurium and its monophasic variant 4,[5],12:i: − by real-time multiplex PCR. Int J Food Microbiol. 193: 8-14.

McConnell JA, and Schaffner DW. 2014. Validation of mathematical models for *Salmonella* growth in raw ground beef under dynamic temperature conditions representing loss of refrigeration. J Food Prot. 77:1110-1115.

McDermid AS, McKee AS, Dowsett AB, and Marsh PD. 1996. The effect of environmental pH on the physiology and surface structures of *Salmonella* serotype Enteritidis phage type 4. J Med Microbiol. 45: 452-458.

Methner U, Haase A, Berndt A, Martin G, Nagy B, and Barrow PA. 2011. Exploitation of intestinal colonization-inhibition between *Salmonella* organisms for live vaccines in poultry: Potential and limitations. Zoonoses Public Health. 58: 540-548.

Meyerholz DK, Stabel TJ, Ackermann MR, Carlson SA, Jones BD, and Pohlenz J. 2002. Early epithelial invasion by *Salmonella enterica* serovar Typhimuium DT104 in swine ileum. Vet Path. 39: 712-720.

Milkman R, and Stoltzfus A. 1988. Molecular evolution of the *Escherichia coli* chromosome II clonal segments. Genetics. 120: 359-366.

Molbak K. 2005. Human health consequences of antimicrobial drug-resistant *Salmonella* and other foodborne pathogens. Clin Infect Dis. 2005. 41: 1613-1620.

Morales CA, Musgrove M, Humphrey TJ, Cates C, Gast R, and Guard-Bouldin J. 2007. Pathotyping of *Salmonella enterica* by analysis of single-nucleotide polymorphism in *cyaA* and flanking 23S ribosomal sequences. Environ Microbiol. 9: 1047-1059.

Morbidity and Mortality Weekly Report (MMWR). 2013. Outbreak of *Salmonella* Heidelberg infections linked to a single poultry producer- 13 states, 2012- 2013. 62: 553- 556.

Muriana PM. 1997. Effect of pH and hydrogen peroxide on heat inactivation of *Salmonella* and *Listeria* in egg white. Food Microbiol. 14: 11-19.

Nordentoft S, Molbak L, Bjerrum L, De-Vylder J, Immerseel F, and Pedersen K. 2011. The influence of the cage system and colonisation of *Salmonella* Enteritidis on the microbial gut flora of laying hens studied by T-RFLP and 454 pyrosequencing. BMC Microbiol. 11: 187.

Onchman H, and Moran NA. 2001. Genes lost and genes found: Evolution of bacterial pathogenesis and symbiosis. Science. 292:1096-1099.

Otto SJG, Carson CA, Finley RL, Thomas MK, Reid-Smith RJ, and McEwen S A. 2014. Estimating the number of human cases of ceftiofur-resistant *Salmonella enterica* serovar Heidelberg in Quebec and Ontario, Canada. Clin Infect Dis. 59:1281-1290.

Park SH, Aydin M, Khatiwara A, Dolan MC, Gilmore DF, Bouldin JL, Ahn S, and Ricke SC. 2014. Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products. Food Microbiol. 38: 250-262.

Parker CT, Liebana E, Henzler DJ, and Guard-Petter J. 2001. Lipopolysaccharide O-chain microheterogeneity of *Salmonella* serotypes Enteritidis and Typhimurium. Environ Microbiol. 3: 332-342.

Philippon A, Arlet G, and Jacoby GA. 2002. Plasmid-determined AmpC-type beta-lactamases. Antimicrob Agents Chemother 46: 1-11.

Rabsch W, Tschäpe H, and Bäumler AJ. 2001. Non-typhoidal salmonellosis: Emerging problems. Microbes Infect. 3: 237–247.

Rashid M-U, Rosenborg S, Panagiotidis G, Löfdal K S, Weintraub A, and Nord CE. 2015. Ecological effects of ceftaroline-avibactam on the normal human intestinal microbiota. Antimicrob Agents Chemother. 59: 4504-4509.

Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonascio R, Granucci F, Kraehenbuhl JP, and Riccicardi-Castagnoli P. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nature Immun 2: 361-367.

Richardson E J, Limaye B, Inamdar H, Datta A, Manjiri AS, Pullinger GD, Thomson NR, Joshi RR, Watson M, and Stevens MP. 2011. Genome sequences of *Salmonella enterica* serovar Typhimurium, Choleraesuis, Dublin, and Gallinarum strains of well- defined virulence in food-poisoning animals. J Bact. 193: 3162-3163.

Richter-Dahlfors A, Buchan AM, and Finlay BB. 1987. Murine salmonellosis studied by confocal microscopy: *Salmonella* Typhimurium resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes *in vivo*. J. Exp Med. 186: 569-580.

Ricke SC, Jones DR, and Gast RK. 2013a. Egg and egg products. Chapter 46. In: Stephanie Doores, Yvonne Salfinger, *and* Mary Lou Tortorello (Eds.), Compendium of Methods for the Microbiological Examinations of Foods, 5th Edition. American Public Health Association, Washington, D.C. pp. 1-11.

Ricke SC, Koo O, Foley SL, and Nayak R. 2013b. *Salmonella*. Chapter 7. In: Ronald G. Labbé and Santos García. (Eds.), Guide to Foodborne Pathogens, 2nd Edition. John Wiley & Sons, Ltd. pp. 112-136.

Rivera Calo J, Baker CA, Park SH, and Ricke SC. 2015. Specificity of *Salmonella* Typhimurium strain (ATCC 14028) growth responses to *Salmonella* serovar-generated spent media. J Envi Health Part B 50: 423–429.

Robertson J, McKenzie J, Duncan N, Vercoe M, Woodward E, Flint M, and Grant G. 2003. Lack of flagella disadvantages *Salmonella enterica* serovar Enteritidis during the early stages of infection in the rat. J Med Microbiol. 52: 91-99.

Rose BE, Hill WE, Umholtz R, Ransom GM, and James WO. 2002. Testing for *Salmonella* in raw meat and poultry products collected at federally inspected establishments in the United States 1998 through 2000. J Food Prot. 65: 937- 947.

Ross IL, and Heuzenroeder MW. 2005. Discrimination within phenotypically closely related definitive types of *Salmonella enterica* serovar Typhimurium by the multiple amplification of phage locus typing technique. J Clin Microbiol. 43: 1604- 1611.

Rosselin M, Abed N, Virloguex-Payant I, Bottreau E, Sizaret PY, Velge P, and Wiedemann A. 2011. Heterogeneity of type III secretion system (T3SS)-1-independent entry mechanisms used by *Salmonella* Enteritidis to invade different cell types. Microbiology. 157: 839-847.

Salcedo SP, Noursadeghi M, Cohen J, and Holden DW. 2001. Intracellular replication of *Salmonella* Typhimurium strains in specific subsets of splenic macrophages in vivo. Cell Microbiol. 3: 587-597.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, and Griffin PM. 2011. Foodborne illness acquired in the United States- major pathogens. Emerg Infect Dis. 17: 7-15.

Schlumberger MC, and Hardt WD. 2006. *Salmonella* type III secretion effectors: pulling the host cell's strings. Curr Opin Microbiol. 9: 46 –54.

Schoeni JL, Glass KA, McDermott JL, and Wong ACL. 1995. Growth and penetration of *Salmonella enteritidis*, *Salmonella heidelberg* and *Salmonella typhimurium* in eggs. Int J Food Microbiol. 24: 385-396.

Shariat N, Sandt C, DiMarizo M, Barrangou R, and Dudley E. 2013. CRISPR-MVLST subtyping of *Salmonella enterica* subsp. *enterica* serovars Typhimurium and Heidelberg and application in identifying outbreak isolates. Biomed Cent Microbiol. 13: 1-31.

Singer, R. S., Mayer, A. E., Hanson, T. E., and Isaacson, R. E. 2009. Do microbial interactions and cultivation media decrease the accuracy of *Salmonella* detection systems and outbreak investigations? J. Food Protection. 72: 707-713.

Smith NS, Beltran P, and Selander RK. 1990. Recombination of *Salmonella* phase 1 flagellin genes generates new serovars. J Bact. 172, 5: 2209-2216.

Smyser CF, Adinarayanan N, Roekel VH, and Snoeyenbos GH. 1965. Field and laboratory observations on *Salmonella* Heidelberg infection in three chicken breeding flocks. The 37th Annual Meeting of the Northeastern Conference on Avian Dis.

Stanley J, Burnens A, Powell N, Chowdry N, and Jones C. 1992. The insertion sequence IS200 fingerprints chromosomal genotypes and epidemiological relationships in *Salmonella* Heidelberg. J Gen Microbiol 138: 2329-2336.

Stevens MP, Humphrey TJ, Maskell DJ. 2009. Molecular insights into farm animal and zoonotic *Salmonella* infections. Philos Trans R Soc Lond Biol Sci. 364: 2709–2723.

Suez J, Portola S, Dagan A, Marcel A, Shirr YI, Desai PT, Gammon V, McClelland M, Rehab G, and Gal-Moor O. 2013. Virulence gene profiling and pathogenicity characterization of non-typhoid *Salmonella* accounted for invasive disease in humans. PLoS ONE 8: e58449.

Taylor AL, Murphree R, Ingram LA, Garman K, Soloman D, Coffey E, Walker D, Rogers M, Marder E, Bottomley M, Woron A, Thomas L, Roberts S, Hardin H, Arjmandi P, Green A, Simmons L, Cornell A, and Dunn J. 2015. Multidrug-resistant *Salmonella* Heidelberg associated with mechanically separated chicken at a correctional facility. Foodborne Pathog Dis. 12: 950-952.

Threlfall EJ, Hall MLM, Ward LR, and Rowe B. 1992. Plasmid profiles demonstrate that an upsurge of *Salmonella* Beria in humans in England and Wales is associated with imported poultry meat. Eur J Epidemiol. 8, 27-33.

Van Asten A., and Van Dijk JE. 2005. Distribution of "classic" virulence factors among *Salmonella* spp. FEMS Immunol Med Microbiol. 44: 251–259.

Wallis TS, Starkey WG, Stephen J, Haddon SJ, Osborne MP, and Candy DC. 1986. The nature and role of mucosal damage in relation to *Salmonella* Typhimurium-induced fluid secretion in the rabbit ileum. J Med Microbiol. 22: 39-49.

Windhorst HW. 2009. Recent patterns of egg production and trade: A status report on regional basis. Worlds Poult Sci J. 65: 685-708.

Withanage GS, Mastroeni P, Brooks HJ, Maskell DJ, and McConnell I. 2005. Oxidative and nitrosative responses of the chicken macrophage cell line MQ-NCSU to experimental *Salmonella* infection. Br Poult Sci. 46: 261-267.

Young C-C, Ross IL, and Heuzenroeder MW. 2012. A new methodology for differentiation and typing of closely related *Salmonella enterica* serovar Heidelberg isolates. Curr Microbiol. 65: 481-487.

III. Chapter 2

Insight into IncI1 Plasmids

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Introduction

Plasmids are linear or circular DNA molecules that exist independently of the host chromosome in microbial cells and can replicate autonomously of the chromosome. Plasmids are seen most often in bacteria, but also seen in archaea and eukaryotic organisms (Athanasopoulos et al, 2017). Plasmids have their own replication origin and can be stably inherited; however they differ from bacterial chromosome in several ways. Compared to bacterial chromosome, plasmids have fewer genes, are not essential for host survival, and typically are present in multiple copies in a cell (Prescott et al, 1996).

Plasmids can be classified in multiple different ways. One way is how they spread, plasmids can be episomal or conjugative. Episomal plasmids can exist independently by themselves or become incorporated into the host chromosome. Conjugative plasmids on other hand undergo conjugation and disperse copies of themselves into another bacterium (Wistreich et al, 1986). Another way to classify plasmids is by their size, they can be distinguished into smaller and larger plasmids. Larger plasmids are generally conjugative and can be classified by their functionality, for example they could be defined fertility (F) plasmids, resistance (R) plasmids, or bacteriocinogenic plasmids (Clewell, 1990). Certain F plasmids can carry resistance genes on them. Bacteriocinogenic plasmids are capable of synthesizing bacteriocins, toxic compounds used by host bacteria to antagonize other bacteria (Miyoshi et al, 1984).

A key method used for the classification of plasmids is compatibility typing (Novick, 1987). Plasmids are assigned to different incompatibility (Inc) groups under this method. Historically, when a given plasmid is introduced into a strain containing a plasmid with a known Inc group and the plasmid is retained, then the plasmid is not incompatible and not assigned to that particular Inc group (Wong et al, 2016; Couturier et al, 1988). This method has been used to

study the dissemination of antimicrobial resistance through plasmid and the study of the evolution of plasmids. Many Inc groups have been identified to date, some key examples are IncI1, IncA/C, IncFIB, and IncN.

IncI1 plasmids are characterized by following distinguishing traits, they typically have a 54kb DNA segment required for mating. This transfer segment is longer than other plasmids, it contains 48 ORFs and has 2.2 kb gap region. The *nik*AB gene oriented right to left, and other genes are oriented left to right (Wilkins et al, 2000). IncI1 plasmids have two types of sex pilli, a thin, flexible pilus required for liquid mating, and a thick, rigid pilus necessary for mating in both liquid and surface (Tetsu et al, 1999). In addition to these IncI1 plasmids carry the *sog* gene, which has DNA primase activity, vital for initial DNA transfer and thin pili formation (Wilkins et al, 2000, Komano et al, 1994). IncI1 plasmids can carry genes responsible for antimicrobial resistance, attachment, virulence, and contribute to stable inheritance during cell division and plasmid maintenance (Riccobono et al, 2015, Smith et al, 2015). This makes them critical for public health concern. This review will focus on IncI1 plasmids, their genetics, phenotypic characteristics associated with them like antimicrobial resistance and virulence.

Genetics

Sampei et al. (2010) sequenced an IncI1 plasmid, R64 and it consists of five different regions: replication, drug resistance, stability, transfer leading, and conjugative transfer regions, oriented in clockwise direction. The replication region (2-3kb) consists of genes pertaining to replication, *inc*, *repY*, *repZ*, and functional DNA sequence. Replication genes codes for replication initiation protein (*repY),* and the origin of replication (*repZ),* and *inc* that encodes the anti-sense RNA which act as a copy number regulator. Functional DNA sequences *ori* and *cis*

are necessary for autonomous replication and copy number control (Mori et al, 1995). The drug resistance region (25-30 kb) contains a variety of genes responsible for resistance to antibiotics. In some cases the resistance gene could be disrupted by an insertion sequence that divides the region into multiple fragments. The stability region (15-20kb) contains genes responsible for maintenance of plasmid, including site-specific recombination (*resD, rfsF*) and the partition of replicated DNA into daughter cells during cell division (*parA, parB*). The transfer leading region or simply called leading region (15-20kb) is conserved among most of the IncI1 plasmids. This region enters first into the recipient cell during transfer. Additionally the *impCAB* genes in this region might be involved in host cell mutagenesis (Sutton et al, 2000). The conjugative transfer region, also referred to as the transfer region (40-50kb) is highly organized into four major gene clusters; *traABCD* regulatory gene cluster, *pil* gene cluster for type IV pilus synthesis, *tra/trb* gene cluster for conjugation, and *oriT* and *nikAB* for conjugative DNA processing. The *tra*B and *tra*C are responsible for conjugative transfer in liquid as well as in solid media.

IncI1 plasmids can also have a shufflon sequence, which acts as a biological switch to select select turn on the seven C-terminal segments of the *pivV* gene. The shufflon is singular DNA rearrangement found in IncI1 plasmids. Komono et al. (1994) noted that the shufflon in R64 is four DNA segments flanked and separated by seven nineteen base pairs repeat sequences and plays a role in recipient specificity during liquid mating of R64. Thus shufflon sequence plays a role in the transfer of plasmid.

Martin et al. (2016) used a curing vector of IncI1 plasmid to evaluate mechanical burden. Their IncI1 curing vector was able to displace the multiple IncI1 plasmid. The IncI1 plasmid did not exhibit a fitness cost in *Salmonella enterica* serotype 4,5,12:i- S348/11, but the IncI1 CTX-

M1 plasmid caused a growth disadvantage in *Klebsiella pneumoniae.* The fitness cost to *K. pneumoniae* might have been due to the recently acquired IncI1 plasmid. This plasmid did not go through co-evolution far enough to give the host growth independence. Differences in the genetics of the plasmids assessed between *K. pneumoniae* and *S. enterica* 4,5,12:i could also be the reason for these different outcomes.

Handel et al. (2015) has studied transfer of IncI1 plasmids. They reported that cell density, energy availability, and growth rate affect plasmid transfer efficacy. For the IncI1 plasmid pESBL-283 in *E. coli* strains; the selective pressure from the antibiotic above the minimum inhibitory concentration reduced transfer rates. Reciprocally, enhanced transfer of resistance genes in the absence of antimicrobial pressure has been reported by some scientists (Laxminarayan et al, 2013).

Antimicrobial Resistance

Most of the bacterial foodborne infections are self-limiting, however some of them lead to severe outcomes such as systemic infections, which are dependent on factors such as infectious dose, bacterial virulence and host immunity. In these more severe cases, antimicrobial agents are used to manage the infection. Additionally, antimicrobials are used as therapeutic as well as preventative measures for human, veterinary, agriculture and food production areas. Over time and exposure, microorganisms have developed resistance to antimicrobial agents used to control them. Different mechanisms are employed by microorganisms to nullify antimicrobials used to manage infection. These include efflux pumps to expel the antimicrobial molecules, synthesis of enzymes to deactivate or modify antimicrobials, competitive inhibition of intermediate molecules and modification of drug targets. The genes responsible are called

antimicrobial resistance genes and they are encoded on plasmids. IncI1 plasmids are known to carry resistance genes (Folster et al, 2017) and hence they have potential to confer multidrug resistance (MDR) characteristic to respective strains. Some of the major class of antimicrobials and associated resistance genes, which are reported to be present on IncI1 plasmids, are listed in Table 1.

Bae et al. (2016) studied *Salmonella* strains in foods imported into the US. They reported *Salmonella* Litchfield strain resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, and trimethoprim-sulmethoxazole. This strain isolated from frozen fish raised and processed in Taiwan was reported to carry the $bla_{\text{TEM-1}}$ gene and a 90 kb, IncI1 plasmid. With globalization of food industry, IncI1 plasmids could become a tool for the spread of resistance across international boundaries.

Beta-lactamases are enzymes that can cause the hydrolysis of oxyimino-β-lactam antimicrobial agents (Bush et al, 2010). Many generations of β-lactam antimicrobial compounds are used in clinical practice. Extended spectrum beta-lactamases (ESBLs) are enzymes that could inhibit broader range of β-lactam antimicrobials (Patterson et al, 2005). Thus strains capable of synthesizing ESBL enzymes are resistant to multiple β-lactam antimicrobials. Many variants of ESBL enzymes have evolved over time through different mutations in the genes coding for these enzymes. The TEM and SHV types of β-lactamase enzyme were prevalent in the 1980s and 1990s respectively. The CTX variant of the β-lactamase (*bla*) gene evolved from TEM and SHV types. Among bla_{CTX} variants $bla_{\text{CTX-M}}$ has been prominent since year 2000. The $bla_{\text{CTX-M}}$ has different lineages including $bla_{\text{CTX-M-1-}}$like, $bla_{\text{CTX-M-2-}}$like, $bla_{\text{CTX-M-8-}}$like, $bla_{\text{CTX-M-9-}}$like, $bla_{\text{CTX-M-25-}}$like, and $bla_{\text{CTX-M-KYLC-}}$like. These lineages are different from each other by

more than 10 % homology (Canton et al, 2006). The *bla*$_{\text{CTX-M}}$ variants have been are detected in enteric organisms including *E. coli*, *Salmonella* from all continents around the world.

The global presence of *bla*$_{\text{CTX-M}}$ variants led to in-depth studies to understand them (Peireno et al, 2010). They are prevalent in both nosocomial and community settings. Strains carrying *bla*$_{\text{CTX-M}}$ variants are associated with plasmids including IncI1, IncFI, IncFII, and IncHI2. Some *bla*$_{\text{CTX-M}}$ variants are associated with insertion sequences (IS) present on the plasmids. The ISEcp1 is associated with *bla*$_{\text{CTX-M-5,}}$ while ISCR1 is linked to *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-9}}$ (Canton et al, 2006). These IS elements are hypothesized to act as promoters for high level of expression of *bla*$_{\text{CTX-M}}$. *bla*$_{\text{CTX-M-15}}$ is the most universal ESBL among *E. coli* strains carrying *bla*$_{\text{CTX-M}}$. These strains typically belong to sequence type (ST)131 and are often resistant to quinolones in addition to cephalosporins (Mathers et al, 2015). The increasing prevalence of *E. coli* causing community-associated and nosocomial infections make this broadening of resistance a public health concern. More research is needed to gain further insight into resistance associated with *bla*$_{\text{CTX-M}}$ variants.

Virulence

IncI1 plasmids are known to carry virulence related genes for antimicrobial peptides and adhesion. Here we will focus on virulence related to bacteriocins and pilli.

Bacteriocins, such as colicins, are toxins generally encoded in Gram-negative bacteria. They mediate competition among neighboring cells, allowing the colicin-encoding bacteria to outcompete the susceptible bacteria (Hayes et al, 2010). They play an important role in the polymicrobial microbiota, as they would enable one bacterium to inhibit others. Colicins are proteins ranging from 30 to 80 kDa in weight. They have three parts namely N-terminal, central

and C-terminal portions. Colicin-encoding genes have been noted to be on IncI1 plasmids, typically adjacent to immunity and lysis genes (Braun et al, 2013). Different types of colicin toxins hsve been reported, with colicin types E and D causing cell lysis and colicin types B, M, and I causing leakage from host cell. Colicin type E has been subtyped further into types such as E1 to E9. Among different colicinE subtypes, E3 and E7 have reported on IncI1 plasmids in multi drug resistant entero toxogenic *E. coli* O141 and O149 strains isolated from swine (Abraham et al, 2014). Once in the target cell, colicins act as nucleases, degrading DNA, tRNA, and rRNA. Colicins also inhibit target cells by causing the formation of pores in cytoplasmic membranes (Cascales et al, 2007). Mechanical breach in the cytoplasmic membrane causes disturbance in electric potential of the membrane. Different plasmid-associated genes have been associated with colicin toxins. These genes are shown to be relatively conserved, having less polymorphism than chromosomal genes (Ziebell et al, 2008). Thus colicin related genes tend to be genetically stable and transmitted by horizontal transmission.

IncI1 plasmids posses to have genes encoding a thin pilus that are required for conjugation (Wilkins et al, 2000). The *pil* family genes are associated with pilus formation. The *pilS* and *pilU* code for prepilin formation. In addition, *pilV* is responsible for cleavage site for nucleotide binding (Komano et al, 2000). *pilQ* has been associated with biogenesis of thin pilus, while *pilN* and *pilR* code for outer and integral membrane protein respectively. In addition to *pil* family genes, *tra* family genes and *rci* gene are essential for pilli formation and function. The *rci* gene modulates the structure of thin pilli and function through rearranging the shufflon (Tetsu et al, 1999). Upto 30% of transfer region of IncI1 plasmids codes for thin pilli implying role of thin pilli in determining specificity of IncI1 plasmids.

Conclusions

IncI1 plasmids due to their genetic composition are critical for public health. Their ability to carry genetic determinants for phenotypic traits such as antimicrobial resistance and virulence make them significant in well-being of food animals. IncI1 plasmids have been noted to spread from one bacterium to another at global level. Comprehensive evaluation of their role in *Salmonella* with respect to antimicrobial resistance and virulence has not been reported. Comparison of IncI1-associated antimicrobial resistance genes and respective susceptibility profile will increase our understanding of functionality of these genes. This understanding will help us to improve our management capability of foodborne illnesses.

Table 1. Antimicrobial agents and respective resistance genes on IncI1 plasmids.

Antimicrobial group	Antimicrobial agents	Resistance gene/s	Mechanism	References
Cephalosporin	cefoxitin, ceftriaxone	bla_{CMY-2} , bla_{TEM}	enzymatic inhibition	Folster et al, 2017
Sulfonamide	sulfisoxazole	*sul1*	nutrient competition	Johnson et al, 2011
Carbapenem	imipenem, meropenem	bla_{IMP} , bla_{NDM}	enzymatic inhibition	Nordmam et al, 2011
Tetracycline	doxycycline, minocycline	*tetA*, *tetR*	efflux pump	Murgia et al, 2016
Aminoglycoside	streptomycin, gentamicin	*aadA1*	enzymatic inhibition	Sanad et al, 2016

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References

Abraham S, Trott DJ, Jordan D, Gordon DM, Groves MD, Fairbrother JM, Smith MG, Zhang R, and Chapman TA. 2014. Phylogenetic and molecular insights into the evolution of multidrug-resistant porcine enterotoxigenic Escherichia coli in Australia. Int J Antimicrob Agents. 44:105-11.

Athanasopoulos T, Munye MM, and Yáñez-Muñoz RJ. 2017. Nonintegrating gene therapy vectors. Hematol Oncol Clin North Am. 5:753-770.

Bae D, Kweon O, and Khan AA. 2016. Isolation and characterization of antimicrobial resistant nontyphoidal *Salmonella enterica* serovars from imported food products. J Food Prot. 79:1348-1354.

Braun V, and Patzer SI. 2013. Intercellular communication by related bacterial protein toxins: colicins, contact-dependent inhibitors, and proteins exported by the type VI secretion system. FEMS Microbiol Lett. 345:13-21.

Carattoli A. 2013. Plasmids and the spread of resistance. Int J Med Microbiol. 303:298-304.

Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, Riley M, Slatin S, and Cavard D. 2007. Colicin biology. Microbiol Mol Biol Rev. 71:158-229.

Clewell DB. 1990. Moveable genetic elements and antibiotic resistance in enterococci. Eur J Microbiol Infect Dis. 9:90-102.

Dhanani A, Block G, Dewar K, Forgetta V, Topp E, Beiko RG, and Diarra MS. 2015. Genomic comparison of non-typhoidal *Salmonella enterica* serovars Typhimurium, Enteritidis, Heidleberg, Hadar and Kentucky isolates form broiler chickens. PLoS ONE. 10:e0128773. doi:10.1371/journal.pone.0128773.

