Salmonella are less likely to develop plasmid mediated drug resistance due to its resident F-like plasmid and specifically the entry exclusion gene, *traS*.

By

Mopelola Olamide Oluwadare

(Under the Direction of John J Maurer)

ABSTRACT

Salmonella enterica are gram-negative intracellular pathogens responsible for foodborne gastroenteritis worldwide. Like other members of the *Enterobacteriaecea*, there has been a marked increase in *Salmonella*s resistance to third generation β-lactams/cephalosporins, antibiotics commonly used to treat salmonellosis in children. However, resistance to this and other antibiotic is not uniformly distributed among *S. enterica* serovars. The virulence of *Salmonella* is due to a low copy number, FII plasmid (65kb - 100 kb) that contains the ADP-ribosylating toxin, SpvB. This virulence plasmid is present in only a few *Salmonella* serovars (*S.* Choleraesuis, *S.* Dublin, *S.* Enteritidis, and *S.* Typhimurium). We believe that the *spvB*-virulence plasmid excludes other large molecular weight plasmids and may explain why antibiotic resistance is slow to develop in certain *Salmonella* serovars (ex. *S.* Enteritidis vs. *S.* Kentucky). The goal of this study is to determine the contribution of the *Salmonella spvB*-virulence plasmid to plasmid exclusion. From conjugation experiments, *S.* Typhimurium exhibits lower conjugation frequency with F plasmids when the *spvB*-virulence plasmid is present. Furthermore, introduction of cloned FI *traS* into "plasmidless" *S. Typhimurium* LT2 strain and

Escherichia coli DH5α excluded FI plasmid. However deletion of the virulence plasmid *traS* did not affect plasmid exclusion significantly compared to control, *spvB* deletion. In addition, differences in F plasmid conjugation in natural *Salmonella* isolates did not correlate with F and virulence plasmid genotype. The slow development of antibiotic resistance in certain *Salmonella* serovars may be attributed to entry exclusion mediated by different mechanisms encoded by either plasmid or chromosomal genes. (248 Words)

KEY WORDS: *Salmonella*, *v*irulence plasmid, antibiotic resistance, entry exclusion

SALMONELLA ARE LESS LIKELY TO DEVELOP PLASMID MEDIATED DRUG RESISTANCE DUE TO ITS RESIDENT F-