Edimanasinghe R, Finley R, Parmeley JE, Avery BP, Carson C, Bekal S, Golding G, and Mulvey MR. 2017. A whole-genome sequencing approach to study cefoxitin-resistant *Salmonella enterica* serovar isolated from various sources. Antimicrob Agents Chemother. 61:e01919-16.

Eswarappa SM, Panguluri KK, Hensel M, and Chakravortty D. 2008. The yejABEF operon of *Salmonella* confers resistance to antimicrobial peptides and contributes to its virulence. Microbiology.154:666–78.

Figueira R, Watson KG, Holden DW, and Helaine S. 2013. Identification of salmonella pathogenicity island-2 type III secretion system effectors involved in intramacrophage replication of *Salmonella enterica* serovar Typhimurium: implications for rational vaccine design. Mol Biol. 4:e00065. doi:10.1128/mBio.00065-13.

Folster JP, Grass JE, Bicknese A, Taylor J, Friedman CR, and Whichard JM. 2017. Characterization of resistance genes and plasmids from outbreaks and illness clusters caused by *Salmonella* resistant ceftriaxone in the United States, 2011-2012. Microb Drug Resist. 23:188-193.

Handel N, Otte S, Jonker M, Brul S, and Kuile BH. 2015. Factors that affect transfer of IncI1 β-lactam resistance plasmid pESBL-283 between *E. coli* strains. PLoS ONE. 10: e0123039.

Hansen-Wester I, and Hensel M. 2001. *Salmonella* pathogenicity islands encoding type III secretion systems. Microb Infect. 3:549-59.

Hayes CS, Aoki SK, and Low DA. 2010. Bacterial contact-dependent delivery systems. Annu Rev Genet. 44:71-90.

Hueck CJ. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev. 62:379-433.
Hunt S, Green J, and Artymiuk PJ. 2010. Hemolysin E (HlyE, ClyA, SheA) and related toxins. Adv Exp Med Biol. 677:116-126.

Johnson TJ, Shepard SM, Rivert B, Danzeisen JL, and Carattoli A. 2011. Comparitive genomics and phylogeny if the IncI1 plasmids: A common plasmid type among porcine enterotoxigenic *Escherichia coli*. Plasmid. 66:144-151.

Jones MA, Hulme SD, Barow PA, and Wingley P. 2007. The *Salmonella* pathogenicity island 1 and *Salmonella* pathogenicity island 2 type III secretion system plays a major role in pathogenesis of systemic disease and gastrointestinal tract colonization of *Salmonella enterica* serivar Typhimurium in the chicken. Avian Pathol. 36:199-203.

Kingsley RA, Santos RL, Keestra AM, Adams LG, and Bäumler AJ. 2002. *Salmonella enterica* serotype Typhimurium ShdA is an outer membrane fibronectin-binding protein that is expressed in the intestine. Mol Microbiol. 43:895-905.

Komano T, Kim SR, Yoshida T, and Nisioka T. 1994. DNA rearrangement of the shufflon determines recipient specificity in liquid mating of IncI1 plasmid R64. J Mol Biol. 243:6-9.

Komano T, Yoshida T, Narhara K, and Furuya N. 2000. The transfer region of IncI1 plasmid R64: similarities between R64 *tra* and *Legionella icm/dot* genes. Mol Microbiol. 35:1348-1359.

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumprdit N. et al. 2013. Antibiotic resistance- the need for global solutions. Lancet Infect Dis. 13:1057-98.

Martin IF, Thomas CM, Laing E, AbuOun M, La Ragione RM, and Woodward MJ. 2016. Curing vector of IncI1 plasmids and its use to provide evidence for a metabolic burden of IncI1 CTX-M-1 plasmid pIFM3791 on *Klebsiella pneumoniae*. J Med Microbiol. 65:611-618.

Miyoshi Y, and Higa A. 1984. Interrelationship between drug resistance and bacteriocinogeny. Microbio Immunol. 28:281-289.

Mori A, Ito K, Mizobuchi K, and Nakamura Y. 1995. A transcription terminator signal necessary for plasmid ColIB-P9 replication. Mol Microbiol. 17:291-301.

Murgia M, Bouchrif B, Timinouni M, Al-Qahtani A, Al-Ahdal MN, Cappuccinelli P, Rubino S, and Paglietti B. 2015. Antibiotic resistance determinants and genetic analysis of *Salmonella enterica* isolated form food in Morocco. Int J Food Microbiol. 215:31-39.

Nordmann P, Naas T, and Poirel L. 2011. Global spread of carbapenemase-producing *Enterobactericeae*. Emerg Infect Dis. 17:1791-1798.

Patterson SK, Kim HB, Borewicz K, and Isaacson RE. 2016. Towards an understanding of *Salmonella enterica* servar Typhimurium persistence in swine. Anim Health Res Rev. 17:159-168.

Prescott LM, Harley JP, and Klein DA. 1996. Microbial Genetics: Recombination and Plasmids. Microbiology. W. C. Brown Publishing. ISBN-13: 978-0697293909 Chp. 14. 3rd Edtn. 305-331.

Raffatellu M, Wilson RP, Chessa D, Andrews-Polymenis H, Tran QT, Lawhon S, Khare S, Adams LG, and Baumler AJ. 2005. SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella enterica* serotype typhimurium invasion of epithelial cells. Infect Immun. 73:146–54.

Riccobono E, Pilato VD, Maggio TD, Revollo C, Bartoloni A, and Pallecchi L. 2015. Characterization of IncI1 sequence type 71 epidemic plasmid lineage responsible for the recent dissemination of CTX-M-65 extended spectrum β-lactamase in the bolivian chaco region. Antimicrob Agents Chemother. 59:5340-5347.

Sampei G, Furuya N, Tachibana K, Saitou Y, Suzuki T, Mizobuchi K, and Komana T. 2010. Complete genome sequence of the incompatibility group I1 plasmid R64. Plasmid. 64:92-103.

Sanad YM, Johnson K, Park SH, Han J, Deck J, Foley SL, Kenney B, Ricke SC, and Nayak R. 2016. Molecular Molecular characterization of *Salmonella enterica* serovars isolated from a turkey production facility in the absence of selective antimicrobial pressure. Foodborne Pathog Dis. 13:80-87.

Severinov K, and Nair SK. 2012. Microcin C: biosynthesis and mechanisms of bacterial resistance. Future Microbiol. 7:281–289.

Shea JE, Beuzon CR, Gleeson C, Mundy R, and Holden DW. 1999. Influence of the *Salmonella* typhimurium pathogenicity island 2 type III secretion system on bacterial growth in the mouse. Infect Immun. 67:213–9.

Smith H, Bossers A, harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodriguez I, Essen-Zandbergen AV, Brouwer M, and Mevius D. 2015. Characterization of epidemic IncI1-Iγ plasmids harboring ambler class A andC genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. Antimicrob Agents Chemother. 59:5357-65.

Sun Y, Ye Q, Wu M, Wu Y, Zhang C, and Yan W. 2016. High yields and soluble expression of superoxide dismutase in *Escherichia coli* due to the HIV-1 Tat peptide via increase in mRNA transcription. Exp Mol Med. 48:e264. doi:10.1038/emm.2016.91.

Sutton MD, Smith BT, Godoy BG, and Walker GC. 2000. The SOS response: recent insights into *umuDC*-dependent mutagenesis and DNA damage tolerance. Annu Rev Genet. 34:479-497.

Testsu Y, Su-Ryang K, and Teruya K. 1999. Twelve *pil* genes are required for biogenesis of the R64 thin pilus. J Bacteriol. 181:2038-43.

Wilkins BA, and Thomas AT. 2000. DNA-independent transport of plasmid primase protein between bacteria by the I1 conjugation system. Mol Microbiol. 38:650-7.

Wistreich GA, and Lechtman MD. 1984. Microbial Genetics. In book Microbiology. MacMillon Publishing. Chp. 8th. 4th Edtn. 170-197.

Wong MH, Kan B, Chan EW, Yan M, and Chen S. 2016. IncI1 plasmids carrying various *bla*CTX-M genes contribute to ceftriaxone resistance in *Salmonella enterica* serovar Enteritidis in China. Antimicrob Agents Chemother. 60:982-989.

Zhang S, Santos RL, Tsolis RM, Stender S, Hardt W, Baumler AJ, and Adams LG. 2002. The *Salmonella enterica* serotype Typhimurium effector proteins SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhea in calves. Infect Immun. 70:3843-55.

Ziebell K, Steele M, Zhang Y, Benson A, Taboada EN, Laing C, McEwen C, Ciebin B, Johnson R, and Gannon V. 2008. Genotypic characterization and prevalence of virulence factors among Canadian *Escherichia coli* O157:H7 strains. Appl Environ Microbiol. 74:4314-4323.

IV. Chapter 3

Evaluation of the Genetics and Functionality of Plasmids in Incompatibility Group I1 (IncI1) Positive *Salmonella enterica*

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Abstract

Salmonella is a predominant foodborne pathogen in the United States and other countries. Mobile genetic elements such as plasmids allow *Salmonella* to adapt to external stress factors such as nutrient deprivation and host factors. IncI1 plasmid-carrying *Salmonella enterica* strains from poultry, bovine, swine and human sources were examined to determine the presence of plasmid-associated genes and their influence on phenotypic characteristics. The objective of this study was to understand the genetic determinants on IncI1 plasmids and their impact on antimicrobial susceptibility, competitive growth inhibition of *Escherichia coli* and plasmid transfer. Primers were designed to detect presence of genes that play a role in virulence, antimicrobial resistance and plasmid transfer based on previously sequenced IncI1 plasmids. PCR assays were conducted on 92 IncI1-positive *Salmonella enterica* strains. Phenotypic expression was measured by conjugation assays, antimicrobial susceptibility testing, and bacteriocin production based on the inhibition of growth of a colicin-negative *E. coli* J53 strain. The antimicrobial resistance genes *aadA1*, *tetA*, *sul1* and bla_{CMY} were detected in 88, 87, 80% and 48% of the strains tested, respectively. Over half of the strains were resistant or intermediately resistant to streptomycin (85%), sulfonamides (76%), tetracycline (74%), and ampicillin (68%) and 57% of the strains inhibited growth of *E. coli* J53 strain. Among putative virulence genes, colicin-associated *colI* and *cib* were detected in 23% and 35% of strains and *imm* and *ccdA* were present in 58% and 54% of strains, respectively. Approximately 61% of strains contained plasmids that conjugally transferred antimicrobial resistance, including 83% where the recipient received IncI1 plasmids. Most of the strains carried an assortment of transfer associated (*pil* and *tra* genes), with between 63 to 99% of strains being positive for individual

genes. Taken together the study affirms that IncI1 plasmids likely play roles in the dissemination of antimicrobial resistance and virulence associated factors among enteric organisms.

Introduction

Salmonella enterica are estimated to account for approximately 1.2 million illnesses per year in the United States (Scallan et al, 2011). Most of these present as self-limiting gastroenteritis, but can lead to more severe disease outcomes. Annually, these infections are responsible for an estimated 23,000 hospitalizations and 450 deaths in the U.S. (Scallan et al, 2011). The source of these illnesses can originate from any one of commonly consumed foods including eggs, meat and poultry products and contaminated fresh produce (Dunkley et al, 2009, Finstad et al, 2012, Foley et al, 2013, Hanning et al, 2009, Howard et al, 2012). *Salmonella* has the capability of adapting to its external environment and out-competing other bacteria in the intestinal microbiota (Jakočiūnė et al, 2014). Certain mobile genetic elements such as plasmids can play critical roles in maintaining this niche within food animal hosts (Han et al, 2012). Plasmids can replicate independently of the bacterial chromosome (Carattoli, 2011) and have evolved to potentially acquire genes for antimicrobial resistance, adhesion, and virulence among other characteristics (Martinez et al, 2002). Plasmids are often defined by their incompatibility (Inc) types. When a strain containing a known Inc group plasmid accepts and retains given plasmid then the given plasmid is considered not incompatible and not assigned to that specific Inc group (Wong et al, 2016). Diverse plasmid types are known to harbor and spread antimicrobial resistance genes (Cambray et al, 2010, Lai et al, 2013, Sanad et al, 2016). Among them, IncI1 plasmids, which typically range from 90 to 110 kb have been noted for being associated with dissemination of ceftriaxone resistance among *Salmonella* strains (Smith et al, 2015). The ability of IncI1 plasmid to carry and spread extended spectrum cephalosporin (ESC)

resistance genes offers a potential explanation for the plasmids' prevalence among multidrug resistant *Salmonella* (Folster et al, 2016). IncI1 plasmids are known for their potential to disseminate among other enteric pathogens (Wong et al, 2016). IncI1 plasmids are known to encode two distinctive types of pili, thin flexible (*pil* locus) and thick rigid (*tra* locus), in their conjugative transfer region; along with multiple inversion regions called shufflons and *sog*, which suppresses mutations (Sampei et al, 2010). These characteristics are unique compared to other plasmid types, such as IncF, that possess a smaller transfer region than IncI1 (Komano et al, 2000).

Salmonella require some metabolic resources (i.e. fitness cost) to retain IncI1 plasmids (Martin el al, 2016). Smith et al. (2015) found that IncI1 plasmids typically carry genes responsible for partitioning and host-addiction systems. These addiction genes (e.g. *ccdAB*) cause the bacterium to retain the plasmid after cell division, facilitating successful inheritance and maintenance of the plasmid (Doumith et al, 2012).

Successful plasmid-gene combination such as IncI1 and $bla_{\text{CMY-2}}$, are prevalent among certain enteric bacteria (Mnif et al, 2010); however, there remains a need to understand the diversity and function of IncI1 plasmids. The major objectives of this study were to detect IncI1 plasmid-associated antimicrobial resistance, putative virulence and transfer-related genes present in IncI1-positive *Salmonella* strains and determine their impact on *Salmonella* phenotypes. Experiments were conducted on isolates from diverse sources, including poultry, cattle, swine and human patients, to determine their phenotypic and genotypic characteristics and the capability of these plasmids to undergo horizontal transfer to *Escherichia coli* J53 strain.

Material and Methods

Bacterial Strains

Ninety-two *Salmonella enterica* isolates previously identified as carrying IncI1 plasmids were selected for the study (Han et al, 2011; Kaldhone et al, 2008; Lynne et al, 2008, 2009; Marrero-Ortiz et al, 2012; Melendez et al, 2010). Strains originated in the U.S. and were collected from chicken (29%, 27/92), bovine (29%, 26/92), turkey (17%, 15/92), porcine (14%, 13/92), and human (12%, 11/92) related sources between the years 1992-2009. The serovar distribution included Heidelberg (40%, 37/92), Typhimurium (23%, 22/92), Kentucky (18%, 16/92), Newport (9%, 8/92) and others (10%, 9/92; Table 1). Strains were frozen at -80°C in Brain Heart Infusion broth with 20% glycerol for archival storage. For use in experiments, strains were streaked on tryptic soy agar containing 5% sheep's blood (blood agar) and incubated at 37°C overnight.

Conjugation Experiments

Salmonella strains served as potential donors and the sodium azide-resistant *Escherichia coli* J53 strain was the recipient for conjugation (Jacoby and Han, 1996). Initial experiments were conducted using a cross-streaking method. Donors and the recipient were streaked across one another on LB agar plate and incubated at 37°C for 18 hrs. At the point of intersection, the equivalent of 3-4 colonies were picked and streaked onto an LB agar plates containing ampicillin (32 µg/mL), gentamicin (10 µg/mL) or streptomycin (32 µg/mL) and sodium azide (350 µg/mL). These selection plates were incubated for up to 48 hours to identify likely transconjugants. Those combinations that did not yield transconjugants were subjected to a broth-mating method (Zheng et al, 2016) at either the ratio of 1:9 (donor: recipient) or 1:1, if the 1:9 ratio was

unsuccessful. In each case, 100 µl of the suspension was spread on LB agar selection plates as described above to identify transconjugants.

PCR for Replicon types, Antimicrobial Resistance, Virulence and Transfer-associated Genes

The DNA template was prepared using a boiling method in which 3-4 bacterial colonies were picked from the blood agar plate, suspended in 200 µl of sterile water and boiled to lyse the cells (Wang et al, 2015). PCR reactions were conducted to confirm the presence of the IncI1 replicon (Carattoli et al, 2005) and for potential IncI1-associated antimicrobial resistance (*sul1, aadA1, aacC, tetA* and bla_{CMY}), putative virulence (*ccdA, ccdB, col1, cib, imm, copD,* and *cusA*) and plasmid transfer-associated genes (*pilJ, pilM, pilP, pilS, traG, traL, traQ,* and *traT*) using the primers listed in Supplemental Table 1. PCR assays included 12.5 µl 2X Master Mix (Promega, Madison, WI), 2.5µl of each primer (10 pmol), 3 µl template and sterile water and were amplified using the following protocol: denaturation at 94°C for 5 mins, 30 cycles of denaturation at 94°C for 30 secs, annealing at optimized temperature (Supplemental Table 1) for 30 secs, and extension at 72°C for 90 secs, and a final extension at 72°C for 7 mins**.** PCR products were separated using 2% E-gels (Invitrogen, Carlsbad, CA) and the bands were visualized under UV-light using a Gel-Doc XR system (Bio-Rad, Hercules, CA). The transconjugants were screened for plasmid replicon types as described by Johnson and Nolan (2009) and for antimicrobial resistance genes as described above.

Antimicrobial Susceptibility Testing (AST)

Strains were previously tested for antimicrobial susceptibility using either broth microdilution or disc diffusion following the Clinical and Laboratory Standards Institute (CLSI) guidelines (Foley et al, 2006; Han et al, 2011; Kaldhone et al, 2008; Lynne et al, 2008, 2009; Marrero-Ortiz et al, 2012; Melendez et al, 2010). AST data from each study were compiled and across the different studies all the strains were tested for susceptibility to ampicillin (Amp), ceftriaxone (Axo), ciprofloxacin (Cip), chloramphenicol (Chl), gentamicin (Gen), kanamycin (Kan), nalidixic acid (Nal), streptomycin (Str), sulfisoxazole (Sul), trimethoprium-sulfamethoxazole (Sxt), and tetracycline (Tet). To verify the earlier results, a subset of the strains (n=35) were re-tested using disc diffusion testing for Amp, Axo, Chl, Gen, Str, Sul, Sxt and Tet, using *E. coli* ATCC 25922 as the quality control strain (Clinical and Laboratory Standards Institute, 2008).

Colicin Inhibition Assay

The ability of *Salmonella* and transconjugant strains to produce colicin was evaluated by assessing growth inhibition of *E. coli* J53, a colicin negative strain. J53 was suspended in sterile water and its bacterial cell concentration adjusted to a 0.5 McFarland standard. The suspension was swabbed for confluence on blood agar plates and 10 µl of a *Salmonella* suspension, prepared by suspending 2 bacterial colonies in 100 µl of sterile water, was spotted on the J53 lawn. The plates were incubated at 37°C for 16 to 18 hrs and then examined for growth inhibition of J53 adjacent to the *Salmonella* growth.

Results

PCR assays were conducted to detect the presence of genes previously identified as being associated with IncI1 plasmids and potentially responsible for antimicrobial resistance, conjugal transfer, and virulence properties. Positive PCR results were identified for *aadA1* in 88% (81/92) of strains, followed by *tetA* (87%, 80/92), *sul1* (80%, 74/92), bla_{CMY} (48%, 44/92) and *aacC* (30%, 28/92) (Table 1). For the corresponding resistance phenotypes, 78% (78/92) of the *Salmonella* strains were resistant or intermediately susceptible to Str (85%, 78/92), followed by Tet (74%, 68/92), Sul (76%, 69/92), Amp (68%, 62/92), Axo 43% (39/92), Gen (35%, 33/92; Table 1). Only three (3%) strains were susceptible to all of the antimicrobials examined in the comparison.

Over half of the strains carried the putative virulence genes *imm* (58%, 53/92) and *ccdA* (53%, 49/92), while *copD* (37%, 34/92), *cib* (35%, 32/92), *cus*A (33%, 30/92) and *col*1 (23%, 21/92) were identified in fewer strains (Table 1). When present, the copper resistance genes, *copD* and *cusA,* were often detected (N=25) together. *col1* and *cib* are two of several known bacteriocin (colicin) encoding genes and *imm* is the host immunity gene for colicin's effects. Over half of the strains (57%, 53/92) were positive for colicin production and inhibited strain J53 growth (Table 1). A positive test was defined as having a clear zone of growth inhibition of the bacterial lawn adjacent to the *Salmonella* spotted on the plate (Supplemental Figure 1).

Among the conjugation-related genes, *pilM* (98%, 91/92), *pilP* (91%, 84/92), and *pilJ* (82%, 76/92) occurred in most of the strains as did *traL* (96%, 90/92), *traQ* (95%, 89/92), and t*raG* (84%, 77/92) (Table 1). Over half of the strains (60%, 56/92) were able to generate transconjugants. Five isolates were susceptible to each of the selection antimicrobials and as such the plasmid transfer was not determined (marked as "ND" in the conjugation results in Table 1).

Some transconjugants (74%, 41/56) were obtained by the plate mating method, while the remaining 15 were obtained by the broth mating method at a 1:9 proportion (n=8) or 1:1 proportion (n=7). When the presence of the IncI1 plasmid in the transconjugants was evaluated, the majority (82%, 46/56), carried IncI1 plasmids, while the remaining strains contained other resistance plasmids (Table 2). PCR for the resistance genes in the transconjugants indicated that 59% (33/56) of the strains were positive for *tetA*, followed by aadA1 (52%, 29/56), *bla*$_{\text{CMY-2}}$ (46%, 27/56), *sul1* (30%, 17/56), and *aacC* (9%, 5/56) (Table 2). In addition, 29% (22/56) of the transconjugants could produce bacteriocins that inhibited the growth of J53 (Table 2).

Discussion

IncI1 plasmids have often been associated with the carriage of antimicrobial resistance genes. Less is known about the potential contribution of IncI1 plasmids to virulence in *Salmonella*. This study was undertaken to assess their potential contribution to virulence through putative virulence gene detection and their production of bacteriocins, which may inhibit bacterial competition for colonization; evaluate their role in antimicrobial resistance; and determine whether the plasmids can be transferred conjugally. Many earlier studies of IncI1 plasmids focused on ESC resistance (Folster et al, 2016; Liakpoulos et al, 2016; Smith et al, 2015). This paper examined several additional resistance genes that have been reported to be present in IncI1 plasmids in *Salmonella* including, *aadA1, aacC, bla*$_{\text{CMY-2}}$, *sul1* and *tetA* (Fricke et al, 2009, Han et al, 2012).

When a resistance gene was detected, the corresponding resistance phenotype was typically observed, with a few noted exceptions. A relatively high percentage of susceptible strains were positive for *tetA* (n=18) and *sul1* (n=17) even after repeated PCR testing. When the

AST for several of the isolates was repeated, they were still identified as susceptible to Tet and Sul. The disparity between presence of *sul1* and lack of Sul resistance was reported in previous studies (Awad et al, 2016). *tetA* encodes an efflux pump that is regulated by the *tetR* gene product (Chopra and Roberts, 2001). The disparity between genotype and phenotype could be due to mutations that reduce or abolish the function of the corresponding proteins or expression of the resistance genes. Both *sul1* and *tetA* were detected in isolates that originated from multiple sources and time periods in the current study, thus highlighting their widespread distribution in *Salmonella* from different food animal populations. Tet resistance genes have been identified on a wide range of transmissible resistance plasmids, indicating their importance due to their distribution among bacteria and potential for horizontal gene transfer. Another resistance gene with relatively high prevalence was *aad*A1. *aadA1* was detected in 78 strains, 71 (88%) of which displayed resistance or intermediate susceptibility to Str. $bla_{\text{CMY-2}}$ is associated with resistance to multiple β-lactam antimicrobials, including Axo, a drug used for the treatment of severe *Salmonella* infections. In this study, 87% of strains resistant to Axo were positive for the $bla_{\text{CMY-2}}$ gene. $bla_{\text{CMY-2}}$ is known to be distributed among several IncI1 and IncA/C plasmids (Welch et al, 2007, Fricke et al, 2009). Liakopoulos et al. (2016) concluded that emergence of ESC-resistant *Salmonella* in the Netherlands was due to the presence of bla_{CMY} gene on IncI1 plasmids, potentially originating from imported poultry from South America. This example highlights the global concern of ESC-resistant *Salmonella* and the potential for their widespread dissemination.

The ability to transfer antimicrobial resistance provides the capability of pathogens to maintain their presence in animal production environments (Anacarso et al, 2016; Aviv et al, 2016). A major concern is that food animals could become a reservoir of these resistant bacteria

and subsequent infections would lead to illnesses that would be difficult to treat with conventional antibiotics (Aviv et al, 2016). Most of the strains in the current study were positive for the *tra* and *pil* genes associated with the IncI1 conjugation machinery (Table 1) and more than half produced transconjugants when mated with the sodium azide resistant *E. coli* J53. The majority (82%) of the transconjugants generated carried the IncI1 plasmid transferred from the donor. When the transconjugants were examined for the resistance genes, it was observed that not all of the resistance genes were able to be transferred (Table 2), which may be due to the genes being on a plasmid that was not transferred or located on the chromosome. In several cases it was difficult to determine whether the resistance genes were on the IncI1 plasmids or another plasmid, such the IncA/C plasmids which often carry multiple resistance genes. Twelve of the 46 IncI1-positive transconjugants contained IncA/C plasmids, thus the plasmids often co-transfer. In 24 cases, the transconjugants appeared to only receive an IncI1 plasmid and in these cases there was a diversity of resistance genes transferred to the recipient (Table 2), including $bla_{\text{CMY-2}}$, *aadA1, aacC, sul1* and *tetA*.

To further evaluate the genetics of IncI1 positive strains, nine of the isolates (142, 143, 144, 146, 397, 991, 1148, N36 and N89) underwent DNA sequencing analyses as parts of parallel studies (Kaldhone et al, 2017, Khajanchi et al, 2017). The results of the sequencing indicated a diversity of resistance genes on the IncI1 and other plasmids within the strains. For example, both isolate 142 from swine, and 1148 from a human patient, had large contigs containing the IncI1 replicon-associated sequence and *aadA1, aacC* and *sul1*. Isolate 142, contained additional resistance genes that are likely associated with different plasmid types, while 1148 did not (Kaldhone et al, 2017). With isolate N36, the IncI1 replicon contained the

bla$_{\text{CMY-2}}$ gene. Many other strains had multiple plasmid contigs, which were difficult to assign the resistance genes to a particular plasmid type.

Initial attempts to evaluate the conjugative ability of plasmids were conducted on solid plate media using a cross streaking method. A phenotype detected for several donor-recipient interactions was the apparent inhibition of the *E. coli* recipient strain by the donor. Many of the available sequences for IncI1 plasmids indicate the presence of the colicin genes, such as *cib* or *col1* and host immunity gene *imm* (Smith et al, 2015). Thirty-two strains were positive for *cib*, of these, 25 could inhibit *E. coli* (Table 1). In addition, 21 strains were positive for *col1*, and 12 displayed growth inhibition of *E. coli*. Nedialkova et al. (2014) used a mouse model to examine production of colicin by *S.* Typhimurium and demonstrated that colicin could inhibit growth of other enteric bacteria under gut inflammatory conditions (Nedialkova et al, 2014). Avirulent strains that did not produce colicin did not exhibit a competitive advantage. In the current study, the *Salmonella* serovars associated with growth inhibition included Newport, Heidelberg, Typhimurium and Kentucky. Of the strains from different sources, 10 of the 11 (91%) *S.* Heidelberg isolated from human patients demonstrated an ability to inhibit *E. coli* growth. Interestingly, a relatively low percentage (8%, 2/25) of the isolates from cattle, regardless of serotype, demonstrated the inhibitory phenotype, which may indicate that the production of colicins may have a selective advantage in other hosts, such as poultry and swine.

Just under half (26/57) of the strains that demonstrated growth inhibition had plasmids that could be transferred using the methods employed in this study. The broth-culture mating was used to attempt to dilute the potential colicin effect; however, even in the broth culture, the presence of colicin may limit conjugation. This phenomenon warrants further exploration to evaluate the effect of colicin on conjugation. Future studies may need to be done with recipient

strains that are more resistant to colicin inhibition. Interestingly, of the colicin positive strains that could transfer plasmids, 22 transconjugants also demonstrated the inhibitory phenotype (Table 2), this includes 13 strains that appeared to only an IncI1 plasmid.

There were also several instances where a colicin inhibitory phenotype was observed, but the strains were negative for *cib* and/or *col1*. This finding was not overly surprising as there are several different classes of bacteriocin genes that have been identified among enteric bacteria (Cascales et al, 2007) and will warrant further study to elucidate a comprehensive understanding of colicin production and their mechanisms of action. Conversely, some strains examined in the current studies possessed the *cib* and/or *col1* but failed to show inhibition. Spriewald et al. (2015) reported that iron limitation could influence *cib* gene expression and our studies were not conducted under iron-limited conditions, which could have negatively impacted the toxin production.

The ability of IncI1 plasmids to encode factors that contribute to antimicrobial resistance and virulence is important to public health. Monitoring these plasmids could serve as guide for detecting and potentially preventing spread of resistant and virulent strains and allow for targeted interventions, such as effective antimicrobial therapy, to effectively limit loss arising from outbreaks. Approaches should be undertaken along the farm-to-fork continuum to prevent cross-contamination reduce specific risks and minimize the spread of clinically important *Salmonella* and lead to improved public health.

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References

Anacarso I, Iseppi R, Sabia C, Messi P, Condo C, Bondi M, and De Niederhäusern S. 2016. Conjugation-mediated transfer of antibiotic-resistance plasmids between Enterobacteriaceae in the digestive tract of *Blaberus craniifer* (Blattodea: Blaberidae). J Med Entomol. 53:591-597.

Aviv G, Rahav G, and 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:e01395-16.

Awad A, Arafat N, and Elhadidy M. 2016. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. Ann Clin Microbiol Antimicrob. 15:59.

Cambray G, Guerout AM, and Mazel D. 2010. Integrons. Annu Rev Genet. 44:141–166.

Carattoli A. 2011. Plasmids in gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol. 301:654-658.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, and Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol Methods. 63:219-228.

Chen W, Fang T, Zhou X, Zhang D, Shi X, and Shi C. 2016. IncHI2 plasmids are predominant in antibiotic-resistant *Salmonella* isolates. Front Microbiol. 7:1566.

Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, Riley M, Slatin S, and Cavard D. 2007. Colicin biology. Microbiol. Mol. Biol. Rev. 71:158-229.

Chopra I, and Roberts M. 2001. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 2001; 65:232-260.

Clinical and Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement (M100-S18). Clinical and Laboratory Standards Institute, Wayne, PA.

Doumith M, Dhanji H, Ellington MJ, Hawkey P, and Woodford N. 2012. Characterization of plasmids encoding extended-spectrum beta- lactamases and their addiction systems circulating among *Escherichia coli* clinical isolates in the UK. J Antimicrob Chemother. 67:878-885.

Dunkley KD, Callaway TR, Chalova VI, McReynolds JL, Hume ME, Dunkley CS, Kubena LF, Nisbet DJ, and Ricke SC. 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe. 15:26-35.

Finstad S, O'Bryan CA, Marcy JA, Crandall PG, and Ricke SC. 2012. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. Food Res Int. 45:789-794.

Foley SL, Johnson TJ, Ricke SC, Nayak R, and Danzeisen J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev. 77:582-607.

Foley SL, White DG, McDermott PF, Walker RD, Rhodes B, Fedorka-Cray P, Simjee S, and Zhao S. 2006. Comparison of subtyping methods for differentiating *Salmonella enterica* serovar Typhimurium isolates obtained from food animal sources. J Clin Microbiol. 44:3569-3577.

Folster JP, Grass JE, Bicknese A, Taylor J, Friedman C, and Whichard JM. 2016. Characterization of resistance genes and plasmids from outbreaks and illness clusters caused by *Salmonella* resistant to ceftriaxone in the United States, 2011–2012. Microbial Drug Res.23:188-193.

Fricke WF, McDermott PF, Mammel MK, Zhao S, Johnson TJ, Rasko DA, Fedorka-Cray PJ, Pedroso A, Whichard JM, Leclerc JE, White DG, Cebula TA, and Ravel J. 2009. Antimicrobial resistance-conferring plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella enterica* serovar Kentucky isolates from poultry. Appl Environ Microbiol. 75:5963-5971.

Han J, David DE, Deck J, Lynne AM, Kaldhone P, Nayak R, Stefanova R, Foley SL. 2011. Comparison of *Salmonella enterica* serovar Heidelberg isolates from human patients with those from animal and food sources. J Clin Microbiol. 49:1130-1133.

Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, Kaldhone P, Logue CM, and Foley SL. 2012. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PLoS ONE. 7:e51160.

Hanning IB, Nutt JD, and Ricke SC. 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog Dis. 6:635-648.

Howard ZR, O'Bryan CA, Crandall PG, and Ricke SC. 2012. *Salmonella* Enteritidis in shell eggs: Current issues and prospects for control. Food Res Int. 45:755-764.

Jacoby GA, and Han P. 1996. Detection of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J Clin Microbiol. 34:908-911.

Jakočiūnė D, Bissgaard M, Pedersen K, and Olsen JE. 2014. Demonstration of persistent contamination of a cooked egg product production facility with *Salmonella enterica* serovar Tennessee and characterization of the persistent strain. J Appl Microbiol. 117:547-553.

Johnson TJ, and Nolan LK. 2009. Plasmid replicon typing. Methods Mol Biol. 551:27-35.

Kaldhone P, Nayak R, Lynne AM, David DE, McDermott PF, Logue CM, and Foley SL. 2008. Characterization of *Salmonella enterica* serovar Heidelberg from turkey-associated sources. Appl Environ Microbiol. 74:5038-5046.

Kaldhone P, Khajanchi BK, Han J, Nayak R, Ricke, SC, and Foley SL. 2017. Draft genome sequences of *Salmonella enterica* isolates containing incompatibility group I1 plasmids from swine, poultry and human sources. Genome Announc. 5:e01056-17.

Khajanchi BK, Hassan NA, Choi SY, Han J, Zhao S, Colwell RR, Cerniglia CE, and Foley SL. 2017. Comparative genomic analysis and characterization of incompatibility group FIB plasmid encoded virulence factors of *Salmonella enterica* isolated from food sources. BMC Genomics. 18:570.

Komano T, Yoshida T, Narahara K, and Furuya N. 2000. The transfer region of IncI1 plasmid R64: similarities between R64 *tra* and *Legionella icm/dot* genes. Mol Microbiol. 35:1348-1359.

Lai J, Wang Y, Shen J, Li R, Han J, Foley SL, and Wu C. 2013. Unique class I integron and multiple resistance genes co-located on IncHI2 plasmid is associated with the emerging multidrug resistance of *Salmonella* Indiana isolated from chicken in China. Foodborne Pathog Dis. 10:581–588.

Liakopoulos A, Geurts Y, Dierikx CM, Brouwer MS, Kant A, Wit B, Heymans R, Van Pelt W, and Mevius DJ. 2016. Extended-spectrum cephalosporin-resistant S*almonella enterica* serovar Heidelberg strains, the Netherlands. Emerg Infect Dis. 22:1257-1261.

Lynne AM, Kaldhone P, David D, White DG, and Foley SL. 2009. Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. Foodborne Pathog Dis. 6:207-215.

Lynne AM, Rhodes-Clark BS, Bliven K, Zhao S, and Foley SL. 2008. Antimicrobial resistance genes associated with *Salmonella enterica* serovar Newport isolates from food animals. Antimicrob Agents Chemother. 52:353-356.

Marrero-Ortiz R, Han J, Lynne AM, David DE, Stempe, ME, Farmer D, Burkhardt III W, Nayak R, and Foley SL. 2012. Genetic characterization of antimicrobial resistance in *Salmonella enterica* serovars isolated from dairy cattle in Wisconsin. Food Res Int. 45:962-967.

Martin IF., Thomas CM, Laing E, AbuOun M, La Regione RM, and Woodward MJ. 2016. Curing vector for IncI1 plasmids and its use to provide evidence for a metabolic burden of IncI1 CTX-M-1 plasmid pIFM3791 on *Klebsiella pneumoniae*. J Medical Microbiol. 65:611-618.

Martinez JL, and Bauero F. 2002. Interactions among strategies associated with bacterial infections: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbial Rev 15:647-679.

Melendez SN, Hanning I, Han J, Nayak R, Clement AR, Wooming, A, Hererra P, Jones FT, Foley SL, and Ricke SC. 2010. *Salmonella enterica* isolates from pasture-raised poultry exhibit antimicrobial resistance and class I integrons. J Appl Microbiol. 109:1957-1966.

Mnif B, Vimont S, Boyd A, Bourit E, Picard B, Branger C, Denamur E, and Arlet G. 2010. Molecular characterization of addiction systems of plasmids encoding extended-spectrum beta-lactamases in *Escherichia coli*. J Antimicrob Chemother. 65:1599-1603.

Nedialkova LP, Denzler R, Koeppel MB, Diehl M, Ring D, Wille T, Gerlach RG, and Stecher B. 2014. Inflammation fuels colicin Ib-dependent competition of *Salmonella* serovar Typhimurium and *E. coli* in enterobacterial blooms. PLoS Pathog. 10:e1003844.

Sampei G, Furuya N, Tachibana K, Saitou Y, Suzuki T, Mizobuchi K, and Komano T. 2010. Complete genome sequence of the incompatibility group I1 plasmid R64. Plasmid. 64:92-103.

Sanad Y, Johnson K, Park SH, Han J, Deck J, Foley SL, Kenney B, Ricke SC, and Nayak R. 2016. Molecular characterization of *Salmonella enterica* serovars isolated from a turkey production facility in the absence of selective antimicrobial pressure. Foodborne Pathog Dis. 13:80-87.

Scallan E, Hoelstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Jones JL, and Griffin PM. 2011. Foodborne illnesses acquired in the United States- major pathogens. Emerg Infect Dis. 17:7-15.

Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodriguez I, Essen-Zandbergen AV., Brouwer M, and Mevius D. 2015. Characterization of epidemic IncI1-Iγ plasmids harboring ambler class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. Antmicrob Agents Chemother. 59:5357-5365.

Spriewald S, Glaser J, Beutler M, Koeppel MB, and Stecher B. 2015. Reporters for single-cell analysis of colicin Ib expression in *Salmonella enterica* serovar Typhimurium. PLoS ONE. 10:e0144647.

Wang H, Gill VS, Cheng CM, Gonzalez-Escalona N, Irvin KA, Zheng J, Bell RL, Jacobson AP, and Hammack TS. 2015. Evaluation and comparison of rapid methods for the detection of *Salmonella* in naturally contaminated pine nuts using different pre enrichment media. Food Microbiol. 46:58-65.

Welch TJ, Fricke WF, McDermott PF, White DG, Rosso ML, Rasko DA, Mammel MK, Eppinger M, Rosovitz MJ, Wagner D, Rahalison L, Leclerc JE, Hinshaw JM, Lindler LE, Cebula TA, Carniel E, and Ravel J. 2007. Multiple antimicrobial resistance in plague: An emerging public health risk. PLoS ONE. 2:e309.

Wong MH, Kan B, Chan EW, Yan M, and Chen S. 2016. IncI1 plasmids carrying various *bla*CTX-M genes contribute to ceftriaxone resistance in *Salmonella enterica* serovar Enteritidis in China. Antimicrob Agents Chemother. 60:982-989.

Zheng R, Zhang Q, Guo Y, Feng Y, Liu L, Zhang A, Zhao Y, Yang X, and Xia X. 2016. Outbreak of plasmid-mediated NDM-1-producing *Klebsiella pneumoniae* ST105 among neonatal patients in Yunnan, China. Ann Clin Microbiol Antimicrob. 15:10.

Table 1: Isolate information and results of antimicrobial resistance, virulence and transfer phenotypic and PCR-based testing

Isolate	Serotype	Source	Year	State	Replicon Types Detectected	Chl	Tet	Axo	Amp	Gen	Kan	Str	Sul	Sxt	*tetA*	*bla* CMY	*aacC*	*aadA1*	*sul1*	*ccdB*	*ccdA*	*copD*	*cusA*	*imm*	*col1*	*cib*	Bacterial Inhibition?	*pilS*	*pilP*	*pilM*	*pilJ*	*traG*	*traL*	*traQ*	*traT*	Conjugation?
891	Anatum	Cattle	2006	WI	I1																						No									Yes
856	Cerro	Cattle	2006	WI	I1																						No									Yes
1073	Cerro	Cattle	2007	WI	I1																						No									No
1078	Cerro	Cattle	2007	WI	I1																						No									Yes
849	Dublin	Cattle	2005	AZ	I1, A/C, FIIA																						No									Yes
111	Heidelberg	Cattle	2001	OH	I1, A/C, HI2																						No									Yes
114	Heidelberg	Cattle	2002	IA	I1, A/C, HI2																						No									Yes
115	Heidelberg	Cattle	2002	IN	I1, A/C, HI2																						No									Yes
116	Heidelberg	Cattle	2002	IN	I1, A/C, B/O, HI2																						No									Yes
121	Heidelberg	Cattle	2002	N/A	I1																						No									Yes
122	Heidelberg	Cattle	2002	VA	I1, A/C, HI2																						No									Yes
1017	Infantis	Cattle	2006	WI	I1, A/C																						No									No
860	Kentucky	Cattle	2006	WI	I1, FIA																						No									Yes
1053	Kentucky	Cattle	2007	WI	I1																						No									No
1076	Kentucky	Cattle	2007	WI	I1																						No									Yes
1116	Kentucky	Cattle	2007	WI	I1																						No									Yes
1118	Kentucky	Cattle	2007	WI	I1																						No									No
1012	Mbandaka	Cattle	2006	WI	I1																						No									ND**
880	Montevideo	Cattle	2006	WI	I1																						No									Yes
67	Newport	Cattle	2002	IA	I1, A/C																						No									Yes
74	Newport	Cattle	2002	WA	I1, A/C																						Yes									No
1057	Subsp. Diarizonae (IIIB)	Cattle	2007	WI	I1, A/C, FIC																						No									Yes
855	Typhimurium	Cattle	2006	WI	I1, FIA, FIB, W																						Yes									Yes
859	Typhimurium	Cattle	2006	WI	I1, FIB, W																						No									Yes
1011	Typhimurium	Cattle	2006	WI	I1																						No									No
1079	Typhimurium	Cattle	2007	WI	I1, FIA, W																						No									Yes
N859	Kentucky	Chicken	2008	AR	I1																						No									No
N860	Kentucky	Chicken	2008	AR	I1																						No									ND
N865	Kentucky	Chicken	2008	AR	I1																						No									No
N100	Kentucky	Chicken		WV	I1, FIB																						Yes									No
N52	Kentucky	Chicken		WV	I1																						Yes									No
N55	Kentucky	Chicken		WV	I1																						No									No
N96	Kentucky	Chicken		WV	I1, FIB																						Yes									No
76	Newport	Chicken	2001	GA	I1, A/C, B/O, FIIA, K/B, T, W																						No									Yes
397	Typhimurium	Chicken	1999	N/A	I1, FIB																						Yes									No
N118	Typhimurium	Chicken		WV	I1, A/C, N																						Yes									No
N119	Typhimurium	Chicken		WV	I1, FIB																						Yes									No
N140	Typhimurium	Chicken		WV	I1, FIB																						Yes									ND
N36	Typhimurium	Chicken		WV	I1, FIB																						Yes									Yes
N53	Typhimurium	Chicken		WV	I1, A/C																						Yes									Yes
N74	Typhimurium	Chicken		WV	I1, FIB																						Yes									Yes
N97	Typhimurium	Chicken		WV	I1, A/C																						Yes									No
N99	Typhimurium	Chicken		WV	I1, A/C																						Yes									No
692	Heidelberg	Chicken Farm	2004	MN	I1																						No									Yes
N822	Kentucky	Chicken Farm	2008	AR	I1																						No									ND
N845	Kentucky	Chicken Farm	2008	AR	I1																						Yes									No

Isolate	Serotype	Source	Year	State	Replicon Types Detectected	Chl	Tet	Axo	Amp	Gen	Kan	Str	Sul	Sxt	*tetA*	*bla* CMY	*aacC*	*aadA1*	*sul1*	*ccdB*	*ccdA*	*capD*	*cusA*	*imm*	*col1*	*cib*	Bacterial Inhibition?	*pilS*	*pilP*	*pilM*	*pilJ*	*traG*	*traL*	*traQ*	*traT*	Conjugation?
N854	Kentucky	Chicken Farm	2008	AR	I1		■					■						■	■	■	■	■	■				Yes		■	■		■	■	■	■	No
N89	Kentucky	Chicken Farm		WV	I1				■			■	■		■	■		■	■	■	■	■		■	■	■	Yes	■	■	■	■	■	■	■	■	Yes
N134	Typhimurium	Chicken Farm		WV	I1, FIB			■	■				■			■			■	■	■	■		■	■	■	Yes	■	■	■	■	■	■	■	■	No
N136	Typhimurium	Chicken Farm		WV	I1, FIB	■	■	■	■	■					■	■		■	■	■	■	■	■	■		■	■	Yes	■	■	■	■	■	■	■	■	No
N139	Typhimurium	Chicken Farm		WV	I1, A/C		■	■	■				■		■	■		■	■		■	■	■	■		■	■	Yes	■	■	■	■	■	■	■	■	No
N82	Typhimurium	Chicken Farm		WV	I1	■	■	■	■		■	■	■		■	■		■	■	■	■		■	■	■	■	Yes	■	■	■	■	■	■	■	■	Yes
N91	Typhimurium	Chicken Farm		WV	I1, A/C		■	■	■		■		■		■	■		■	■	■	■		■	■	■	■	Yes	■	■	■	■	■	■	■	■	No
990	Heidelberg	Human	2008	AR	I1, X		■			■	■	■	■	■	■		■	■	■	■	■	■	■	■			Yes		■	■	■	■	■	■	■	No
991	Heidelberg	Human	2009	AR	I1		■			■	■	■	■		■			■	■		■	■	■		■		No	■	■	■	■	■	■	■	■	Yes
1000	Heidelberg	Human	2009	NY	I1, B/O		■		■	■	■	■	■		■	■		■				■		■	■		Yes	■	■	■	■	■	■	■	■	Yes
1031	Heidelberg	Human	2009	NY	I1, X		■			■	■	■	■				■	■	■		■	■	■	■	■		Yes	■	■	■	■	■	■	■	■	No
1147	Heidelberg	Human	2007	WI	I1			■	■						■	■		■	■	■	■						Yes		■	■	■	■	■	■	■	Yes
1148	Heidelberg	Human	2007	WI	I1							■	■		■		■	■	■					■	■		Yes	■	■	■	■	■	■	■	■	No
1149	Heidelberg	Human	2007	WI	I1, X		■	■	■		■	■			■	■		■	■	■	■	■	■	■		■	Yes	■	■	■	■	■	■	■	■	Yes
1154	Heidelberg	Human	2007	WI	I1, A/C	■	■	■	■	■		■	■	■	■			■	■	■	■	■	■	■	■	■	Yes	■	■	■		■	■	■	■	Yes
1160	Heidelberg	Human	2007	WI	I1			■	■							■			■	■	■	■	■	■	■		Yes	■	■	■	■	■	■	■	■	Yes
1162	Heidelberg	Human	2007	WI	I1, B/O, X		■	■	■		■	■			■	■		■	■	■	■	■	■		■	■	Yes	■	■	■	■	■	■	■	■	Yes
1163	Heidelberg	Human	2007	WI	I1		■	■	■				■			■		■	■	■	■				■	■	Yes	■	■	■	■	■	■	■	■	Yes
142	Heidelberg	Swine	2002	IN	I1, A/C, HI2	■	■	■	■	■	■	■	■		■	■	■	■	■	■		■		■			Yes	■	■	■	■	■	■	■	■	Yes
143	Heidelberg	Swine	2002	MN	I1, A/C, HI1, HI2	■	■	■	■		■	■	■	■	■	■	■		■			■		■			Yes	■	■	■		■	■	■	■	Yes
144	Heidelberg	Swine	2002	MN	I1, A/C, HI2	■	■	■	■	■	■	■	■	■	■	■		■		■							No		■	■	■	■	■	■	■	Yes
146	Heidelberg	Swine	2002	MN	I1, A/C	■	■	■	■		■	■	■	■	■	■		■		■		■	■				Yes	■	■	■	■	■	■	■	■	Yes
148	Heidelberg	Swine	2002	MO	I1, X		■	■	■		■	■			■	■		■									Yes	■	■	■	■	■	■	■	■	Yes
151	Heidelberg	Swine	2002	NE	I1, A/C, X	■	■	■	■		■	■	■	■	■		■	■		■	■	■	■		■	■	No	■	■	■	■	■	■	■	■	Yes
86	Newport	Swine	2001	MO	I1, A/C		■	■	■		■	■	■	■	■	■		■	■								No		■	■		■	■	■	■	Yes
89	Newport	Swine	2001	UT	I1, A/C	■	■	■	■			■	■		■	■		■	■								Yes	■	■	■		■	■	■	■	No
91	Newport	Swine	2002	IA	I1, A/C	■	■	■	■			■	■	■	■	■		■	■								Yes		■	■		■	■	■	■	No
93	Newport	Swine	2002	KS	I1, A/C	■	■	■	■			■	■		■	■		■	■					■			Yes		■	■	■		■		■	Yes
470	Typhimurium	Swine	1999	N/A	I1, HI2	■	■		■		■	■	■	■	■			■	■	■		■	■	■		■	Yes	■	■	■	■	■	■	■	■	No
471	Typhimurium	Swine	1999	N/A	I1					■		■	■		■		■	■	■	■				■			Yes	■	■	■	■	■	■	■	■	Yes
474	Typhimurium	Swine	1999	N/A	I1		■								■			■						■			No	■	■	■	■	■	■	■	■	No
159	Heidelberg	Turkey	2002	NC	I1, HI2		■	■	■		■	■			■	■	■	■	■	■	■	■	■	■	■	■	Yes	■	■	■	■	■	■	■	■	Yes
695	Heidelberg	Turkey	2000	MW*	I1, X	■	■	■	■		■		■		■		■	■	■	■	■	■	■	■			Yes	■	■	■	■	■	■	■	■	Yes
703	Heidelberg	Turkey	2000	MW	I1		■		■		■	■	■					■	■	■	■				■	■	No		■	■	■	■	■	■	■	Yes
705	Heidelberg	Turkey	2000	MW	I1		■		■		■	■	■		■				■	■				■		■	Yes	■	■	■	■	■	■	■	■	Yes
706	Heidelberg	Turkey	2000	MW	I1		■		■	■	■	■	■		■		■	■						■			Yes	■	■	■	■	■	■	■	■	Yes
709	Heidelberg	Turkey	1992	ND	I1		■		■	■	■	■	■		■					■				■			Yes	■		■	■	■	■	■	■	Yes
713	Heidelberg	Turkey	1995	ND	I1		■			■	■	■	■		■			■	■							■	No		■	■	■	■	■	■	■	Yes
714	Heidelberg	Turkey	2000	MW	I1		■			■	■	■	■		■			■	■					■		■	Yes	■	■	■	■	■	■	■	■	No
715	Heidelberg	Turkey	2000	MW	I1		■		■	■	■	■	■		■			■	■					■			Yes	■	■	■	■	■	■	■	■	Yes
824	Heidelberg	Turkey	2003	GA	I1, X		■		■	■	■	■	■		■		■	■	■		■	■	■	■		■	Yes	■	■	■	■	■	■	■	■	Yes
827	Heidelberg	Turkey	2003	MN	I1		■		■	■	■	■	■		■			■	■		■		■	■		■	Yes	■	■	■		■	■	■	■	No
828	Heidelberg	Turkey	2003	MN	I1		■		■	■	■	■	■		■			■	■					■		■	Yes	■	■	■		■	■	■	■	No
830	Heidelberg	Turkey	2003	GA	I1, HI2		■			■	■	■	■		■			■	■			■		■			Yes	■	■	■	■	■	■	■	■	No
100	Newport	Turkey	2001	ND	I1				■	■	■		■					■	■					■			No		■			■	■	■	■	Yes
482	Typhimurium	Turkey	1999	N/A	I1		■		■	■	■	■	■		■			■	■	■							Yes	■	■	■	■	■	■	■	■	Yes

Note: For antimicrobial susceptibility testing results, a white box is susceptible, a grey box is intermediate and a black box is resistant. A black box indicates a positive PCR result. *MW: Midwestern state, not further defined. **ND: Not determined due to the donor strain being susceptible to all selection agents.

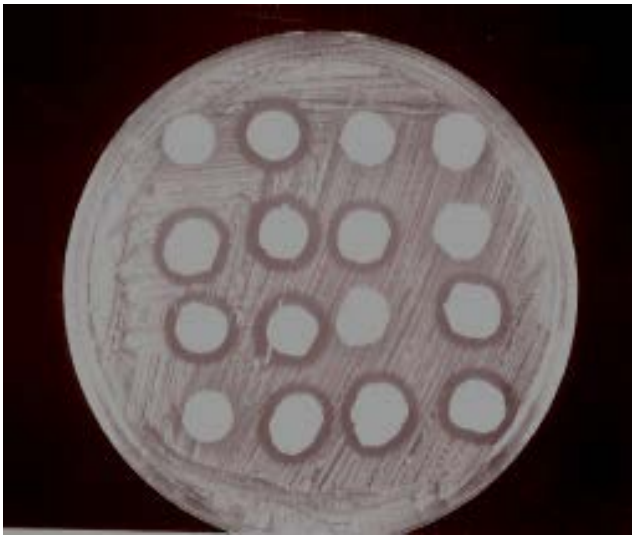
Table 2. Evaluation of Transconjugants Generated in the Study

Transconjugant	Donor Serotype	Donor Source	Donor Replicon Types	Transconjugant Replicon Types	Donor					Transconjugant					Colicin Inhibition
					tetA	*bla* CMY	*aacC*	*aadA1*	*sul1*	*tetA*	*bla* CMY	*aacC*	*aadA1*	*sul1*	
X67	Newport	Cattle	I1, A/C	I1, A/C	■	■	■	■	■	■	■				
X76	Newport	Chicken	C, B/O, FIIA, K/B	I1, A/C, W	■			■		■					
X86	Newport	Swine	I1, A/C	I1, A/C	■	■		■	■	■	■		■	■	
X93	Newport	Swine	I1, A/C	I1, A/C	■	■		■	■	■	■		■		■
X100	Newport	Turkey	I1	I1				■	■				■	■	
X111	Heidelberg	Cattle	I1, A/C, HI2	I1, A/C	■	■	■	■		■	■		■		
X114	Heidelberg	Cattle	I1, A/C, HI2	I1, A/C	■	■		■		■	■				
X115	Heidelberg	Cattle	I1, A/C, HI2	I1, A/C	■	■				■	■				
X116	Heidelberg	Cattle	I1, A/C, B/O, HI2	I1	■			■	■						
X121	Heidelberg	Cattle	I1	I1	■	■		■	■	■	■				
X122	Heidelberg	Cattle	I1, A/C, HI2	A/C	■	■	■	■	■	■	■				
X142	Heidelberg	Swine	I1, A/C, HI2	I1, A/C	■	■	■	■	■	■	■	■	■	■	■
X143	Heidelberg	Swine	I1, A/C, HI1, HI2	I1, A/C	■	■	■		■	■	■			■	■
X144	Heidelberg	Swine	I1, A/C, HI2	I1, A/C	■	■		■		■	■		■		
X146	Heidelberg	Swine	I1, A/C	ND*	■	■		■	■		■				
X148	Heidelberg	Swine	I1, X	I1	■	■		■		■	■				
X151	Heidelberg	Swine	I1, A/C, X	I1, A/C	■		■	■		■					
X159	Heidelberg	Turkey	I1, HI2	I1	■	■	■	■			■				■
X471	Typhimurium	Swine	I1	I1	■		■	■	■	■		■	■	■	■
X482	Typhimurium	Turkey	I1	I1	■		■	■	■						■
X692	Heidelberg	Chicken Farm	I1	ND	■		■		■	■					
X695	Heidelberg	Turkey	I1, X	I1	■		■	■	■	■		■	■	■	■
X703	Heidelberg	Turkey	I1	ND				■	■						
X705	Heidelberg	Turkey	I1	I1	■			■	■	■					■
X706	Heidelberg	Turkey	I1	I1	■		■	■		■			■		■
X709	Heidelberg	Turkey	I1	ND	■					■					
X713	Heidelberg	Turkey	I1	ND	■		■	■	■	■		■	■	■	
X715	Heidelberg	Turkey	I1	I1	■		■	■		■		■	■		■
X824	Heidelberg	Turkey	I1, X	I1	■		■	■	■				■	■	■
X849	Dublin	Cattle	I1, A/C, FIIA	I1, A/C	■	■		■	■	■	■		■	■	■
X855	Typhimurium	Cattle	I1, FIA, FIB, W	I1, FIA, W	■	■		■	■	■	■			■	
X856	Cerro	Cattle	I1	I1	■		■	■		■			■		■
X859	Typhimurium	Cattle	I1, FIB, W	FIB, W			■	■	■				■		
X860	Kentucky	Cattle	I1, FIA	ND	■	■		■		■	■		■		
X880	Montevideo	Cattle	I1	ND	■			■	■	■			■	■	
X891	Anatum	Cattle	I1	I1	■			■	■	■			■		
X991	Heidelberg	Human	I1	ND	■			■	■				■	■	
X1000	Heidelberg	Human	I1, B/O	I1	■	■		■		■			■		■
X1011	Typhimurium	Cattle	I1	I1	■			■	■				■	■	
X1057	ıbsp. Diarizonae (III	Cattle	I1, A/C, FIC	I1, FIC	■	■		■	■	■			■		
X1076	Kentucky	Cattle	I1	I1	■	■									
X1078	Cerro	Cattle	I1	I1	■			■		■			■		
X1079	Typhimurium	Cattle	I1, FIA, W	I1, FIA, W	■			■	■				■		
X1116	Kentucky	Cattle	I1	I1	■			■	■				■	■	
X1147	Heidelberg	Human	I1	I1	■	■		■	■		■		■		■
X1149	Heidelberg	Human	I1, X	I1	■	■		■	■		■				■
X1154	Heidelberg	Human	I1, A/C	I1	■			■	■				■		
X1160	Heidelberg	Human	I1	I1		■			■						■
X1162	Heidelberg	Human	I1, B/O, X	I1	■	■		■	■		■				
X1163	Heidelberg	Human	I1	I1		■		■	■		■				■
XN36	Typhimurium	Chicken	I1, FIB	I1, FIB		■		■	■		■				■
XN53	Typhimurium	Chicken	I1, A/C	I1	■	■		■	■		■		■	■	■
XN74	Typhimurium	Chicken	I1, FIB	I1, FIB	■	■		■	■		■			■	■
XN82	Typhimurium	Chicken Farm	I1	I1	■	■		■	■	■	■			■	■
XN89	Kentucky	Chicken Farm	I1	I1	■	■		■	■		■		■		
XN822	Kentucky	Chicken Farm	I1	I1	■				■						

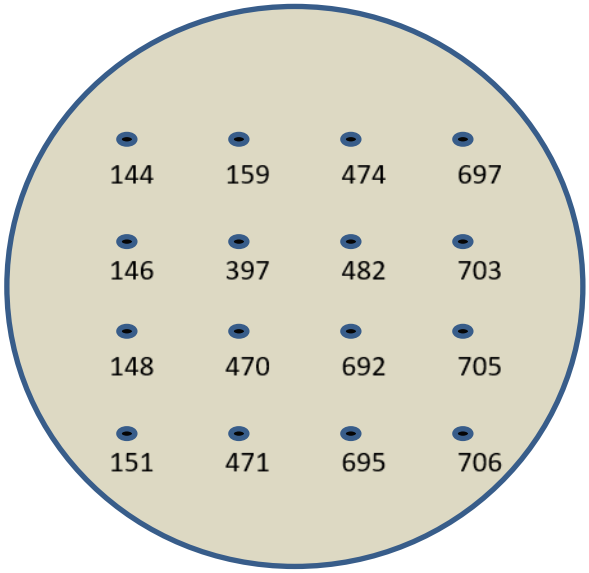
A black box indicates a positive result in the test. *ND: indicates no plasmid type was detected by PCR screening.

Supplemental Information

Figure S1: Example results of the colicin inhibition assay. A) A zone of clearing around the growth of *Salmonella* (bright white spots) was interpreted as being positive for colicin production. In this experiment 10/16 strains were interpreted as being positive. **B)** The panel below denotes the identification numbers and positon of respective isolates.



A



B

Table S1: Primers used in this study for the detection of antimicrobial resistance, plasmid transfer and virulence associated genes for PCR reactions.

	aadA1	TATCAGAGGTAGTTGGCGTCAT	GTTCCATAGCGTTAAGGTTTCATT	55	Lynne et al.,2008
	aacC	GGCGCGATCAACGAATTTATCCGA	CCATTCGATGCCGAAGGAAACGAT	58	Lynne et al.,2008
	bla_{CMY}	GACAGCCTCTCTTTCTCCACA	TGGAACGAAGGCTACGTA	48	Lynne et al.,2008
	sul1	TCACCGAGGACTCCTTCTTC	AATATCGGGATAGAGCGCAG	60	Lynne et al.,2008
	tetA	GCTACATCCTGCTTGCCTTC	CATAGATCGCCGTGAAGAGG	55	Lynne et al.,2008
	*pil*S	GTTCAGGGTAATGCCGTTAGT	TAGTCGTTGTTCTGGGTAGTTTG	60	This study
	*pil*P	CATGCGAGAACGGCATTAAG	GCAACAACACAACCTCTTGTC	60	This study
	*pil*M	GACGACAGAACCAGCAGTAAT	CACAAACGCCCAGCAATATG	60	This study
	*pil*J	CACCATTATTTCCACAGGCTTAC	CATCACGGTGGTGGTGTTCATAGA	60	This study
	*tra*G	TCCATAACGACGGGTTCTTTAC	GGTAGCGGCAGTGACAAA	60	This study
	*tra*L	GTCCTTCCAGGGATCAATATTCC	GCTCGGCCTTATCCTGATTT	60	This study
	*tra*Q	GCATTGAGGACACGATCGATAA	GAGGCGTACAAATGGGATTCA	60	This study
	*tra*T	TGTCAGAATCAGGGCAATCC	GGCCACAGAACATCTGGATAA	60	This study
	*ccd*B	GCTGAGATCAGCCACTTCC	GAGAGCCGTTATCGTCTGTTT	60	This study
	*ccd*A	CCTGTTCTCGTCGGCAAA	AAGCAGCGCATTACAGTCA	60	This study
	*col*1	CGAGATTTGCCGGTACGATAA	CGGTGACAGCCATCAGTAAA	60	This study
	cib	GGATGTGGAAGGTGACAAGAA	CACTCACAGCCGCCATAATA	60	This study
	imm	GGCAACCACAGGAACTGATA	GGATGGAAAGATAGCCAGGAAA	60	This study
	*cop*D	AATTCCCTTCCCTGACCATAAC	CATGACGGTCTGCATTACTATCT	60	This study
	*cus*A	CCTGCGGAATGCCAGATATAA	GAGTATCGAAGCAGTCGCTAAA	60	This study

V. Chapter 4

Draft Genome Sequences of *Salmonella enterica* Isolates Containing Incompatibility Group I1 Plasmids from Swine, Poultry and Human Sources

Running Title: IncI1 Plasmid Carrying *Salmonella* strains

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Abstract:

The draft genome sequences of the eight *Salmonella enterica* isolates were evaluated for the influence of incompatibility group (Inc)-I1 plasmids on virulence from various sources. Strains SE142, SE143, SE144, and SE146 originated from swine, SE36N and SE89N from poultry-related sources and SE991 and SE1148 from human patients.

Salmonella enterica is one of the top five bacterial pathogens contributing to foodborne illnesses. *Salmonella* is also a leading foodborne pathogen associated with hospitalizations and deaths in the United States (CDC, 2012). Food products originating from diverse sources like poultry, swine, and cattle are commonly associated with outbreaks caused by *Salmonella enterica* (Scallan et al, 2011). Some serotypes of *Salmonella* including Enteritidis, Typhimurium, and Heidelberg are more prevalent as foodborne pathogens, than others such as Kentucky (Foley et al, 2013). Isolates containing certain mobile genetic elements such as plasmids have been associated with clinical manifestations of *Salmonella* (Han et al, 2011). Plasmids can encode genes responsible for antimicrobial resistance and virulence which may have clinical significance associated with severe manifestations of the diseases (Han et al, 2012). Incompatibility group 1 (IncI1) plasmids have been reported to carry genes related to antimicrobial resistance and virulence (Gokulan et al, 2013).

Eight strains of *Salmonella enterica*, containing IncI1 plasmids, were sequenced (Table 1). Four of these strains were isolated from swine, two from poultry-related sources and two from human patients. Previous studies showed that SE1148 and SE146 carried IncI1 plasmids and antimicrobial resistance genes (Smith et al, 2015). In addition to resistance genes SE146 also contained an IncX4 plasmid which encodes a VirB/D4 type 4 secretion system that is likely involved in the increased virulence potential of this strain (Lynne et al, 2009). Strains SE142, SE143, SE144, and SE146 were found to contain one or more plasmids and were resistant to commonly used antimicrobial agents (Khajanchi et al, 2016). Overall analysis of the whole genome sequences of these strains will improve our current understanding of the potential role of IncI1 plasmids on pathogenicity of *Salmonella enterica* isolated from various foods and hosts.

To conduct the sequencing, total DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). Nextera XT DNA sample kits (Illumina, San Diego, CA, USA) were used to construct DNA library. Sequencing reactions were carried out at the DNA Sequencing Core at the University of Arkansas Medical Sciences (UAMS, Little Rock, AR, USA) and the Division of Microbiology, National Center for Toxicological Research (NCTR, Jefferson, AR, USA) on an Illumina MiSeq to generate 2 X 250 (UAMS) or 2 X 300 (NCTR) paired-end reads. Trimming and *de novo* assembly of the paired-end reads was performed using CLC Genomic Workbench (Qiagen, Germantown, MD, USA; ver. 8.5.1 and 9). The Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al, 2008), the Pathosystems Resource Integration Center (PATRIC) (Wattam et al, 2014), and the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (Angiuoli et al, 2008) were employed to annotate the draft genomes of these strains (Table 1). The average G+C content of these strains is estimated to be 51.81% as examined by PATRIC. Table 1 enlists individual G+C content (%), number of contigs, coding sequences, and functional proteins for respective strains. Sequencing files obtained in FASTA format were analyzed using various bioinformatics tools that are described in Supplement A along with the results of these analyses.

Accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers listed in the Table 1.

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References

Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, and White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. Omics. 12:137-41.

Aziz RK., Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein ., Wilke A, and Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics. 9:75.

Centers for Disease Control and Prevention. 2012. National *Salmonella* surveillance overview. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/nationalsurveillance/salmonella_surveillance.html

Foley SL, Johnson TJ, Ricke SC, Nayak R, and Danzeisen J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev. 77:582-607.

Gokulan K, Khare S, Rooney AW, Han J, Lynne AM, and Foley SL. 2013. Impact of plasmids, including those encodingVirB4/D4 type IV secretion systems, on *Salmonella enterica* serovar Heidelberg virulence in macrophages and epithelial cells. PLoS One. 8:e77866.

Han J, David D, Deck J, Lynne AM, Kaldhone P, Nayak R, Stefanova R, and Foley SL. 2011. Comparison of *Salmonella enterica* serovar Heidelberg isolated from human patients with those from animal and food sources. J Clin Microbiol. 49:1130-33.

Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, Kaldhone P, Logue CM, and Foley SL. 2012. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PLoS ONE. 7**:**e51160.

Khajanchi BK, Han J, Gokulan K, Zhao S, Gies A, and Foley SL. 2016. Draft genome sequences of four *Salmonella enterica* strains isolated from turkey-associated sources. Genome Announc 4:e01122-16.

Lynne AM, Kaldhone P, David D, White DG, and Foley SL. 2009. Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. Foodborne Pathog Dis. 6:7-15.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, and Griffin PM. 2011. Foodborne illnesses acquired in the Unites States- major pathogens. Emerg Infect Dis. 17:7-15.

Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwartz S, Guerra B, Rodriguez I, Essen-Zandbergen AV, Brouwer M, and Mevis D. 2015. Characterization of epidemic IncI1-Iγ plasmids harboring ambler class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. Antimicrob Agents Chemother. 59:5357-65.

Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJ, Yoo HS, Zhang C, Zhang Y, and Sobral B. W. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. Nucleic Acids Res. 42:D581–D591.

Table 1. Summary of the genome sequence analysis of *Salmonella enterica* strains containing IncI1 plasmids.

Strain	Serovar	Source	Location, Year	Contigs	Assembly size (bp)	G+C content (%)	CDS	Functional proteins	Accession no.
SE142	Heidelberg	Swine	Indiana, 2002	205	5,197,369	51.85	5450	4683	NPFC00000000
SE143	Heidelberg	Swine	Minnesota, 2002	306	5,361,922	51.69	5718	4779	NPEL00000000
SE144	Heidelberg	Swine	Minnesota, 2009	111	5,279,737	51.82	5488	4673	NPEQ00000000
SE146	Heidelberg	Swine	Minnesota, 2002	221	5,356,597	51.59	5704	4801	NPEM00000000
SE36N	Typhimurium	Chicken	West Virginia, 2000	158	5,160,965	51.9	5358	4705	NPER00000000
SE89N	Kentucky	Poultry house water	West Virginia, 2000	120	5,146,652	51.69	5799	4811	NPES00000000
SE991	Heidelberg	Human feces	Arkansas, 2009	98	5,053,493	51.82	5161	4493	NPEP00000000
SE1148	Heidelberg	Human blood	Wisconsin, 2007	166	4,867,737	52.17	4995	4435	NPEO00000000

Note: CDS- coding sequences.

Supplement A

This supplement provides data obtained by using FASTA files from whole genome sequencing reactions described in the body of the chapter and analyzing them with various bioinformatics tools designed to genomic data analyses. The approaches and outcomes obtained from PlasmidFinder, ResFinder and PATRIC databases are described below.

Plasmid Replicon Type Detection: FASTA files from the assembled contigs described in Table 1 were uploaded into the PlasmidFinder database (Center for Genomic Epidemiology, Danish Technical University) to identify the predicted incompatibility (Inc) groups of the plasmids from WGS data based on their replication-associated sequences (Carattoli et al, 2014). The results of the replicon tying analyses are shown in **Supplemental Table 1.** The results shown in the table displays sequenced strain identity (Strain), the Inc groups of the plasmids detected (Plasmid), the percent identity to the reference sequence (Percent Identity), and the GenBank Accession Numbers of the plasmid reference sequence (Accession Number). The reference sequences for several of the different Inc groups is based on the targets used for PCR-based replicon typing and because some plasmids have multiple replicon-associated genes, there is the potential for a single plasmid to be identified as multiple plasmids in the PlasmidFinder output.

Antimicrobial Resistance Gene Detection: FASTA files from the assembled contigs described in Table 1 were uploaded into the ResFinder database (Center for Genomic Epidemiology, Danish Technical University) to identify the antimicrobial resistance genes that are present in the different strains (Zankari et al, 2012). The results of the detected antimicrobial resistance genes are shown in **Supplemental Table 2,** with results displayed show the identified resistance genes (Resistance Gene), percent identity to the reference gene in the database (Percent Identity), the

predicted resistance phenotype encode (Encodes Resistance to;) and the GenBank Accession Numbers of the reference genes (Accession Number).

Virulence Factor Detection: FASTA files from the assembled contigs were uploaded to the PATRIC Bacterial Bioinformatics Resource Center's database (Wattam et al, 2017) and the sequences were searched against the Virulence Factor Database (VFDB) (Chen et al, 2005) for the presence of predicted virulence factors. **Supplemental Tables 3A-H** describe the putative virulence factors for each of the strains sequenced by providing the corresponding VFDB identifier (Source ID), predicted gene product (Product) and the predicted functional classification of the gene product (Classification).

Supplement References

Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, and Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 58:3895-903.

Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, and Jin Q. 2005. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 33:D325-8.

Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, and Stevens RL. 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res. 45:D535-D542

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, and Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 67:2640-4.

Supplemental Table 1: Predicted plasmid incompatibility groups

Strain	Plasmid	%Identity	Accession Number
SE142	IncHI2A	99.52	BX664015
	IncA/C2	100	JN157804
	IncHI2	100	BX664015
	IncI1	100	AP005147
SE143	IncA/C2	100	JN157804
	IncI1	100	AP005147
	IncHI2	100	BX664015
	IncHI2A	99.52	BX664015
SE144	IncI1	97.89	AP005147
	IncA/C2	100	JN157804
	IncHI2	100	BX664015
	IncHI2A	99.52	BX664015
SE146	Col(BS512)	100	NC_010656
	IncHI2A	99.52	BX664015
	IncX4	100	CP002895
	IncI2	97.52	AP002527
	IncA/C2	100	JN157804
	IncHI2	100	BX664015
	IncI1	100	AP005147
SE36N	IncFIC(FII)	95.59	AP001918
	ColpVC	95.85	JX133088
	ColpVC	98.96	JX133088
	IncI1	100	AP005147
	IncX1	98.66	EU370913
	IncFII	95.42	AJ851089
	IncFIB	98.39	AP001918
SE89N	IncX1	100	CP001123
	ColpVC	96.64	JX133088
	IncHI2	100	BX664015
	IncHI2A	99.52	BX664015
	IncI1	100	AP005147
SE991	IncHI2	100	BX664015
	IncI1	99.3	AP005147
	IncHI2A	99.52	BX664015
SE1148	IncI1	100	AP005147

Supplemental Table 2: ResFinder prediction of resistance genes

Resistance Gene	Percent Identity	Encodes Resistance to:	Accession Number
SE142			
aph(3')-IIa	99.75	Aminoglycoside	X57709
strB	100	Aminoglycoside	M96392
aph(6)-Ic	100	Aminoglycoside	X01702
aadA1	99.75	Aminoglycoside	FJ591054
aac(3)-VIa	99.78	Aminoglycoside	M88012
strA	100	Aminoglycoside	AF321551
blaCMY-2	100	Beta-lactam	X91840
floR	98.27	Phenicol	AF118107
sul2	100	Sulphonamide	GQ421466
sul1	100	Sulphonamide	CP002151
tet(A)	100	Tetracycline	AJ517790
tet(B)	100	Tetracycline	AF326777
SE143			
aph(6)-Ic	100	Aminoglycoside	X01702
aph(3')-IIa	99.75	Aminoglycoside	X57709
aph(3')-Ia	100	Aminoglycoside	V00359
strA	100	Aminoglycoside	AF321551
aac(3)-VIa	99.51	Aminoglycoside	M88012
strB	100	Aminoglycoside	M96392
blaCMY-2	100	Beta-lactam	X91840
floR	98.35	Phenicol	AF118107
sul2	100	Sulphonamide	GQ421466
sul1	100	Sulphonamide	CP002151
tet(A)	100	Tetracycline	AJ517790
tet(B)	100	Tetracycline	AF326777
dfrA1	100	Trimethoprim	X00926
SE144			
dfrA12	100	Trimethoprim	AB571791
aadA1	100	Aminoglycoside	JQ414041
sul2	100	Sulphonamide	GQ421466
aph(3')-IIa	99.75	Aminoglycoside	X57709
mph(A)	100	Macrolide	D16251
aph(3')-Ia	100	Aminoglycoside	V00359
sul3	100	Sulphonamide	AJ459418

blaCMY-2	100	Beta-lactam	X91840
blaTEM-1B	100	Beta-lactam	JF910132
strB	100	Aminoglycoside	M96392
floR	98.35	Phenicol	AF118107
aadA2	100	Aminoglycoside	X68227
tet(A)	100	Tetracycline	AJ517790
sul1	100	Sulphonamide	AY224185
aph(6)-Ic	100	Aminoglycoside	X01702
tet(B)	100	Tetracycline	AF326777
strA	100	Aminoglycoside	M96392
cmlA1	99.92	Phenicol	M64556
aadB	100	Aminoglycoside	JN119852
SE146			
aadA2	100	Aminoglycoside	JQ364967
aph(6)-Ic	100	Aminoglycoside	X01702
aph(3')-IIa	99.75	Aminoglycoside	X57709
strB	100	Aminoglycoside	M96392
aph(3')-Ia	99.51	Aminoglycoside	V00359
strA	100	Aminoglycoside	AF321551
blaCMY-2	100	Beta-lactam	X91840
mph(A)	100	Macrolide	D16251
sul1	100	Sulphonamide	CP002151
sul2	100	Sulphonamide	GQ421466
tet(A)	100	Tetracycline	AJ517790
tet(B)	100	Tetracycline	AF326777
tet(M)	99.17	Tetracycline	U58985
dfrA12	100	Trimethoprim	AB571791
SE36N			
blaCMY-2	99.83	Beta-lactam	X91840
SE89N			
blaCMY-2	99.91	Beta-lactam	X91840
strB	100	Aminoglycoside	M96392
strA	100	Aminoglycoside	M96392
SE991			
aph(6)-Ic	100	Aminoglycoside	X01702
strB	100	Aminoglycoside	M96392
strA	100	Aminoglycoside	M96392

aph(3')-IIa	99.75	Aminoglycoside	X57709
tet(B)	100	Tetracycline	AF326777
SE1148			
aac(3)-VIa	99.78	Aminoglycoside	M88012
aadA1	100	Aminoglycoside	JQ414041
sul1	100	Sulphonamide	AY224185

Supplemental Table 3A: Virulence factors predicted for strain SE142

Source ID	Product	Classification
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0793	Mobile element protein	Protease, Autotransporter
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion

VFG1626	Mobile element protein	Adherence
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG0578	Integral membrane protein	Magnesium uptake
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0470	SifA protein	Secretion, Type III secretion system
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG1038	Right origin-binding protein	Protease, Serine protease
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system

VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG1031	Mobile element protein	Protease, Serine protease
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG1602	Mobile element protein	Adherence
VFG2322	Flagellin (FliC)	Secretion, Invasion, Motility
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG1698	Mobile element protein	Adherence
VFG0670	Bactoprenol glucosyl transferase	Endotoxin

VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0643	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1029	Tn21 protein of unknown function Urf2	Protease, Serine protease
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG1033	FIG00731654: hypothetical protein	Protease, Serine protease
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system

VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion

VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG1036	Tetracycline resistance, MFS efflux pump => Tet(B)	Protease, Serine protease
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial

VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0579	Agglutination protein	Magnesium uptake
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1654	Glycosyltransferase IroB	Adherence
VFG0480	Putative amino acid permease	Regulation
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG1583	D-serine permease DsdX	Adherence
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG1030	Mobile element protein	Protease, Serine protease
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system

VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG1037	Transcriptional regulator, AcrR family	Protease, Serine protease
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG1718	Transposase	Adherence
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system

VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0483	Putative ribokinase	Regulation
VFG1653	ABC transporter protein IroC	Adherence
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG1034	Transcriptional regulator, ArsR family	Protease, Serine protease
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG1035	Tetracycline resistance regulatory protein TetR	Protease, Serine protease

VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion

Supplemental Table 3B: Virulence factors predicted for strain SE143

Source ID	Product	Classification
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG1034	Transcriptional regulator, ArsR family	Protease, Serine protease
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0470	SifA protein	Secretion, Type III secretion system
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG1032	Chloramphenicol O-acetyltransferase (EC 2.3.1.28) => CatA1/CatA4 family	Protease, Serine protease
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake

VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG0480	Putative amino acid permease	Regulation
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG1037	Transcriptional regulator, AcrR family	Protease, Serine protease
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG1626	Mobile element protein	Adherence
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial

VFG2322	Flagellin (FliC)	Secretion, Invasion, Motility
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0483	Putative ribokinase	Regulation
VFG1031	Mobile element protein	Protease, Serine protease

VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG1035	Tetracycline resistance regulatory protein TetR	Protease, Serine protease
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system

VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export	Secretion, Type III secretion system, Invasion

	components); Surface presentation of antigens protein SpaO	
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1653	ABC transporter protein IroC	Adherence
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG1038	Right origin-binding protein	Protease, Serine protease
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0491	Transcriptional regulator STM1390	Regulation
VFG1654	Glycosyltransferase IroB	Adherence
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation

VFG1033	FIG00731654: hypothetical protein	Protease, Serine protease
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG1718	Transposase	Adherence
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG1030	Mobile element protein	Protease, Serine protease
VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0579	Agglutination protein	Magnesium uptake
VFG0578	Integral membrane protein	Magnesium uptake
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation

VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0483	Putative ribokinase	Regulation
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG1583	D-serine permease DsdX	Adherence
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore

VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG1036	Tetracycline resistance, MFS efflux pump => Tet(B)	Protease, Serine protease
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG1698	Mobile element protein	Adherence
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0793	Mobile element protein	Protease, Autotransporter
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG1030	Mobile element protein	Protease, Serine protease
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG1031	TnpA transposase	Protease, Serine protease
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion

VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation

Supplemental Table 3C: Virulence factors predicted for strain SE144

Source ID	Product	Classification
VFG0633	Transposase	Protease, Serine protease, Autotransporter
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG1035	Tetracycline resistance regulatory protein TetR	Protease, Serine protease
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG1038	Right origin-binding protein	Protease, Serine protease
VFG1034	Transcriptional regulator, ArsR family	Protease, Serine protease
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG1654	Glycosyltransferase IroB	Adherence
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides

VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG1030	Mobile element protein	Protease, Serine protease
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG1626	Mobile element protein	Adherence
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG0482	FIG074102: hypothetical protein	Regulation
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake

VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0491	Transcriptional regulator STM1390	Regulation
VFG1037	Transcriptional regulator, AcrR family	Protease, Serine protease
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0483	Putative ribokinase	Regulation
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of	Secretion, Type III secretion system, Invasion

	translocator pore); Cell invasion protein sipC (Effector protein SipC)	
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG0480	Putative amino acid permease	Regulation
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0582	Putative type-1 secretion protein	Magnesium uptake

VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0594	Putative dipeptidase	Magnesium uptake
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0581	Large repetitive protein	Magnesium uptake
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion

VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG1036	Tetracycline resistance, MFS efflux pump => Tet(B)	Protease, Serine protease
VFG1033	FIG00731654: hypothetical protein	Protease, Serine protease
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG0470	SifA protein	Secretion, Type III secretion system
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG1031	TnpA transposase	Protease, Serine protease
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG1698	Mobile element protein	Adherence
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG0453	Protein LpfD	Adherence, Fimbrial

VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0578	Integral membrane protein	Magnesium uptake
VFG0793	Mobile element protein	Protease, Autotransporter
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG1026	Aminoglycoside 3"-nucleotidyltransferase (EC 2.7.7.-) => APH(3")-Ia (AadA family)	Protease, Serine protease
VFG1653	ABC transporter protein IroC	Adherence
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system

VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG1583	D-serine permease DsdX	Adherence
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1718	Transposase	Adherence
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0579	Efflux transport system, outer membrane factor (OMF) lipoprotein	Magnesium uptake
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance

VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1029	BsuBI-PstI family restriction endonuclease (PF06616)	Protease, Serine protease
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0452	Protein LpfE	Adherence, Fimbrial

VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation

Supplemental Table 3D: Virulence factors predicted for strain SE146

Source ID	Product	Classification
VFG1626	Mobile element protein	Adherence
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0482	FIG074102: hypothetical protein	Regulation
VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG1030	Mobile element protein	Protease, Serine protease

VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG1583	D-serine permease DsdX	Adherence
VFG0793	Mobile element protein	Protease, Autotransporter
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG1654	Glycosyltransferase IroB	Adherence
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system

VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG1718	Transposase	Adherence

VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG1033	FIG00731654: hypothetical protein	Protease, Serine protease
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0480	Putative amino acid permease	Regulation
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system

VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG1037	Transcriptional regulator, AcrR family	Protease, Serine protease
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin

VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0578	Integral membrane protein	Magnesium uptake
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG0470	SifA protein	Secretion, Type III secretion system
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion

VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0483	Putative ribokinase	Regulation
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG1698	Mobile element protein	Adherence
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG1031	TnpA transposase	Protease, Serine protease
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG1034	Transcriptional regulator, ArsR family	Protease, Serine protease
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system

VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG1036	Tetracycline resistance, MFS efflux pump => Tet(B)	Protease, Serine protease
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG1038	Right origin-binding protein	Protease, Serine protease
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion

VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1035	Tetracycline resistance regulatory protein TetR	Protease, Serine protease
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial

VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG0579	Agglutination protein	Magnesium uptake
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG1653	ABC transporter protein IroC	Adherence
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion

Supplemental Table 3E: Virulence factors predicted for strain SE36N

Source ID	Product	Classification
VFG1513	Mobile element protein	Invasion, Antiphagocytosis
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG1728	Mobile element protein	Adherence
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0578	Integral membrane protein	Magnesium uptake
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG1485	Transposase InsN for insertion sequence element IS911	Invasion, Antiphagocytosis
VFG0483	Putative ribokinase	Regulation
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0488	Tetrathionate reductase two-component response regulator	Regulation

VFG1691	Mobile element protein	Adherence
VFG0579	Agglutination protein	Magnesium uptake
VFG1626	Mobile element protein	Adherence
VFG1627	Mobile element protein	Adherence
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0582	Type I secretion system ATPase, LssB family LapB	Magnesium uptake
VFG0785	Transposase InsO for insertion sequence element IS911	Protease, Autotransporter
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0612	IS1 protein InsB	Adherence, Nonfimbrial
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0937	L-lysine 6-monooxygenase [NADPH] (EC 1.14.13.59), aerobactin biosynthesis protein IucD Siderophore biosynthesis protein, monooxygenase	Iron uptake, Siderophore
VFG0612	IS1 protein InsB	Adherence, Nonfimbrial
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG1665	Mobile element protein	Adherence
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system

VFG0938	Aerobactin synthase (EC 6.3.2.39), aerobactin biosynthesis protein IucC	Iron uptake, Siderophore
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG1729	Mobile element protein	Adherence
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0939	N6-hydroxylysine O-acetyltransferase (EC 2.3.1.102), aerobactin biosynthesis protein IucB Siderophore synthetase small component, acetyltransferase	Iron uptake, Siderophore
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0784	Transposase InsN for insertion sequence element IS911	Protease, Autotransporter

VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG1565	Mobile element protein	Adherence
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG0627	putative DNA helicases	Iron uptake, Siderophore
VFG0643	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0614	Possible H+-antiporter clustered with aerobactin genes	Adherence, Nonfimbrial
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion

VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG1717	Mobile element protein	Adherence
VFG0605	Mobile element protein	Adherence, Nonfimbrial
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation

VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG0480	Putative amino acid permease	Regulation
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial

VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG1583	D-serine permease DsdX	Adherence
VFG0612	IS1 protein InsB	Adherence, Nonfimbrial
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0785	Transposase InsO for insertion sequence element IS911	Protease, Autotransporter
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion

VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0581	Large repetitive protein	Magnesium uptake
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0638	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0936	Aerobactin siderophore receptor IutA TonB-dependent siderophore receptor	Iron uptake, Siderophore
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility

VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG0785	Transposase InsO for insertion sequence element IS911	Protease, Autotransporter
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG1654	Glycosyltransferase IroB	Adherence
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG0451	putative fimbrial protein	Adherence, Fimbrial
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0648	Transposase InsC for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0670	Bactoprenol glucosyl transferase	Endotoxin

VFG0470	SifA protein	Secretion, Type III secretion system
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG1445	IncF plasmid conjugative transfer regulator TraJ	Invasion
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0940	N(2)-citryl-N(6)-acetyl-N(6)-hydroxylysine synthase (EC 6.3.2.38), aerobactin biosynthesis protein IucA	Iron uptake, Siderophore
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion

VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system

Supplemental Table 3F: Virulence factors predicted for strain SE89N

Source ID	Product	Classification
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0480	Putative amino acid permease	Regulation
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG1074	Mobile element protein	Protease, Serine protease
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0612	IS1 protein InsB	Adherence, Nonfimbrial
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system

VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG1665	Mobile element protein	Adherence
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG0579	Agglutination protein	Magnesium uptake
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0648	Transposase InsC for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of	Secretion, Type III secretion system, Invasion

	translocator pore); Cell invasion protein sipC (Effector protein SipC)	
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG1082	N6-hydroxylysine O-acetyltransferase (EC 2.3.1.102), aerobactin biosynthesis protein IucB Siderophore synthetase small component, acetyltransferase	Protease, Serine protease
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG1732	Transposase InsD for insertion element IS2	Adherence
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG1732	Transposase InsD for insertion element IS2	Adherence
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG1654	Glycosyltransferase IroB	Adherence
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion

VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0578	Integral membrane protein	Magnesium uptake
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG2362	Phosphomannomutase (EC 5.4.2.8) => Colanic acid	
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0648	Transposase InsC for insertion element IS2	Toxin, A-B type, Enterotoxin

VFG0483	Putative ribokinase	Regulation
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG0793	Mobile element protein	Protease, Autotransporter
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0940	N(2)-citryl-N(6)-acetyl-N(6)-hydroxylysine synthase (EC 6.3.2.38), aerobactin biosynthesis protein IucA	Iron uptake, Siderophore
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG1627	Mobile element protein	Adherence
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG1504	Co-activator of prophage gene expression IbrA	Invasion, Antiphagocytosis
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin

VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0614	Possible H+-antiporter clustered with aerobactin genes	Adherence, Nonfimbrial
VFG1698	Mobile element protein	Adherence
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG1733	Transposase InsC for insertion element IS2	Adherence
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0581	Large repetitive protein	Magnesium uptake
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility

VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0938	Aerobactin synthase (EC 6.3.2.39), aerobactin biosynthesis protein IucC	Iron uptake, Siderophore
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG1485	Transposase InsN for insertion sequence element IS911	Invasion, Antiphagocytosis
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG1513	Mobile element protein	Invasion, Antiphagocytosis
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG1484	Transposase InsO for insertion sequence element IS911	Invasion, Antiphagocytosis
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG1732	Transposase InsD for insertion element IS2	Adherence
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion

VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB @ Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG1505	Co-activator of prophage gene expression IbrB	Invasion, Antiphagocytosis
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0936	Aerobactin siderophore receptor IutA TonB-dependent siderophore receptor	Iron uptake, Siderophore
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion

VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG1658	Mobile element protein	Adherence
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1664	Mobile element protein	Adherence
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial

VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG1626	Mobile element protein	Adherence
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG1513	Mobile element protein	Invasion, Antiphagocytosis
VFG0618	L-lysine 6-monooxygenase [NADPH] (EC 1.14.13.59), aerobactin biosynthesis protein IucD Siderophore biosynthesis protein, monooxygenase	Iron uptake, Siderophore
VFG1583	D-serine permease DsdX	Adherence
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0470	SifA protein	Secretion, Type III secretion system
VFG0486	Tetrathionate reductase subunit B	Regulation

VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG1732	Transposase InsD for insertion element IS2	Adherence
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG1513	Mobile element protein	Invasion, Antiphagocytosis
VFG0649	Transposase InsD for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG1665	Mobile element protein	Adherence
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG1653	ABC transporter protein IroC	Adherence
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion

Supplemental Table 3G: Virulence factors predicted for strain SE991

Source ID	Product	Classification
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG1626	Mobile element protein	Adherence
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG0470	SifA protein	Secretion, Type III secretion system
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake

VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG1037	Transcriptional regulator, AcrR family	Protease, Serine protease
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0639	Retron-type RNA-directed DNA polymerase (EC 2.7.7.49)	Toxin, A-B type, Enterotoxin
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0578	Integral membrane protein	Magnesium uptake
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0581	Large repetitive protein	Magnesium uptake
VFG1583	D-serine permease DsdX	Adherence
VFG1698	Mobile element protein	Adherence
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG1038	Right origin-binding protein	Protease, Serine protease
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG1654	Glycosyltransferase IroB	Adherence
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0583	Uncharacterized protein YjcB	Magnesium uptake

VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0480	Putative amino acid permease	Regulation
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB @ Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG0579	Agglutination protein	Magnesium uptake
VFG0621	Transposase InsD for insertion element IS2	Iron uptake, Siderophore
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion
VFG0647	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion

VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0605	Mobile element protein	Adherence, Nonfimbrial
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system

VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG1653	ABC transporter protein IroC	Adherence
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG1033	FIG00731654: hypothetical protein	Protease, Serine protease
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0594	Putative dipeptidase	Magnesium uptake
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG0590	Putative inner membrane protein	Magnesium uptake
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion

VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0648	Transposase InsC for insertion element IS2	Toxin, A-B type, Enterotoxin
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system

VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG1627	Mobile element protein	Adherence
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG1036	Tetracycline resistance, MFS efflux pump => Tet(B)	Protease, Serine protease
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0643	Mobile element protein	Toxin, A-B type, Enterotoxin
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG0793	Mobile element protein	Protease, Autotransporter
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG1718	Transposase	Adherence
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion

VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion

VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG1035	Tetracycline resistance regulatory protein TetR	Protease, Serine protease
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG1602	Mobile element protein	Adherence
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG0451	Fimbriae W protein	Adherence, Fimbrial
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG1633	Bacteriocin/lantibiotic efflux ABC transporter, permease/ATP-binding protein	Adherence
VFG0483	Putative ribokinase	Regulation
VFG1034	Transcriptional regulator, ArsR family	Protease, Serine protease
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system

VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility

Supplemental Table 3H: Virulence factors predicted for strain SE1148

Source ID	Product	Classification
VFG0521	Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0562	DNA mismatch repair protein MutS	Secretion, Type III secretion system, Invasion
VFG0510	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system
VFG2319	RNA polymerase sigma factor for flagellar operon	Secretion, Invasion, Motility
VFG0592	FIG01045843: hypothetical protein	Magnesium uptake
VFG0575	Mg(2+)-transport-ATPase-associated protein MgtC	Magnesium uptake
VFG0460	Curli production assembly/transport component CsgE	Adherence, Fimbrial
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0490	COG1683: Uncharacterized conserved protein / FIG143828: Hypothetical protein YbgA	Regulation
VFG0548	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components); Surface presentation of antigens protein SpaS	Secretion, Type III secretion system, Invasion
VFG0482	FIG074102: hypothetical protein	Regulation
VFG0584	c-di-GMP phosphodiesterase (EC 3.1.4.52) => PdeC	Magnesium uptake
VFG2331	Flagellum-specific ATP synthase FliI	Secretion, Invasion, Motility
VFG1631	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence
VFG0519	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components)	Secretion, Type III secretion system
VFG1718	Transposase	Adherence
VFG0585	DNA-binding transcriptional dual regulator SoxS	Magnesium uptake
VFG2346	Flagellar basal-body rod protein FlgG	Secretion, Invasion, Motility

VFG0453	Protein LpfD	Adherence, Fimbrial
VFG0550	Type III secretion inner membrane protein (YscS, homologous to flagellar export components); Surface presentation of antigens protein SpaQ	Secretion, Type III secretion system, Invasion
VFG2358	Flagellar transcriptional activator FlhC	Secretion, Invasion, Motility
VFG0537	Type III secretion transcriptional regulator HilD	Secretion, Type III secretion system, Invasion
VFG0581	Large repetitive protein	Magnesium uptake
VFG0541	Chaperone protein SicP	Secretion, Type III secretion system, Invasion
VFG0452	Protein LpfE	Adherence, Fimbrial
VFG0499	Type III secretion effector SseB	Secretion, Type III secretion system
VFG0448	Uncharacterized fimbrial-like protein SfmF	Adherence, Fimbrial
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0551	Type III secretion inner membrane protein (YscR, SpaR, HrcR, EscR, homologous to flagellar export components); Surface presentation of antigens protein SpaP	Secretion, Type III secretion system, Invasion
VFG0517	Type III secretion protein (YscP)	Secretion, Type III secretion system
VFG2345	Flagellar L-ring protein FlgH	Secretion, Invasion, Motility
VFG0458	Major curlin subunit precursor CsgA	Adherence, Fimbrial
VFG0538	Type III secretion transcriptional activator HilA	Secretion, Type III secretion system, Invasion
VFG0508	Type III secretion protein SsaH	Secretion, Type III secretion system
VFG1440	Phosphoethanolamine transferase EptC	Invasion
VFG0545	Type III secretion negative modulator of injection (YopK, YopQ, controls size of translocator pore); Cell invasion protein sipC (Effector protein SipC)	Secretion, Type III secretion system, Invasion
VFG0552	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components); Surface presentation of antigens protein SpaO	Secretion, Type III secretion system, Invasion
VFG0502	Secretion system effector SseD	Secretion, Type III secretion system

VFG0925	Ferric enterobactin transport ATP-binding protein FepC (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0488	Tetrathionate reductase two-component response regulator	Regulation
VFG0486	Tetrathionate reductase subunit B	Regulation
VFG0511	Type III secretion protein SsaK	Secretion, Type III secretion system
VFG0462	Curli production assembly/transport component CsgG	Adherence, Fimbrial
VFG0472	Sensor protein BasS (activates BasR)	Secretion, Type III secretion system
VFG0477	RNA polymerase sigma factor RpoS	Regulation, Sigma factor
VFG1443	Outer membrane protein A precursor	Invasion, Serum resistance
VFG0443	Uncharacterized fimbrial-like protein SfmA	Adherence, Fimbrial
VFG0455	Chaperone protein LpfB	Adherence, Fimbrial
VFG0564	ATP-binding protein	Secretion, Type III secretion system, Invasion
VFG0491	Transcriptional regulator STM1390	Regulation
VFG0542	Acyl carrier protein	Secretion, Type III secretion system, Invasion
VFG0557	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV); Invasion protein InvA	Secretion, Type III secretion system, Invasion
VFG0544	Type III secretion host injection protein (YopB); Cell invasion protein SipD (Salmonella invasion protein D)	Secretion, Type III secretion system, Invasion
VFG0549	Surface presentation of antigens protein SpaR; Type III secretion inner membrane protein (YscT, HrcT, SpaR, EscT, EpaR1, homologous to flagellar export components)	Secretion, Type III secretion system, Invasion
VFG0601	Transposase InsF for insertion sequence IS3 Mobile element protein	Adherence, Nonfimbrial
VFG0470	SifA protein	Secretion, Type III secretion system
VFG2338	Flagellar biosynthesis protein FliP	Secretion, Invasion, Motility
VFG0449	Transcriptional regulator of fimbriae expression FimZ (LuxR/UhpA family)	Adherence, Fimbrial
VFG0484	Tetrathionate reductase subunit A	Regulation
VFG1653	ABC transporter protein IroC	Adherence

VFG0554	Surface presentation of antigens protein SpaM	Secretion, Type III secretion system, Invasion
VFG0933	Isochorismatase (EC 3.3.2.1) [enterobactin] siderophore / Apo-aryl carrier domain of EntB Isochorismatase (EC 3.3.2.1) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0932	2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) [enterobactin] siderophore 2, 3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0580	Putative type-I secretion protein	Magnesium uptake
VFG1654	Glycosyltransferase IroB	Adherence
VFG2329	Flagellar motor switch protein FliG	Secretion, Invasion, Motility
VFG0547	Chaperone protein SicA (Salmonella invasin chaperone)	Secretion, Type III secretion system, Invasion
VFG0524	Formate hydrogenlyase transcriptional activator	Secretion, Type III secretion system
VFG0586	Redox-sensitive transcriptional activator SoxR	Magnesium uptake
VFG0533	Type III secretion bridge between inner and outermembrane lipoprotein (YscJ, HrcJ, EscJ, PscJ)	Secretion, Type III secretion system, Invasion
VFG2356	Flagellar biosynthesis protein FlhA	Secretion, Invasion, Motility
VFG1582	D-serine ammonia-lyase (EC 4.3.1.18)	Adherence
VFG2307	adherence and invasion outermembrane protein (Inv, enhances Peyer's patches colonization)	Adherence, Nonfimbrial
VFG1029	Tn21 protein of unknown function Urf2	Protease, Serine protease
VFG1650	Outer Membrane Siderophore Receptor IroN	Adherence
VFG0576	Single-stranded DNA-binding protein	Magnesium uptake
VFG0931	Isochorismate synthase (EC 5.4.4.2) [enterobactin] siderophore @ Isochorismate synthase (EC 5.4.4.2) of siderophore biosynthesis	Iron uptake, Siderophore
VFG1030	Mobile element protein	Protease, Serine protease
VFG0468	leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0589	Pentapeptide repeat family protein	Magnesium uptake
VFG0495	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG)	Secretion, Type III secretion system
VFG0500	Secretion system chaparone SscA	Secretion, Type III secretion system

VFG0539	Invasion protein IagB precursor	Secretion, Type III secretion system, Invasion
VFG0574	Mg(2+) transport ATPase, P-type (EC 3.6.3.2)	Magnesium uptake
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0444	Fimbriae-like adhesin FimI	Adherence, Fimbrial
VFG1031	Mobile element protein	Protease, Serine protease
VFG0459	Putative curli production protein CsgC	Adherence, Fimbrial
VFG0570	Putative transcriptional regulator MarT	Secretion, Type III secretion system, Invasion
VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0504	Secretion system chaparone SscB	Secretion, Type III secretion system
VFG2302	Transcriptional regulator	Resistance to antimicrobial peptides
VFG0509	Type III secretion protein SsaI	Secretion, Type III secretion system
VFG0530	SPI1-associated transcriptional regulator SprB	Secretion, Type III secretion system, Invasion
VFG0513	Type III secretion protein SsaM	Secretion, Type III secretion system
VFG0471	Secreted effector J SseJ	Secretion, Type III secretion system
VFG0556	Type III secretion system protein BsaR; Surface presentation of antigens protein SpaK (Invasion protein InvB)	Secretion, Type III secretion system, Invasion
VFG2306	Putative outer membrane protein	Adherence, Nonfimbrial
VFG1584	D-serine dehydratase transcriptional activator	Adherence
VFG0934	2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) [enterobactin] siderophore 2, 3-dihydro-2, 3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) of siderophore biosynthesis	Iron uptake, Siderophore
VFG0483	Putative ribokinase	Regulation
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0498	Secretion system effector SseA	Secretion, Type III secretion system
VFG0532	OrgB protein, associated with InvC ATPase of type III secretion system	Secretion, Type III secretion system, Invasion

VFG0457	Minor curlin subunit CsgB, nucleation component of curlin monomers	Adherence, Fimbrial
VFG0534	Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0496	Type III secretion protein SsaD	Secretion, Type III secretion system
VFG0518	Type III secretion inner membrane protein (YscQ, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0489	Uncharacterized protein YdhZ	Regulation
VFG0450	Transcriptional regulator of fimbriae expression FimY	Adherence, Fimbrial
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG0559	Type III secretion outermembrane pore forming protein (YscC, MxiD, HrcC, InvG); Protein InvG precursor	Secretion, Type III secretion system, Invasion
VFG0461	Curli production assembly/transport component CsgF	Adherence, Fimbrial
VFG0579	Agglutination protein	Magnesium uptake
VFG0565	FIG01046146: hypothetical protein	Secretion, Type III secretion system, Invasion
VFG0487	Tetrathionate reductase sensory transduction histidine kinase	Regulation
VFG0479	Pyruvate kinase (EC 2.7.1.40)	Regulation
VFG0516	Type III secretion spans bacterial envelope protein (YscO)	Secretion, Type III secretion system
VFG0577	Putative inner membrane or exported protein	Magnesium uptake
VFG0555	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN); Probable ATP synthase SpaL (EC 3.6.3.14) (Invasion protein InvC)	Secretion, Type III secretion system, Invasion
VFG0928	Ferric enterobactin transport system permease protein FepG (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0543	Type III secretion injected virulence protein (YopE)	Secretion, Type III secretion system, Invasion
VFG1028	Integron integrase IntI1	Protease, Serine protease
VFG0528	Manganese ABC transporter, inner membrane permease protein SitD	Secretion, Type III secretion system
VFG0480	Putative amino acid permease	Regulation

VFG0553	Type III secretion host injection and negative regulator protein (YopD); Surface presentation of antigens protein SpaN (Invasion protein InvJ)	Secretion, Type III secretion system, Invasion
VFG0569	YqeJ protein	Secretion, Type III secretion system, Invasion
VFG0514	Type III secretion inner membrane channel protein (LcrD, HrcV, EscV, SsaV)	Secretion, Type III secretion system
VFG2335	Flagellar motor switch protein FliM	Secretion, Invasion, Motility
VFG0454	Outer membrane usher protein LpfC	Adherence, Fimbrial
VFG0536	MxiG protein; Pathogenicity 1 island effector protein	Secretion, Type III secretion system, Invasion
VFG0512	Type III secretion cytoplasmic protein (YscL)	Secretion, Type III secretion system
VFG0526	Manganese ABC transporter, ATP-binding protein SitB	Secretion, Type III secretion system
VFG0474	Sensor histidine kinase PhoQ (EC 2.7.13.3)	Regulation, Two-component system
VFG0595	Putative two component system histidine kinase YedV	Magnesium uptake
VFG0583	Uncharacterized protein YjcB	Magnesium uptake
VFG0505	Type III secretion effector SseF	Secretion, Type III secretion system
VFG0475	Transcriptional regulatory protein PhoP	Regulation, Two-component system
VFG0591	Invasion gene E protein (Pathogenicity island encoded protein: homologous to ipgE of Shigella)	Magnesium uptake
VFG0466	Secreted protein	Secretion, Type III secretion system, Invasion
VFG0515	Type III secretion cytoplasmic ATP synthase (EC 3.6.3.14, YscN, SpaL, MxiB, HrcN, EscN)	Secretion, Type III secretion system
VFG0567	RmbA	Secretion, Type III secretion system, Invasion
VFG0492	Secretion system regulator of DegU/UvrY/BvgA type	Regulation
VFG1026	Aminoglycoside 3''-nucleotidyltransferase (EC 2.7.7.-) => APH(3'')-Ia (AadA family)	Protease, Serine protease
VFG0478	Ferric uptake regulation protein FUR	Regulation
VFG0445	Probable fimbrial chaperone SfmC	Adherence, Fimbrial

VFG0467	G-nucleotide exchange factor SopE	Secretion, Type III secretion system, Invasion
VFG0923	TonB-dependent receptor; Outer membrane receptor for ferric enterobactin and colicins B, D	Iron uptake, Siderophore
VFG0456	Long polar fimbria protein A	Adherence, Fimbrial
VFG1583	D-serine permease DsdX	Adherence
VFG0603	Transposase InsE for insertion sequence IS3E	Adherence, Nonfimbrial
VFG2305	AIDA autotransporter-like protein	Adherence, Nonfimbrial, Autotransporter
VFG0469	Leucine-rich repeat protein	Secretion, Type III secretion system, Invasion
VFG0463	Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)	Stress protein
VFG0447	Uncharacterized fimbrial-like protein SfmH	Adherence, Fimbrial
VFG0566	Uncharacterized protein clustered with Type I restriction-modification system	Secretion, Type III secretion system, Invasion
VFG0529	Type III secretion injected virulence protein (YopP, YopJ, induces apoptosis, prevents cytokine induction, inhibits NFkb activation)	Secretion, Type III secretion system, Invasion
VFG2350	Flagellar basal-body rod protein FlgC	Secretion, Invasion, Motility
VFG0594	Putative dipeptidase	Magnesium uptake
VFG0473	Two-component transcriptional regulatory protein BasR (activated by BasS)	Secretion, Type III secretion system
VFG0535	MxiH protein; Type III secretion cytoplasmic protein (YscF)	Secretion, Type III secretion system, Invasion
VFG0503	Secretion system effector SseE	Secretion, Type III secretion system
VFG0926	Ferric enterobactin transport system permease protein FepD (TC 3.A.1.14.2)	Iron uptake, Siderophore
VFG0525	Manganese ABC transporter, periplasmic-binding protein SitA	Secretion, Type III secretion system
VFG0506	Type III secretion effector SseG	Secretion, Type III secretion system
VFG0465	Inositol phosphate phosphatase sopB (EC 3.1.3)	Secretion, Type III secretion system, Invasion
VFG0527	Manganese ABC transporter, inner membrane permease protein SitC	Secretion, Type III secretion system
VFG0446	Outer membrane usher protein SfmD	Adherence, Fimbrial
VFG0546	Cell invasion protein SipB	Secretion, Type III secretion system, Invasion

VFG0540	Type III secretion injected virulence protein (YopH, tyrosine phosphatase of FAK and p130cas, prevents phagocytosis)	Secretion, Type III secretion system, Invasion
VFG0494	Type III secretion protein SsaB	Secretion, Type III secretion system
VFG0531	Type III secretion transcriptional regulator HilC (= SirC)	Secretion, Type III secretion system, Invasion
VFG0493	Secretion system regulator: Sensor component (EC 2.7.3.-) (EC 2.7.1.40)	Regulation
VFG0572	Nicotinamidase family protein YcaC	Secretion, Type III secretion system, Invasion
VFG2304	autotransporter	Adherence, Nonfimbrial, Autotransporter
VFG0670	Bactoprenol glucosyl transferase	Endotoxin
VFG0481	Proline iminopeptidase (EC 3.4.11.5)	Regulation
VFG0596	Putative two-component system response regulator YedW	Magnesium uptake
VFG0522	Type III secretion inner membrane protein (YscU, SpaS, EscU, HrcU, SsaU, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0507	Type III secretion protein SsaG	Secretion, Type III secretion system
VFG0582	Putative type-1 secretion protein	Magnesium uptake
VFG0588	Pathogenicity island encoded protein: SPI3	Magnesium uptake
VFG0501	Secretion system effector SseC	Secretion, Type III secretion system
VFG1511	ISSod13, transposase	Invasion, Antiphagocytosis
VFG0558	Type III secretion outermembrane contact sensing protein (YopN, Yop4b, LcrE); Invasion protein InvE	Secretion, Type III secretion system, Invasion
VFG0464	secreted effector protein	Secretion, Type III secretion system, Invasion
VFG0520	Type III secretion inner membrane protein (YscS, homologous to flagellar export components)	Secretion, Type III secretion system
VFG0560	Type III secretion thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid)	Secretion, Type III secretion system, Invasion
VFG0578	Integral membrane protein	Magnesium uptake
VFG0497	Secretion system effector SsaE	Secretion, Type III secretion system

VFG0573	Putative inner membrane protein	Secretion, Type III secretion system, Invasion
VFG0485	Tetrathionate reductase subunit C	Regulation
VFG0561	Invasion protein invH precursor	Secretion, Type III secretion system, Invasion
VFG0451	Fimbriae W protein	Adherence, Fimbrial

VI. Chapter 5

Virulence Evaluation of Incompatibility group 1 (IncI1) Plasmids Containing *Salmonella enterica*

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Abstract

Salmonella enterica is a leading foodborne pathogen in the United States. Mobile genetic elements such as plasmids can potentially increase their ability to infect and persist in to hosts. IncI1 plasmids are widely distributed in food animal sources and associated with clinically important strains. We have evaluated potential virulence of *Salmonella* isolates from human and animal sources using the Caco-2 human intestinal epithelial cell culture model. Transconjugants were generated in a two-step process to evaluate virulence potential of transferred IncI1 plasmids. First, IncI1 plasmids were transferred from *Salmonella* isolates to an *Escherichia coli* recipient and their plasmid content assessed. Plasmids from the *E. coli* transconjugants were subsequently transferred back into a *Salmonella* to assess the impact of the plasmids on invasion and persistence within the Caco-2 cell line. The Caco-2 cells were grown to confluence, and were infected with representative *Salmonella* isolates in media with and without antimicrobial agents and incubated for both one and 48 hours for the invasion and persistence assays, respectively. All isolates, wild-type and *Salmonella* transconjugants infected Caco-2 cells after one hour incubation and were able to persist in the cells at 48 hrs. In some cases, persistent cell counts were observed to be one log higher than invasion assay cell counts among our isolates. Most *Salmonella* transconjugants showed persistence greater than that of recipient. In the presence of antimicrobial agents reduction in both invasion and persistence was noted in most cases. In this study virulence potential was assessed and IncI1 plasmids appear to contribute to the overall ability of *Salmonella* to invade and persist in the intestinal epithelial cell model. This study lay the foundation for additional studies to refine the contribution of the specific plasmid-associated genes to virulence.

Introduction

Salmonella enterica is predicted to cause more than one million enteric infections resulting in 400 deaths per year in the U.S. (Scallan et al, 2011). Most *Salmonella* infections are self-limiting and resolve by themselves (Acheson et al, 2001). In some cases, such as co-infection with other bacteria, infection of an immunocompromised host and/or infection by highly virulent strains of *Salmonella* can lead to a fatal infection (Gordon, 2008). The economic impact of all *Salmonella* infections has been estimated to be up to 9 billion dollars due to the cost of treatment, loss of wages, and quality of life (Scharff, 2010). Most of the *Salmonella* infections are enteric in nature and foodborne salmonellosis is an important economical and public health concern (Ricke et al, 2017). The origin of foodborne salmonellosis can be eggs, poultry products, meat and fresh produce (Howard et al, 2012, Finstand et al, 2012, Foley et al, 2013, Ricke et al, 2017). *Salmonella* is widespread in poultry and other food animals (Dunkley et al, 2009).

Salmonella maintains its high presence in diverse hosts thorough genetic plasticity (Jakočiūnė et al, 2014). Genetic plasticity allows *Salmonella* to change its genetic composition to adapt to changing environmental conditions. This plasticity can be achieved with help of mobile genetic elements such as plasmids. Plasmids that have been characterized in *Salmonella* are known to carry genes associated with increased antimicrobial resistance and virulence for their hosts (Pulcrano et al, 2016, Martinez et al, 2002). A bacterium can receive plasmids from other bacterium, which leads to the potential for rapid spread of genes among bacteria in a relatively short duration. If these genes influence phenotypic characteristics such as antimicrobial resistance and virulence, then plasmid-mediated spread of genetic determinants

could be vital to the management of foodborne illnesses arising from *Salmonella* (Foley et al, 2013).

Plasmids can be grouped based on their incompatibility to co-exist (Sanad et al, 2016). Incompatibility based typing methods are based on phenomena that prevents coexistence of plasmids with the same replication and division mechanisms in the same bacterium (Han et al, 2012). Incompatibility group 1 (IncI1) plasmids are commonly found in food animal sources and associated with clinically relevant strains. They are known for their potential to carry and disseminate antimicrobial resistance and virulence genes among enteric pathogens (Mo et al, 2017, Wong et al, 2016). Dissemination of genes encoding resistance to ceftriaxone, an antimicrobial agent used in management of severe *Salmonella* infections has been reported in several strains (Smith et al, 2015). Many of these strains are reported to have IncI1 plasmids carrying genes responsible for the resistance.

Similarly, the spread of virulence-related genes could lead to *Salmonella*-related illnesses difficult to manage. Genes potentially associated with virulence have been identified on IncI1 plasmids (Johnson et al, 2002) however, very few, if any studies have been conducted to directly evaluate IncI1 plasmids and their virulence potential in *Salmonella*. This information should help to understand role of IncI1 plasmids in the virulence in *Salmonella*. Thus, the objective of this study was to assess to the impact of IncI1 plasmids on the ability of *Salmonella* to invade and persist human in intestinal epithelial cells.

Material and Methods

Bacterial isolates: Thirty-two *Salmonella enterica* isolates carrying IncI1 plasmids were selected for this study from a larger set of previously characterized IncI1-positive isolates that were evaluated for antimicrobial resistance, conjugal transfer ability and ability to inhibit the growth of other bacteria (Kaldhone et al, 2017). Isolates selected belong to serovars Heidelberg (n=11, 34%), Typhimurium (n=9, 28%), Newport (n=6, 18%), Kentucky (n=4, 13%) and Anatum and Infantis (n=1,3%) (Table 1). Isolates originated from poultry (n=15, 46%), swine (n=7, 21%), cattle (n=6, 18%), and human patients (n=4, 13%) with in the U.S. from 1999 to 2009. *E. coli* isolate J53 (Jacoby and Han, 1996, Yi et al, 2012) and *Salmonella enterica* serovar Newport isolate 1087 (Marrero-Ortiz et al, 2012) was used as recipients for conjugation reactions, both recipients are IncI1 plasmid negative.

Conjugation: The first sets of transconjugants were obtained by using *E. coli* J53 as a recipient as described in Kaldhone et al. (2017). These *E. coli* recipients were used as donors for transfer experiments into 1087. Recipient and donor were grown separately in LB broth overnight. The recipient and donor were subsequently mixed together in 1:1 proportion and centrifuged to obtain the pellet. The pellets were dispersed in 250 µl of LB broth and spotted on to LB agar plates. The plates were incubated for 5 hrs at 37°C in upright position. The growth seen was dissolved in 1 ml PBS and 100 µl of cell suspension was plated on to LB agar selection plates containing ampicillin (32 µg/mL), gentamicin (10 µg/mL) or streptomycin (32 µg/mL). After overnight incubation, single colonies were picked up and streaked onto MacConkey selective agar plates (Remel, Lenexa, KS) and incubated overnight at 37°C. Resultant *Salmonella* colonies were picked further studies.

PCR: PCR reactions were carried out to determine the replicon types of transconjugants (Carattoli et al, 2005). Transconjugants were streaked on LB agar plates and incubated overnight at 37ºC. Three to four colonies were collected from LB agar plates and suspended in 200 µl of sterile water. Cells were lysed by boiling method (Wang et al, 2015). PCR reactions included 12.5 µl of 2X Master Mix (Promega, Madison, WI), 2.5µl of each primer (10 pmol) (Carattoli et al, 2005), 3 µl template and 4.5 µl sterile water and were amplified with the following steps: denaturation at 94°C for 5 mins, 30 cycles of denaturation at 94°C for 30 secs, annealing at 60°C for 30 secs, and extension at 72°C for 90 secs, and a final extension at 72°C for 7 mins**.** The resulting PCR products were separated on 2% E-gels (Invitrogen, Carlsbad, CA) and visualized under UV-light using a Gel-Doc XR system (Bio-Rad, Hercules, CA).

Tissue Culture: A flow diagram describing the tissue culture experiments is shown in Figure 1. Caco-2 cells were grown in Modified Eagle Medium (MEM) supplemented with 10% FBS, 1% of Pen/Strep/Amphotericin B, amino acids, and Glutamax. Cells were grown in a 37ºC incubator with 5% CO_2 atmosphere. Prior to infection, *Salmonella* isolates were grown in LB broth overnight. The following day, the optical density of cell suspension was measured at 600 nm and the predicted number of bacteria was calculated. Caco-2 cells were trypsin treated and dispersed into 24-well culture plates and grown to confluence. The antibiotic-containing media was removed, the culture cells washed with sterile media and representative wells enumerated to determine the numbers of Caco-2 cells/well. The Caco-2 cells were then infected with a 10 times greater number of *Salmonella* (i.e. multiplicity of infection 10:1). Each experiment was done with three replicates and repeated for a total of six infections.

Invasion assay: After infection, cells were incubated at 37^0C in 5% CO_2 for one hour. Gentamicin (200 µg/ml) was subsequently added to each well, and incubated for another hour.

After which, cells were washed three times with PBS and then lysed with 0.1% Triton-X and suspensions were serially diluted and the dilutions plated on LB agar plates. After overnight incubation at 37^0C, the plates were counted to enumerate the number of bacteria that were able to invade the Caco-2 cells.

Persistence assay: After initial one hour incubation, gentamycin (100 μg/ml) was added per well, and incubated for 48 hrs. After which, the cells were washed, lysed and plated as done for invasion assay.

Tissue culture assay with antimicrobial agents: For a subset of the *Salmonella* isolates (n=11), the tissue culture invasion and persistence assays were carried out as mentioned earlier, except Caco-2 cells were infected with *Salmonella* isolates in the presence of MEM media containing antimicrobial agents (penicillin 80 units/mL and streptomycin 90 μg/mL).

Statistical analyses: For each of the invasion and persistence experiments, statistical analyses were conducted using Microsoft Excel (ver. 2016, Redmond, WA); the average of the six replicates was calculated along with the standard deviation for each set. Differences between the counts of invasion and persistence were evaluated using two-tailed paired T-test and significance was set at a $p<0.05$.

Results

Thirty-two *Salmonella enterica* isolates, represented by six different serovars were examined under this study. As reported in our earlier studies (Kaldhone et al, 2017) replicon types of these isolates are listed in Table 1 and all of the isolates in the current study contain IncI1 plasmids. Isolate 76 contains seven different replicon plasmid types. Three of isolates

contain four different replicon plasmid types, whereas six isolates contain three different replicon types. The results of the replicon typing PCR of the *Salmonella* transconjugants are shown in Table 2. All of these transconjugants carry IncA/C plasmids, while 76% of them (16/21) carry IncI1 plasmids. The presence of the IncA/C plasmid is due to the recipient 1087, carrying an IncA/C plasmid.

The results of invasion and persistence assay are displayed in Figure 2. All isolates tested could invade Caco-2 cells. Fifteen of thirty-two (46%) isolates demonstrated higher bacterial cell counts after 48 hrs of infection. Two of the isolates examined, 93 and 142, yielded persistent cell counts significantly lower than those of the invasion assays ($p<0.05$). In general, *S.* Typhimurium isolates gave the higher persistent cell count as compared to other serotypes. *S.* Heidelberg and *S.* Kentucky isolates expressed a medium range of persistence cell count, while *S.* Newport isolates were observed to have lower persistent cell counts.

Figure 3 shows the outcome of tissue culture assay of transconjugants and *S.* Newport, 1087, the recipient used for conjugation experiment. Most of the transconjugants have invasion cell counts higher than that of 1087 (Figure 3). Overall, persistent cell counts were higher than respective invasion cell counts. Each of the transconjugants who acquired IncI1 plasmids showed higher persistent cell counts compared to that of 1087. One of the transconjugants, that also received IncW plasmid (XX76) exhibited higher virulence as defined by increased invasion and persistence, while the other transconjugant which received IncW plasmid did not result in persistent levels statistically higher than recipient. Transconjugants that did not receive an IncI1 plasmid, showed persistent levels higher than recipient, but generally lower than that of transconjugants with IncI1.

The results of tissue culture assays for isolates in presence and absence of antimicrobial agents are shown in Figure 4. Among isolates tested for these experiments, isolates 67, 114, 116, 143, 146 and 159 were resistant to both ampicillin and streptomycin. Isolates 100 and N74 were resistant to ampicillin, while susceptible to streptomycin. Isolate 1188 was susceptible to ampicillin, while resistant to streptomycin, while isolate 891 was susceptible to ampicillin and has intermediate resistance to streptomycin (Kaldhone et al, 2017). For some of the isolates, the persistent cell counts decreased in the presence of antimicrobial agents, which may correspond to a reduction in cells that invaded the Caco-2 cells. Isolates 143 and 1148 were the exception to this, where cell counts in the presence of antimicrobial agents were higher than those of without antimicrobial agents.

Discussion

Salmonella enterica is an enteric pathogen that can infect several different hosts (Foley et al, 2013) Fecal-oral transmission is the main mode of transmission for *Salmonella*. After ingestion, *Salmonella* enters the intestinal tract and can cause gastroenteritis, the most common illness caused by *Salmonella* infection. Intestinal epithelial cells provide *Salmonella* a niche for colonization into their host (Wrande et al, 2016). After entering epithelial cells *Salmonella* become engulfed into *Salmonella*-containing vacuole (SCV). Within the SCV, *Salmonella* can move towards basal side of the epithelium where then can be taken up by macrophages or dendritic cells. Through these immune cells, *Salmonella* can enter into the lymphatic and systemic circulation leading to systemic infection (Foley and Lynne, 2008). Therefore, intestinal epithelial cells play an important role in the pathogenesis of *Salmonella* infection. The Caco-2

cell line, is derived from human intestinal epithelial cells and has been commonly used to study *Salmonella* pathogenesis (Balakrishnan et al, 2017, Wrande et al, 2016). Caco-2 cell lines were used in this study to try and better understand host-pathogen interactions. Tissue culture assays using Caco-2 cell lines enabled us to examine the potential for invasion and persistence of *Salmonella* with intestinal epithelium.

In the virulence assay, we noticed that persistent cell counts after 48 hrs of infection were higher in many of our isolates (Figure 2). Among different serovars tested, isolates from serovar Typhimurium appear to have higher average persistent cell counts, in general. *Salmonella* Typhimurium is known to be able to persist in the SCV, as well as the host cell cytosol (Knodler et al, 2010, 2014). These characteristics could give *S.* Typhimurium an advantage for persistence within intestinal epithelial cells, potentially allowing for more efficient multiplication than other serovars.

To more specifically target to role of IncI1 plasmids in virulence, a series of *Salmonella* transconjugants were generated. These were obtained by first transferring plasmids from *Salmonella* to *E. coli*, and then from *E. coli* to back into *Salmonella*. This approach was used to ensure that plasmids of interest were transferred from the wild-type *Salmonella* to a second characterized *Salmonella* strain for use in the invasion and persistence assays. Initial transconjugants in *E. coli* were generated using the well characterized J53 strain (Jacoby and Han, 1996, Yi et al, 2012). These *E. coli* transconjugants were screened for the plasmid content and subsequent transferred in to an IncI1-negative *S.* Newport strain (1087) to allow of the comparative assessment of invasion and persistence associated with differences in plasmid content. The *E. coli* transconjugants were not good candidates for the invasion and persistence

assays because they lack type 3 secretion systems important for entry into intestinal epithelial cells (Foley et al, 2013).

Among the virulence assays for the transconjugants, the persistence and in most cases invasion was higher for the transconjugants compared to that of the recipients (1087) (Figure 3). These findings could be indicative that the plasmids transferred carry virulence factors important for persistence or that the presence of the plasmid could improve the fitness of the organisms within the epithelial cells. Johnson et al. (2010) reported that the acquisition of ColV plasmid by *Salmonella* increased their extra-intestinal fitness. In the current study, the recipient 1087 was sequenced and found to carry an IncA/C plasmid, and the coexistence with the newly acquired IncI1 plasmids, could limit the fitness cost of carrying multiple plasmids. Johnson et al. (2015) showed that the acquisition of IncI1 plasmids did not significantly add to the fitness cost of a host bacterium and in some cases led to a negative fitness cost (i.e. benefit) to the strains that acquired them. In strains carrying IncA/C plasmids, the fitness cost of also acquiring an IncI1 plasmid was no greater than carrying the IncA/C plasmid alone. The fact that some strains carrying both IncI1 and IncA/C plasmids can have a negative fitness cost (Johnson et al, 2015), could help explain the higher persistence cell counts from our transconjugants containing both IncI1 and IncA/C, compared to the recipient carrying only IncA/C. These results seem to indicate that the co-carriage of IncI1 and IncA/C plasmids in strain 1087, does not negatively impact that ability to persist in intestinal epithelial cells.

A third set of comparative tissue culture experiments were carried out to examine the impact of antimicrobial stress on the ability of *Salmonella* strains to invade and subsequently persist within the intestinal epithelial cells. Two of the strains (143 and 1148) were non-susceptible to the antimicrobial agents present in the tissue culture media and were able to show

increased persistence following exposure and invasion, compared to the infections conducted in media without the antimicrobial exposure (Figure 4). This finding concurs with previous studies (Johnson et al, 2015), who reported that in presence of antimicrobial agents can impact bacterial physiology (in their case conjugal transfer of plasmids). Silva et al. (2011) suggested that in presence of antimicrobial agents, sign epistasis occurs between chromosomal antibiotic resistance mutations and conjugative plasmids. During sign epistasis, different mutations lead to reduced metabolic needs for the cell. This reduces nutrients required for cell to express their respective phenotypes. Millan et al. (2014) hypothesized that plasmid stability could be improved by positive epistasis and the co-existence of multidrug resistance and multiple plasmids could reduce fitness cost of the plasmids.

Most of the IncI1 positive isolates carried multiple putative virulence-related genes (Kaldhone et al, 2017). Some of the isolates in this study with the highest levels of invasion and persistence were observed to be from serovar Typhimurium and originated from poultry-related isolates. *S.* Typhimurium has been a prominent serovar in the poultry (Gast et al, 2017). Some of the isolates such as N36, N53, N74, N82 which originated from poultry-related sources, and carried *ccdB, ccdA*, and *imm* genes have demonstrated relatively high persistent cell counts compared to other strains. While, these genes may not be directly related to intestinal cell invasion and persistence, plasmids carrying these genes may have other factors that contribute to the phenotypes; however a direct correlation cannot be established from the data from this study.

Conclusions

Salmonella are important foodborne pathogens in humans and are major concerns for food animal production. Increased virulence of *Salmonella* and the capability to disseminate

virulence determinants rapidly on plasmids, make them a concern for public health. The research presented here explored the virulence of *Salmonella* containing IncI1 plasmids. This study indicates potential role of genetic determinants encoded on IncI1 plasmid for virulence. Although the genetics of IncI1 plasmids have been well studied, there is a need for further work to determine their impact on bacterial pathophysiology and human health.

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References

Acheson D, and Hohmann E. L. 2001. Nontyphoidal salmonellosis. Clin Infect Dis. 32:263-269.

Balakrishnan A, and Chakravortty D. 2017. Epithelial cell damage activates bactericidal/ permeability increasing-protein (BPI) expression in intestinal epithelium. Front Microbiol. 8:1567.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, and Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol Methods. 63:219-228.

Dunkley KD, Callaway TR, Chalova VI, McReynolds JL, Hume ME, Dunkley CS, Kubena LF, Nisbet DJ, and Ricke SC. 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe. 15:26-35.

Finstad S, O'Bryan CA, Marcy JA, Crandall PG, and Ricke SC. 2012. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. Food Res Int. 45:789-794.

Foley, S. L., Johnson, T. J., Ricke, S. C., Nayak, R., and Danzeisen, J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. Microbiol. Mol. Biol. Rev. 77: 582-607.

Foley, S. L. and Lynne A. M. 2008. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. J Anim Sci. 86: E173-187E.

Gast RK, Guraya R, Jones DR, Guard J, Anderson KE, and Karcher DM. 2017. Frequency and duration of fecal shedding of *Salmonella* serovars Heidelberg and Typhimurium by experimentally infected laying hens housed in enriched colony cages at different stocking densities. Avian Dis. 61-366-371.

Gordon M. A. 2008. *Salmonella* infections in immunocompromised adults. *J. Infect.* 56:413-422.

Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, Kaldhone PR, Logue CM, and Foley SL. 2012. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PLoS One. 7:e51160.

Howard ZR, O'Bryan CA, Crandall PG, and Ricke SC. 2012. *Salmonella* Enteritidis in shell eggs: Current issues and prospects for control. Food Res Int. 45:755-764.

Jacoby GA and Han P. 1996. Detection of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J Clin Microbiol. 34:908-911.

Jakočiūnė D, Bissgaard M, Pedersen K, and Olsen JE. 2014. Demonstration of persistent contamination of a cooked egg product production facility with *Salmonella enterica* serovar Tennessee and characterization of the persistent strain. J Appl Microbiol. 117:547-553.

Johnson TJ, Singer RS, Isaacson RE, Danzeisen JL, Lang K, Kobluk K, Rivet B, Borewicz K, Frye JG, Englen M, Anderson J, and Davies PR. 2015. In vivo transmission of an IncA/C plasmid in *Escherichia coli* depends on tetracycline concentration, and acquisition of the plasmid results in a variable cost of fitness. Appl Environ Microbiol. 81:3561–3570.

Johnson TJ, Thorness JL, Anderson CP, Lynne AM, Foley SL, Han J, Fricke WF, McDermott PF, White DG, Khatri M, Stell AL, Flores C, and Sanger RS. 2010. Horizontal gene transfer of a ColV plasmid has resulted in a dominant avian clonal type of *Salmonella enterica* seroovar Kentucky. PLoS One. 5:e15524.

Johnson TJ, and Nolan LK. 2009. Plasmid replicon typing. Methods Mol Biol. 551:27-35.

Johnson TJ, Giddings CW, Horne SM, Gibbs PS, Wooley RE, Skyberg J, Olah P, Kercher R, Sherwood JS, Foley SL, and Nolan LK. 2002. Location of increased serum survival gene and selective virulence traits in an avian *Escherichia coli* isolate. Avian Dis. 46:342-352.

Kaldhone PR, Han J, Deck J, Khajanchi B, Nayak R, Foley SL, and Ricke SC. 2017. Evaluation of the genetics and functionality of plasmids in Incompatibility Group I1 (IncI1) positive *Salmonella enterica.* Foodborne Path Dis. Accepted.

Knodler LA, Nair V, and Steele-Mortimer O. 2014. Quantitative assessment of cytosolic *Salmonella* in epithelial cells. PLoS One. 9:e84681.

Knodler LA, Vallance BA, Celli J, Winfree S, Hansen B, Montero M, and Steele-Mortimer O. 2010. Dissemination of invasive *Salmonella* via bacterial-induced extrusion of mucosal epithelia. Proc Natl Acad Sci. 107:17733–17738.

Marrero-Ortiz, R., Han J, Lynne AM, Stemper ME, David DE, Nayak R, and Foley SL. 2012. Genetic characterization of antimicrobial resistance in *Salmonella enterica* serovars isolated from dairy cattle in Wisconsin. Food Res Int. 45:962-967.

Millan AS, Heilborn K, and MacLean RC. 2014. Positive epistasis between co-infecting plasmids promote plasmid survival in bacterial populations. The ISME Journal. 8:601-612.

Mo SS, Sunday M, Ilag HK, Langsrud S, and Heir E. 2017. Transfer potential of plasmids conferring extended-spectrum-cephalosporin resistance in *Escherichia coli* from poultry. Appl Enviorn Microbiol. 83-e00654-17

Pulcrano G, Pignanelli S, Vollaro A, Esposito M, Iula VD, Roscetto E, Soriano AA, and Catania MR. 2016. Isolation of *Enterobacter aerogenes* carrying bla_{TEM-1} and bla_{KPC-3} genes recovered from a hospital intensive care unit. APMIS. 124:516-521.

Ricke SC, Dawoud TM, Shi Z**,** Kaldhone PR, and Kwon YK. 2017. Foodborne *Salmonella* in laying hens and egg production. In Ricke S. C., Atungulu G. G., Rainwater C. E., and Park S. H. (Eds.), Food and Feed Safety Systems and Analysis. Elsevier Inc., Oxford, UK. pp 156-171.

Sanad Y, Johnson K, Park SH, Han J, Deck J, Foley SL, Kenney B, Ricke SC, and Nayak R. 2016. Molecular characterization of *Salmonella enterica* serovars isolated from a turkey production facility in the absence of selective antimicrobial pressure. Foodborne Pathog Dis. 13:80-87.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, and Griffin PM. 2011. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis. 17:7-15.

Scharff RL. 2010. Health-related costs from foodborne illness in the United States. Georgetown University, Washington, D.C. 3:1-28.

Silva RF, Mendonca SCM, Carvalho LM, Reis AM, Gordo I, Trindade S, and Dionisio F. 2011. Pervasive sign epistasis between conjugative plasmids and drug-resistance chromosomal mutations. PLoS Genet 7: e1002181.

Wang H, Gill VS, Cheng CM, Gonzalez-Escalona N, Irvin KA, Zheng J, Bell RL, Jacobson AP, and Hammack TS. 2015. Evaluation and comparison of rapid methods for the detection of *Salmonella* in naturally contaminated pine nuts using different pre-enrichment media. Food Microbiol; 46:58-65.

Wrande M, Andrews-Polymenis H, Twedt DJ, Steele-Mortimer O, Powollik S, McClelland M, and Kondler LA. 2016. Genetic determinants of *Salmonella enterica* serovar Typhimurium proliferation in the cytosol of epithelial cells. Infect Immun 84:585-95.

Wong MH, Kan B, Chan EW, Yan M, and Chen S. 2016. IncI1 plasmids carrying various *bla*CTX-M genes contribute to ceftriaxone resistance in *Salmonella enterica* serovar Enteritidis in China. Antimicrob Agents Chemother; 60:982-989.

Yi H, Cho Y-J, Yong D, and Chun J. 2012. Genome sequence of *Escherichia coli* J53, a reference strain for genetic studies. Genome Announc. 194:3742-3.

Table 1. Isolate characterization and results of PCR experiment

Isolate	Serotype	Source	Year	State	Replicon Types Detected
891	Anatum	Cattle	2006	WI	I1
115	Heidelberg	Cattle	2002	IN	I1, A/C, HI2
1017	Infantis	Cattle	2006	WI	I1, A/C
67	Newport	Cattle	2002	IA	I1, A/C
74	Newport	Cattle	2002	WA	I1, A/C
855	Typhimurium	Cattle	2006	WI	I1, FIA, FIB, W
N860	Kentucky	Chicken	2008	AR	I1
N865	Kentucky	Chicken	2008	AR	I1
76	Newport	Chicken	2001	GA	I1, A/C, B/O, FIIA, K/B, T, W
N36	Typhimurium	Chicken	NA	WV	I1, FIB
N53	Typhimurium	Chicken	NA	WV	I1, A/C
N74	Typhimurium	Chicken	NA	WV	I1, FIB
N97	Typhimurium	Chicken	NA	WV	I1, A/C
N822	Kentucky	Chicken Farm	2008	AR	I1
N89	Kentucky	Chicken Farm	NA	WV	I1
N134	Typhimurium	Chicken Farm	NA	WV	I1, FIB
N136	Typhimurium	Chicken Farm	NA	WV	I1, FIB
N82	Typhimurium	Chicken Farm	NA	WV	I1
990	Heidelberg	Human	2008	AR	I1, X
991	Heidelberg	Human	2009	AR	I1
1148	Heidelberg	Human	2007	WI	I1
1163	Heidelberg	Human	2007	WI	I1
142	Heidelberg	Swine	2002	IN	I1, A/C, HI2
143	Heidelberg	Swine	2002	MN	I1, A/C, HI1, HI2
144	Heidelberg	Swine	2002	MN	I1, A/C, HI2
146	Heidelberg	Swine	2002	MN	I1, A/C
89	Newport	Swine	2001	UT	I1, A/C
93	Newport	Swine	2002	KS	I1, A/C
470	Typhimurium	Swine	1999	N/A	I1, HI2
159	Heidelberg	Turkey	2002	NC	I1, HI2
695	Heidelberg	Turkey	2000	MW*	I1, X
100	Newport	Turkey	2001	ND	I1

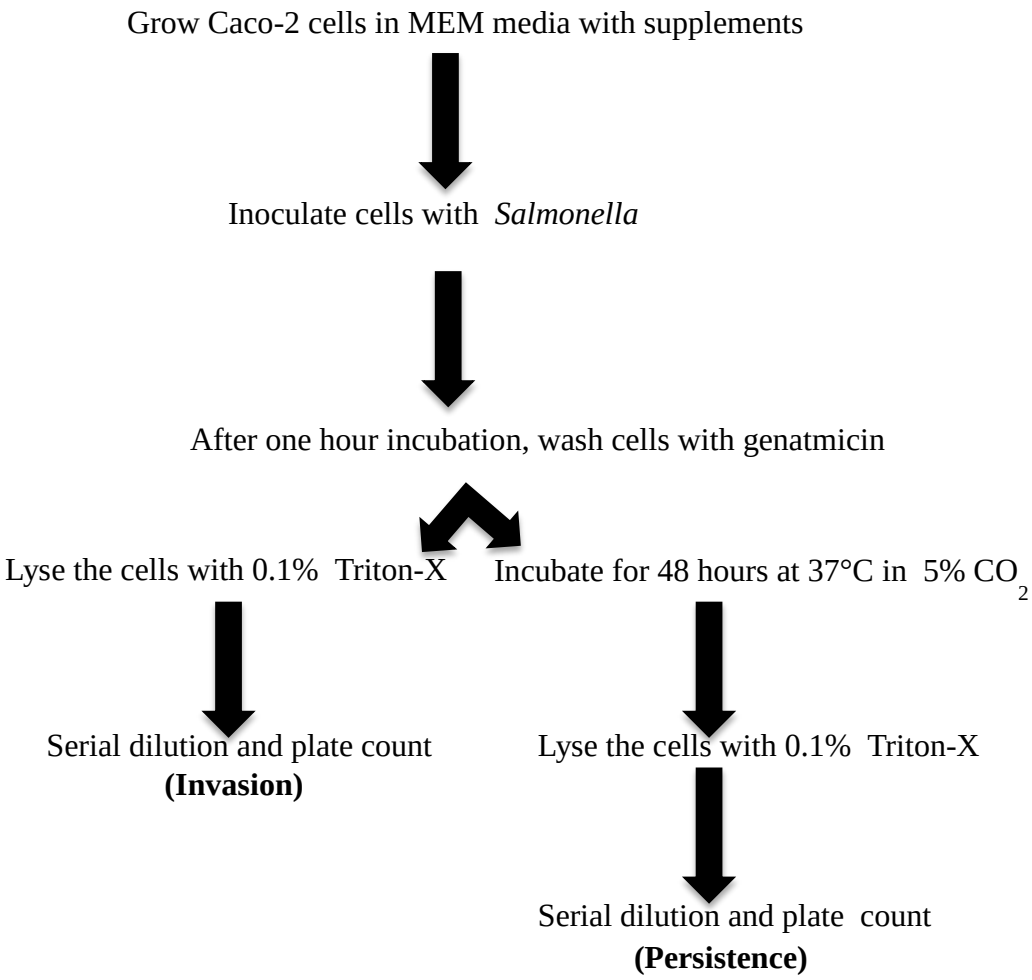
Note: *MW: Midwestern State, not further defined. NA: not available

Table 2. Transconjugant replicon types

Isolate	*E. coli*-transconjugant*	*Salmonella* transconjugant
76	I1,A/C,W	A/C,W
93	I1,A/C	A/C,I1
111	I1,A/C	A/C,I1
114	I1,A/C	A/C,I1
116	I1	A/C,I1
121	I1	A/C,I1
142	I1,A/C	A/C,I1
471	I1	A/C,I1
482	I1	A/C
706	I1	A/C,I1
713	ND	A/C
715	I1	A/C,I1
849	I1,A/C	A/C,I1
855	I1,FIA,W	A/C,I1,W
856	I1	A/C,I1
860	ND	A/C
880	ND	A/C
891	I1	A/C,I1
1000	I1	A/C,I1
N74	I1,FIB	A/C,I1,FIB
N82	I1	A/C,I1

ND: not detected, *Kaldhone et al., 2017

Figure 1. Flow diagram for tissue culture experiment.



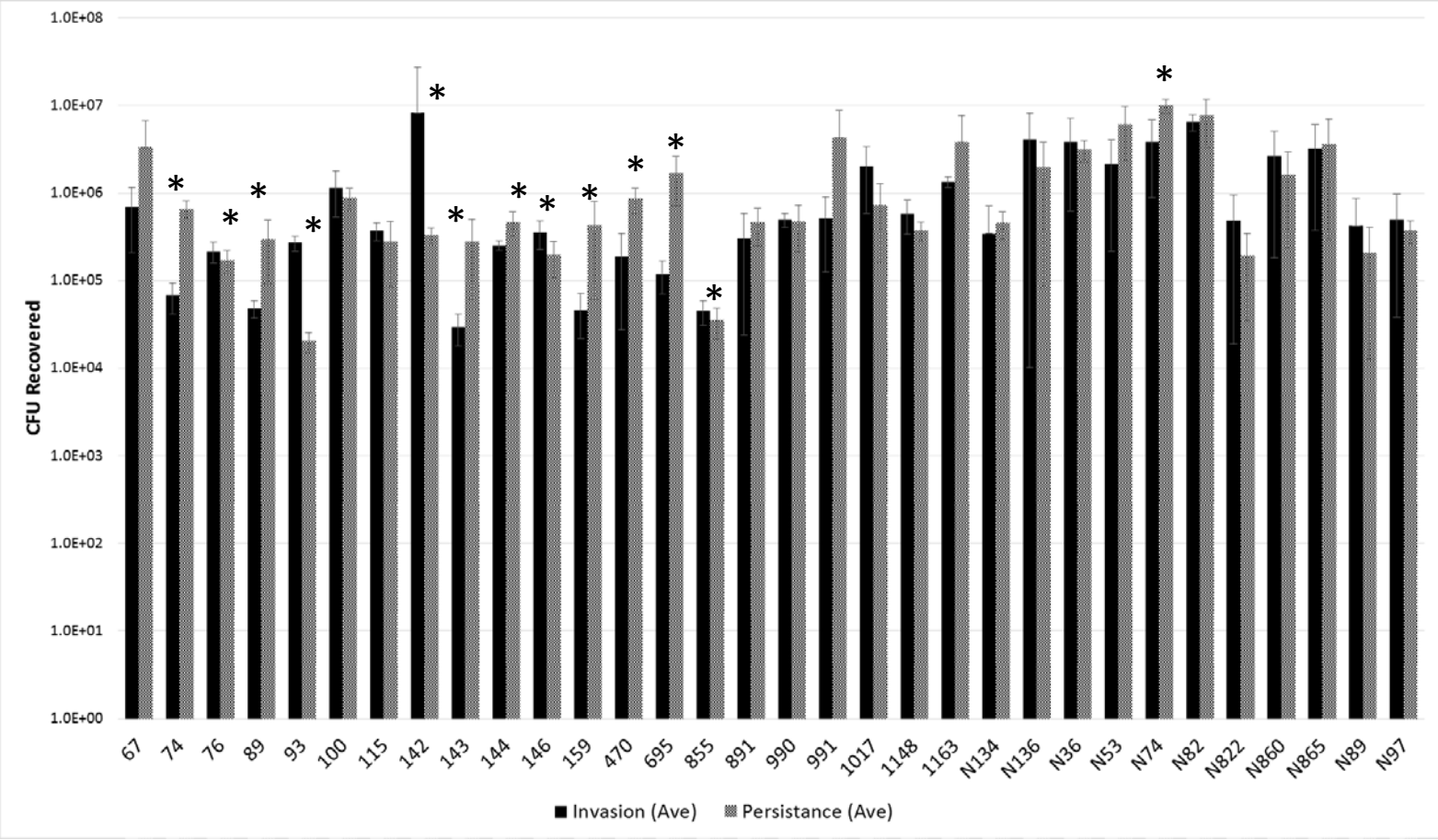


Figure 2. Results of invasion and persistence assays

Note: Invasion bars indicate the average number of colony forming units (CFU) detected after 1 hr invasion period and persistence bars indicate the number of CFU detected following 48 hr incubation period for persistence assay. Each set of experiments was done in triplicate and repeated. The error bars indicate the standard deviation across the 6 counts for each isolate. Isolate numbers are noted on X-axis, while CFU recovered are expressed along Y-axis. *: indicates statistically significant difference between invasion and persistent cell count for given isolate ($p<0.05$).

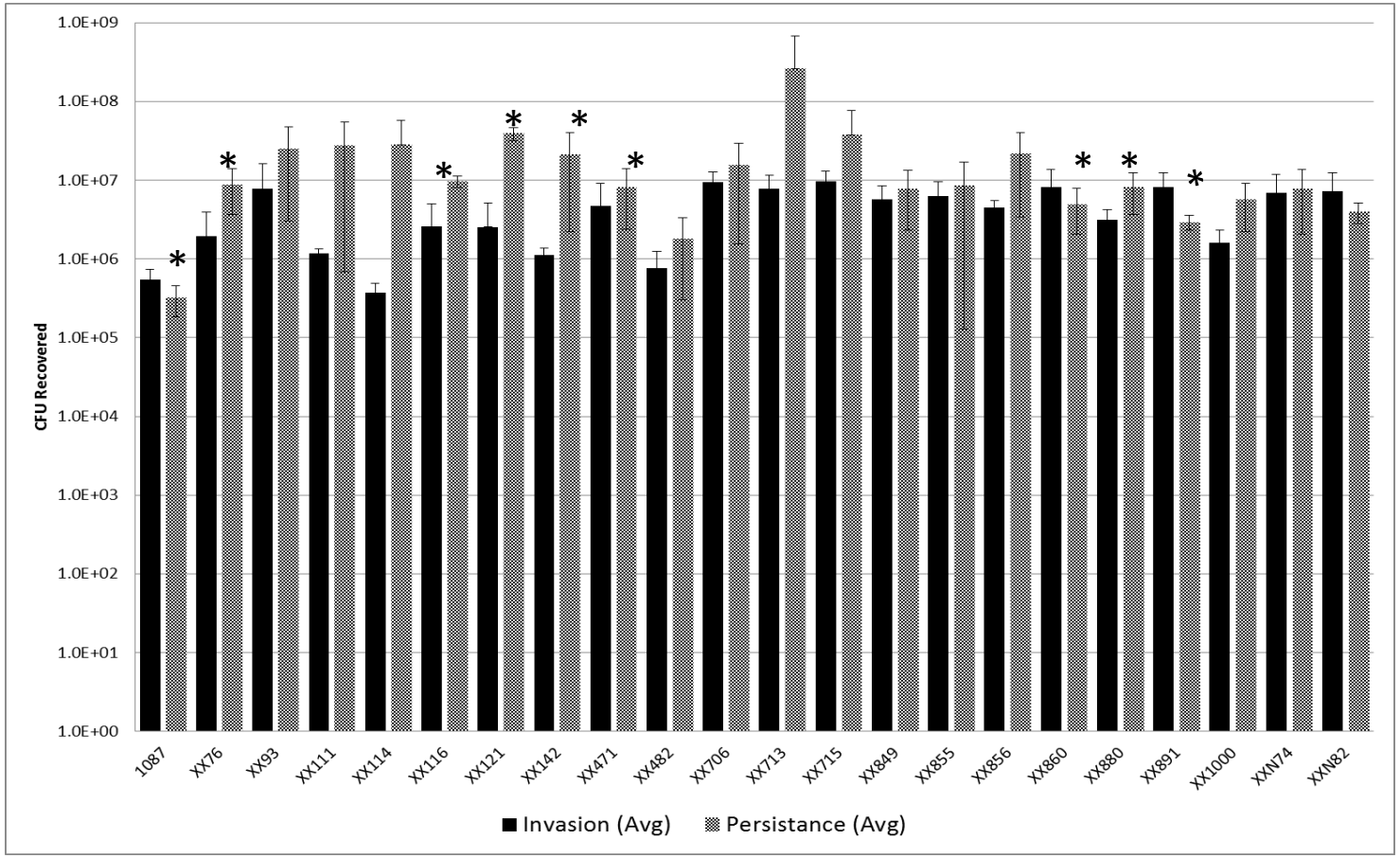


Figure 3. Results of invasion and persistence assays for *Salmonella Newport* (SE1087) and transconjugants.
Note: Each transconjugant is represented by XX prefix of respective donor. The invasion bars indicate the average number of colony forming units (CFU) detected after 1 hr invasion period and persistence bars indicate the number of CFU detected following 48 hr incubation period for persistence assay. Each set of experiments was done in triplicate and repeated. The error bars indicate the standard deviation across the 6 counts for each isolate. Isolate numbers are noted on X-axis, while CFU recovered are expressed along Y-axis. *: indicates statistically significant difference between invasion and persistent cell count for given isolate ($p<0.05$).

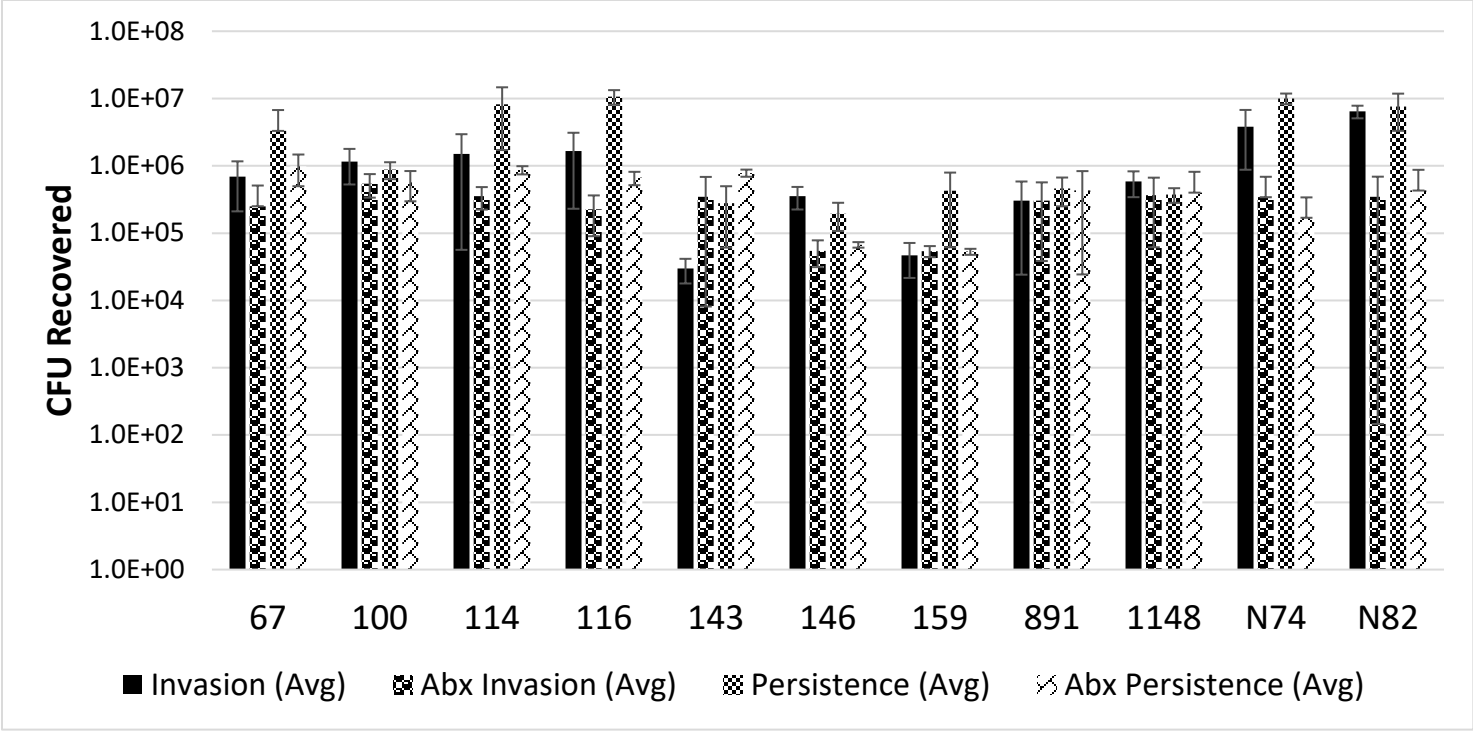


Figure 4. Results of invasion and persistence assays for *Salmonella* isolates with and without antimicrobial agents.
Note: Invasions bars indicate the average number of colony forming units (CFU) detected after 1 hr invasion period in media without antimicrobial agents and Abx Invasion bars represent the number of CFU detected after 1 hr invasion period in media with antimicrobial agents. Persistence bars show the number of CFU detected following 48 hr incubation period for persistence assay in media without antimicrobial agents and Abx Persistence bars denote the number of CFU detected following 48 hr incubation period for persistence assay in media with antimicrobial agents. Each set of experiments was done in triplicate and repeated. The error bars indicate the standard deviation across the 6 counts for each isolate. Isolate numbers are noted on X-axis, while CFU recovered are expressed along Y-axis.

VII. Conclusion and Future Directions

Salmonella is one of the leading foodborne pathogen. It's relatively high prevalence in poultry and other food animal sources make it critical for public health. Plasmids are often present in *Salmonella* and other Enterobacteriaceae. IncI1 group of plasmids have been associated with antimicrobial resistance and virulence. IncI1 Plasmids have played role in dissemination of ceftriaxone resistance among *Salmonella* strains. They have potential to rapidly spread genetic determinants for virulence among other enteric pathogens over a short period.

These studies characterize IncI1 plasmids in the *Sallmonella enterica*, focusing on their ability to carry genetic determinants related to antimicrobial resistance, virulence and the ability to transfer themselves to other enteric microbes. Antimicrobial resistance genes and corresponding resistance phenotype were observed in most of our isolates with a few noted exceptions. We also showed that many IncI1 plasmids are transferable from one bacterium to another. Along with IncI1 plasmids, resistance and virulence also spread among other enteric bacteria. Certain transferred genes were potentially associated with resistance and virulence. A more comprehensive understanding of genetic determinants can be achieved through the use of advanced sequencing method in future experiments. The whole genome sequencing of selected strains using Illumina Miseq platform revealed valuable information pertaining to presence of plasmids, antimicrobial resistance and virulence- associated genes among sequenced strains. Alternative sequencing methods such as PacBio could give more in depth and more comphrehensive analyses of genetic background of these strains. Through their use of long read strategies that facilitate better genome assembly. A colicin inhibition assay showed some of our *Salmonella* isolates were able to inhibit *E. coli* and were able transfer this ability to another

Enterobacteriaceae. During tissue culture assays for virulence using Caco-2 cells, *Salmonella* Typhimurium from poultry appeared to have higher persistent cell counts when compared to other serovars. More work will need to be done to verify this finding and what the mechanics behind are. Tissue culture assays of our transconjugants revealed that persistent cell counts for tranconjugants were typically higher than those of the recipient. We also noted that persistent cell counts for transcojugants carrying IncI1 and IncA/C were higher than those of transconjugants carrying only IncA/C. This might be due to fitness benefit from co-carriage of IncI1and IncA/C plasmids. We also showed that in presence of antimicrobial agents, few *Salmonella* isolates expressed an increase in persistent cell count in Caco-2 cells when compared to other isolates.

Multiple paths could be taken to follow up this project. Comprehensive colicin-related assays would give us more understanding of functional aspect of the colicin toxin and the influence of colicin production on conjugation. Further studies of similar phenotypic assays under stress conditions such as probiotic-like compounds would give us more insight into respective traits of *Salmonella*. Expression studies targeting specific genetic determinants during conjugation and virulence assays might provide more understanding about regulation of these phenotypes. Collectively, these findings could provide us tools to identify clinically important *Salmonella* strains and manage them effectively.

Appendix A

BioSafety Approval Letter

PROTOCOL/ADDENDUM COVER PAGE

☑ Protocol ☐ Addendum ☐ Preliminary Experiment

Project Number: E0759601

Animal requirements	☐ Yes	☑ No
GLP:	☐ Yes	☑ No
Human Subject, Data, Cells, Tissues or Fluids:	☑ Yes	☐ No
Controlled/Hazardous/ Radioactive Substance:	☐ Yes	☑ No
Collaborative Research Effort:	☑ Yes	☐ No

If yes, what agency?

☑ FDA Unit CVM

☐ IAG _____

☐ CRADA _____

☐ Other (Please specify) _____

Title:

Role of Plasmid-encoded Factors in Salmonella enterica Virulence

FDA Goals: **Goal 1: Enhance Oversight of FDA-Regulated Products**

Approved

Director, Office of Research — 11.17.2015 Date

Principal Investigator — 11/17/15 Date

NCTR PROTOCOL/ADDENDUM INTERNAL REVIEW SHEET

☑ Protocol ☐ Addendum ☐ Preliminary Experiment

Initial/Concept Project Number: C14011 **Project Number** *(assigned by GMI)*: E0259601

Master Project Number: _____

Project Title: Role of Plasmid-Encoded Factors in Salmonella enterica Virulence

	Yes	No
Animal Requirements		✓
Human Subjects, Data, Cells, Tissues or Fluids	✓	
GLP		✓
Controlled/Hazardous/Radioactive Substance	✓	

1/27/15

If this is a collaborative research effort indicate agency below.

FDA Unit: Center for Veterinary Medicine

IAG: _____

CRADA: _____

Other (Please specify.): _____

Select one of the FDA Goals:

✓ **Goal 1**

(Enhance Oversight of FDA-Regulated Products)

Goal 2

(Improve and Safeguard Access to FDA-Regulated Products that Benefit Health)

Select one primary NCTR Strategic Objective for the protocol *(see page four for the objective descriptions).*

✓ **Objective 1.1**
(Integrated product assessment)

☐ **Objective 1.2**
(Advance regulatory science through new tools & approaches)

Objective 1.3
(Approaches for promoting individualized health and identifying susceptible subpopulations)

Please indicate the affiliation, sign, date, and forward to next reviewer.

	Name	Signature	Date
Principal Investigator	Steven Foley		6/29/15
Division Director	DM - Cari Cerniglia		7/14/2015
CTP Liaison to NCTR *(if appropriate)*	_____	N/A	_____
NCTR Liaison to CTP *(if appropriate)*		N/A	_____
Office of Planning and Resource Management			11/17/2015
NTP Project Officer (NIEHS) *(if appropriate)*		N/A	_____
NTP Project Officer (NCTR) *(if appropriate)*		N/A	_____
for Director, Office of Research			11-17-2015
IACUC Chairperson			11/17/15
Director, Regulatory Compliance and Risk Management			11/17/2015
for Director, Planning and Resource Management Staff			11/17/2015
for FDA RIHSC Liaison			11-17-2015

NCTR Protocol/Addendum Internal Review Sheet

Co-Investigators, Collaborators, Approving Officials

Please indicate the affiliation, sign, date, and forward to next reviewer.

	Name	Signature	Date
Co-Investigator(s)	Shaohua Zhao		
	Ruby Singh		
	Jeffrey Gilbert	Jeffrey M. Gilbert, Ph.D.	
	Rajesh Nayak		6-29-15
	Sangeeta Khare		6/29/2015
	Kuppan Gokulan		6/29/2015
	Joanne Dorlo		6-29-2015
Consultant(s)			

Director's Signature for Co-Investigators that are not part of Primary Investigator's Division

	Name	Signature	Date
Division Director			
Division Director			
Division Director			

Collaborating FDA or other Agency Approving Official (if appropriate)

Name	Title	Agency	Signature	Date
John Graham			John Graham -S	
Kevin Greenlees	Sr Advisor Sci. & Policy	FDA	Kevin J. Greenlees -S	7/20/2015

NCTR Toxic/Hazardous Materials Use Form

NOTE: This form must be completed by the Principal Investigator and attached to the study protocol. Please contact the Regulatory Compliance and Risk Management (RCRM) Staff regarding questions or assistance concerning this form.

SECTION 1. STUDY INFORMATION

Protocol#: (14011

Protocol Title: Role of Plasmid-Encoded Factors in Salmonella enterica Virulence

Principal Investigator: Steven Foley

Division: Microbiology

SECTION 2. HAZARDOUS CHEMICAL IDENTIFICATION

In the table below identify the hazardous chemicals to be used in the protocol. Hazard classification information may be obtained from Safety Data Sheets (SDSs) and other references listed in the NCTR Environmental, Safety, and Health (ESH) manual. http://inside.fda.gov:9003/PolicyProcedures/GuidanceRegulations/Safety/ucm019052.htm

Radioactive chemicals, controlled substances, and biohazardous materials should be specifically identified in the "Hazard" column. Address special safety procedures and personnel safety training requirements in Sections 5, 6, and 7.

	Chemical Name (as listed on SDS)	Maximum Qty (designate unit)	Location (Building/Rm #)	*Hazard(s)
	Ethidium Bromide Solution	5 ml	51/116	mutagen,acute toxicity
	Gentamicin sulfate	25 g	51/127	irritant,sensitizer
	Triton X-100	500 ml	51/116	irritant,sensitizer
	Sodium Azide	25 g	51/127	poison,toxic to aquatic life
	Kanamycin Sulfate	25 g	51/127	irritant
	Tetracycline	25 g	51/127	harmful if swallowed
	Triton X-100	1 L	60/113	poi son,irritant,toxic to aquatic life
	Tri-Reagent	2 L	60/113	poision,irritant, corrosive
	Ampicillin	10 g	51/127	irritant
	Ceftiofur hydrochloride	5g	51/127	irritant/allergen
	Chloramphenicol	10 g	51/116	potential carsinogen
	Sulfamethoxazole	10 g	51/127	irritant/allergen
	Tylosin tartrate	5g	51/116	irritant/allergen

*examples ofhazards: flammable, pyrophoric, oxidizer, organic peroxide, sensitizer, carcinogen, mutagen, reproductive toxicity, irritant, acute toxicity, corrosive, explosive.

SECTION 3. HAZARD CHEMICAL LIST

Verify and/or complete the following requirements: The hazardous chemicals to be used in the protocol have been added ore will be added upon receipt to the "Hazardous Chemical List" for your division/area. The SDSs are readily available to laboratory staff, and have been provided or will be provided upon receipt to the Occupational Health Unit (OHU).

SECTION 4. EXPERIMENAL PROCEDURES

Briefly describe how the material will be used.

Salmonella strains with plasmids carrying genes that may contribute to virulence will be selected based on DNA sequence analysis for further study. Plasmids of interest will either be cured from strains or moved into an avirulent recipient and compared to the parental strains to determine differences in virulence due to the plasmids. Individual genes on those plasmids likely to contribute to increased virulence will be evaluated using expression studies and mutation analysis to determine their impacts on the host cells. The studies will include an in vitro analysis of the host-pathogen interactions, looking at both the pathogen and host immune responses to gain a more holistic picture of the infectious process. Additionally,because plasmids are potentially mobile genetic elements, it is also important to understand the factors that may facilitate the transfer of virulenee and antimicrobial resistanee plasmids among bacterial isolates. Factors such as exposure to different types and concentrations of antimicrobial agents, including certain disinfectants and sanitizers, will be evaluated to determine if exposure to the compounds could contribute to an increase in plasmid dissemination. These findings will provide data on the roles of antimicrobial compounds in the spread of virulence and resistance plasmids,which can lead to a more judicious use of these compounds.

SECTION 5. LABORATORY SAFETY PLAN

Complete the following questions to address the hazards associated with the chemicals used in this protocol. Include any other safety measures that are required for work on this protocol.

a) What safety Engineering Controls will be required/used? (ex. fume hood, biological safety cabinet, autoclave, laboratory design)

Culture and molecular biology work will be carried out either on the benchtop or in biological safety cabinet (BSC) in accordance with established BSL-2 guidelines. When there is a chanee for the generation of aerosols, all work will be done in a certified BSC. Waste materials associated with these experiments will either be autoclaved to sterilize the contaminated waste prior to disposal in accordance with NCTR and State of Arkansas guidelines.

b) What safety Administrative and SOP Controls will be required/used? (ex. laboratory specific SOPs, use of chemical in designated areas)

The staff who be working on the project will have experience working with microorganisms and will receive laboratory safety training at NCTR. The staff will work during normal business hours, unless prior approval is granted and the security staff is notified to ensure monitoring of the safety of the worker. During normal business hours,the laboratories have numerous people around them and the labs have glass in the doors which will allow for monitoring of the personnel's safety.

c) What Personal Protective Equipment (PPE) Controls will be required/used? (ex. gloves, respirators, Tyvek suits)

Personnel working in the laboratory with microbiological samples will wear proper PPE,including gloves, laboratory coats and eye protection. Areas where work is being carried out will be sanitized after use.

d) Document the decontamination and clean-up procedures to be used, including the decontamination material.

Areas where samples are being used will be disinfected using isopropanol,bleach or other commercially available microbiocide. Waste will placed in biohazard containers and autoclaved to sterilize before final disposal. In addition, since the work will be done in biological safety cabinets,the cabinets will be disinfected with isopropanol before and after use.

e) Describe any special handling procedures for this material, including any personnel or area monitoring procedures that are required.

In the event that there is potential for the generation on aerosols,the microbiological work will be completed in a biological safety cabinet,with the researcher wearing their required PPE.

f) Describe the emergency procedures to be used in case of a spill or personnel exposure.

Spills will be cleaned up immediately and the clean materials placed in a biohazardous waste container and the area decontaminated with disinfectants. Personnel exposed to the potential microorganisms will wash there hands (or other body part exposed) with copious amounts of water along with disinfectant soap. If the samples get into the eye,the person will wash their eye(s) in one of the eye wash stations and see the medical staff for further evaluation.

g) Describe the emergency response equipment that is required and will be available. (ex. eye wash, emergency shower, spill control equipment)

Within the laboratories,eye wash stations,fire blankets and extinguishers and hand washing stations will be available. In the hallways outside of the laboratories, safety showers are available for an emergency. Spill kits will also be available to clean small to moderate size spills.

SECTION 6. HAZARDOUS WASTE

Describe the types and amounts of wastes to be generated and waste disposal procedures to be used.

The primary types of waste that will be generated is biological waste,which will be autoclaved to sterilize the materials and disposed of according to NCTR guidelines.

SECTION 7. PERSONNEL/TRAINING

All laboratory staff must complete laboratory safety training prior to beginning work in the laboratory and attend annual laboratory safety refresher training. The PI must provide laboratory staff with protocol-specific training, including specific hazards and control measures.

a) Provide the names of personnel or support groups involved in handling the materials and their division/area.

Joanna Deck,Jing Han,Yasser Sanad,Rajesh Nayak,Sangeeta Khare,Kuppan Gokulan, Steven Foley/ All in the Division of Microbiology.

b) List the type of safety training that will be required. (ex. Radiation Safety, Bloodborne Pathogen, Nonhuman Primate Emergency Procedures)

Basic Laboratory Safety training and annual refresher training and BSL-2 training.

c) List the protocol-specific safety training that will be provided by the PI and how it will be documented.

Staff/fellows working on the project will receive specific instruction on proper use of PPE,working with Salmonella,and basic molecular biology techniques that may involve potentially hazardous chemicals.

NCTR Toxic/Hazardous Materials Use Form

SECTION 8. SIGNATURE

"All information in this form is correct to the best of my knowledge. If the information in this form is revised I will notify RCRM and provide a new form with signature and current date."

Steven Foley -S

Signature Date

*Electronic signature should be updated if revisions are made to the form.

Appendix B

CURRICULUM VITAE

PRAVIN KALDHONE

Adderss

3900 NCTR Road
Apartment 2B
Microbiology Division
Jefferson, AR, 72079
Cell: 1(773) 403-5285
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Edication

Ph.D. (Expected completion in December 2017), Food Science- emphasis on Food Safety and Microbiology, Center for Food Safety, Department of Food Science, University of Arkansas, Fayetteville, AR. August 2014- December 2017.

M.S., Biological Sciences- emphasis on Microbiology and Molecular Biology, Department of Biological Sciences, University of Central Arkansas, Conway, AR, August 2004- August 2006.

M.B.B.S. (Bachelor of Medicine and Bachelor of Surgery), Thane Municipal College, University of Mumbai, Thane, India, June 1996- June 2002.

Current Position

Graduate Assistant, Microbiology Division, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, May 2017- current.

Previous Positions

Graduate Assistant, Department of Food Science, University of Arkansas, Fayetteville, AR, August 2014- May 2017.

Teaching Assistant, Division of Health Sciences, Taylor Business Institute, Chicago, IL, April 2012- July 2014.

Volunteer Research Microbiologist, Joint Venture Laboratory, Marshfield Clinic Research Foundation, Marshfield, WI, April 2011- April 2012.

Research Associate, Clinical Research Center, Marshfield Clinic Research Foundation, Marshfield, WI, October 2007- March 2011.

Research Associate, National Farm Medicine Center, Marshfield Clinic Research Foundation, Marshfield, WI, October 2006- September 2007.

Graduate Research Assistant, National Farm Medicine Center, Marshfield Clinic Research Foundation, Marshfield, WI, August 2005- August 2006.

Graduate Teaching Assistant, Department of Biology, University of Central Arkansas, Conway, AR, August 2004- May 2005.

Observer, Department of Oncology, Tata Memorial Center, Mumbai, India, June 2004- August 2004.

Safety Officer, Offshore Section, Bombay High Region, Oil and Natural Gas Corporation, Mumbai, India, September 2003- June 2004.

Research Associate, Cyto Tech Laboratory, Anusaya Medical Trust, Thane, India, July 2002- August 2003.

Intern, Chhatrapati Shivaji Maharaj Hospital, Thane Municipal Corporation, Thane, India, June 2001- June 2002.

Membership in professional Organizations

American Society of Microbiology, 2005- present.

American Association for the Advancement of Sciences, 2006- present.

Arkansas Association for Food Protection, 2014- present.

Ozark Institute of Food Technologists, 2014- present.

Activities

Assistant, Pulse Polio Immunization Program and Blood Donation Camp, Department of Health, Thane Municipal Corporation, 1996-2000.

Coordinator, Student Council, Thane Municipal College, Thane, India, 1998-1999.

Health Division In-charge, National Himalayan Winter Trekking Expedition, Youth Hostel Association of India, Dal housie, Himachal Pradesh, India, 2001.

Supervisor, Community Health Education Camp and Primary Health School Health Check-up Program, Primary Health Care centers, Department of Health, Thane Municipal Corporation, 2001-2002.

Certificate Program in Yoga, Department of Philosophy, University of Mumbai, Mumbai, India, 2002.

Certificate Program in Personality Development, Narse Monje Institute of Management Studies, Mumbai, India, 2002.

Secretary, Cricket Association, University of Central Arkansas, Conway, AR, 2005.

Service-In-Charge, Thanks giving dinner, Upham Village Assisted Living Facility, Marshfield, WI, November, 2008.

Volunteer, Culinary Festival, Chicago Cultural Center, Chicago, IL, 2010.

Inter-Cultural Secretary, Taylor Business Institute, Chicago, IL, 2012.

Vice-President, Service, Alpha Phi Omega, Beta-Rho chapter, University of Arkansas, Fayetteville, AR, 2014.

Volunteer-In-Charge, Jane B. Gearhart Full Circle Campus Food Pantry, University of Arkansas, Fayetteville, AR, 2014-2016.

President, Friends of India, University of Arkansas, Fayetteville, AR, 2016-2017.

Contuning Education

Health Care Providers Program, Basic Life Support, American Heart Association, 2006-present.

Advance Training

Light Cycler 480 instrument/ software 1.4 training for Real-time PCR by Roche Diagnostic, University of Wisconsin, Madison, Spring, 2006.

Comparative Genomic Web based Tools training (ASAP, Mauve) by Dr. Guy Plunkett III/ Dr. Bob Mau, Laboratory of Genetics, Henry Mall, University of Wisconsin, Madison, Summer, 2009.

QIIME software training for sequence analysis by Dr. Si Hong Park, Department of Food Science, University of Arkansas, Fayetteville, AR, Summer, 2015.

Bio Safety Lab Training by Dr. Tucker Patterson, Division of Compliance, National Center for Toxicological Research, Jefferson, AR, Summer, 2016.

NCBI Workshop on Genome analysis. National Center for Toxicological Research, Jefferson, AR, June, 2017.

Safe-T-Pack Training for transportation of hazardous substances. National Center for Toxicological Research, Jefferson, AR, September, 2017.

FDA Annual Records Management Training, National Center for Toxicological Research, Jefferson, AR, October, 2017.

Publications

Nayak R, Call V, **Kaldhone PR**, Tyler C, Anderson G, Phillips S, Kerdahi K, and Foley SL. 2007. Comparison of *Salmonella enterica* serovar Heidelberg susceptibility testing results. Clin Med Res. 5:98-105.

Kaldhone PR, Nayak R, Lynn AM, David DE, McDermott PF, Logue CM, and Foley SL. 2008. Characterization of *Salmonella enterica* serovar Heidelberg from turkey-associated sources. Appl Environ Microbiol. 74:5038-5046.

Lynne AM, **Kaldhone PR**, David D, White DG, and Foley SL. 2009. Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. Foodborne Pathog Dis. 6:207-215.

Han J, David DE, Deck J, Lynne AM, **Kaldhone PR**, Nayak R, Stefanova R, and Foley SL. 2011. Comparison of *Salmonella enterica* serovar Heidelberg isolated from human patients with those from animal and food sources. J Clin Microbiol. 49:1130-1133.

Lin Y, Barker E, Kislow J, **Kaldhone PR**, Stemper M, Pantrangi M, Moore FM, Hall M, Fritsche TR, Novicki T, Foley SL, and Shukla SK. 2011. Evidence of multiple virulence subtypes in nosocomial and community associated MRSA genotypes in companion animals from the upper midwestern and northeastern United States. Clin Med Res. 9:7-16.

Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, **Kaldhone PR**, Logue CM, and Foley SL. 2012. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PLOS One. 7:e51160.

Roto SM, Park SH, Lee SI, **Kaldhone PR**, Pavlidis HO, Frankenbach SB, McIntyre DR, Striplin

K, Brammer L, and Ricke SC. 2017. Effects of feeding original XPC^{TM} to broilers with a live coccidiosis-vaccine under industry conditions: Part 1. Growth performance and *Salmonella* inhibition. Poult Sci. 96:1831-1837.

Kaldhone PR, Khanjanchi BK, Han J, Nayak R, Ricke SC, and Foley SL. 2017. Draft genome sequences of *Salmonella enterica* isolates containing the incompatibility group I1 plasmids form swine, poultry and human sources. Genome Announc. 5:e01056-17.

Kaldhone PR, Han J, Deck J, Khajanchi B, Nayak R, Foley SL, and Ricke SC. 2017. Evaluation of the genetics and functionality of Incompatibility 1 (IncI1) plasmids from *Salmonella enterica.* Foodborne Pathogen and Diseases. Accepted. DOI:10.1089/fpd.2017.2332.

Book Chapters

Ricke SC, Calo JR, and **Kaldhone PR.** 2015. *Salmonella* control in food production: current issues and perspective in the United States. In: Ricke SC, Donaldson JR, and Phillips CA. (Eds.), Food Safety: Emerging Issues, Technologies and Systems. Elsevier Inc., Oxford, UK. pp 107-133.

Kaldhone PR, Foley SL, and Ricke SC. 2016. *Salmonella* Heidelberg occurrence in foods and egg production. In: Ricke SC, and Gast RK. (Eds.), Producing Safe Eggs. Elsevier Inc., Oxford, UK. pp 235-256.

Ricke SC, Dawoud TM, Shi Z**, Kaldhone PR**, and Kwon YK. 2017. Foodborne *Salmonella* in laying hens and egg production. In Ricke SC, Atungulu GG, Rainwater CE, and Park SH. (Eds.), Food and Feed Safety Systems and Analysis. Elsevier Inc., Oxford, UK. pp 156-171.

Poster Presentations

Kaldhone PR (presenter), Nayak R, White DG, Logue CM, and Foley SL. 2006. Characterization of Antimicrobial Resistance in *Salmonella Heidelberg* from Pre-harvest and Post-harvest Turkey sources. Abstracts of ASM General Meeting, Orlando, FL.

Foley SL, Call V, **Kaldhone PR**, Tyler C, Potter L, Anderson G, Phillips S, Kerdahi K, and Nayak R. 2006. Comparison of Antimicrobial Susceptibility Testing Methods for *Salmonella enterica* serotype Heidelberg isolates. Proceedings of the 2006 FDA Science Forum, Washington, DC.

Lynne AM, **Kaldhone PR**, and Foley SL. 2007. Plasmid mediated antimicrobial resistance in *Salmonella Heidelberg* from turkeys. Poster presented at AVMA/AAAP Annual Meeting, Washington, D.C.

Kaldhone PR (presenter), Nayak R, Lynne AM, White DG, Logue CM, and Foley SL. 2007. Characterization of Antimicrobial Resistance in *Salmonella enterica* serovar Heidelberg from Turkey-Associated Sources. Abstracts of the 67th North Central Branch Meeting of ASM, Marshfield, WI.

Foley SL, **Kaldhone PR**, David DE, White DG, and Lynne AM. 2008. Genetic Characterization of Antimicrobial Resistance in *Salmonella* Serovars Associated with Food Animals and Invasive Human Infection. Abstracts of the ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, Copenhagen, Denmark.

Han J, David D, **Kaldhone PR**, Nayak R, Stefanova R, and Foley SL. 2010. Comparison of *Salmonella Heidelberg* Isolated from Human Patients with those from Animal and Food Sources. Abstracts of the 2010 ASM General Meeting. San Diego, CA.

Kaldhone PR (presenter), Rubinelli P, Baker A, and Ricke SC. 2015. Quantification of short chain fatty acids produced by *Salmonella* under anaerobic condition. Arkansas Association of Food Protection, Fayetteville, AR.

Baker C, Roto S, **Kaldhone PR**, Miller M, and Ricke SC. 2015. Evaluation of two antibody sera against *Salmonella* serovars that exhibit a broad range of surface antigens by flow cytometry. International Poultry Scientific Forum, Georgia World Congress Center, Atlanta, GA.

Kaldhone PR (presenter), Nayak R, Foley SL, and Ricke SC. 2016. Evaluation of Incompatibility group 1 (IncI1) plasmids from *Salmonella enterica.* Arkansas Association of Food Protection, Fayetteville, AR.

Kaldhone PR (presenter), Khajanchi BK, Han J, Ricke SC, and Foley SL. 2017. Virulence evaluation of Incompatibility group 1 (IncI1) plasmids containing *Salmonella enterica*. Abstracts of the South Central Branch Meeting of ASM, Little Rock, AR.

Oral Presentations

Kaldhone PR 2006. Farm to fork look at antimicrobial resistance in *Salmonella enterica* Serotype Heidelberg. Thesis Presentation, University of Central Arkansas, Conway, AR.

Kaldhone PR. 2015. Application of transposon mutant libraries from different *Salmonella* serovars to various stress factors. Graduate Seminar, Department of Food Science, University of Arkansas, Fayetteville, AR.

Kaldhone PR. 2016. Role of Incompatibility group 1 (IncI1) plasmids on *Salmonella enterica* Antimicrobial Resistance and Virulence. Graduate Seminar, Department of Food Science, University of Arkansas, Fayetteville, AR.

Kaldhone PR. 2017. Role of Incompatibility group 1 (IncI1) plasmids on *Salmonella enterica* Antimicrobial Resistance and Virulence. Defense Presentation, Department of Food Science, University of Arkansas, Fayetteville, AR.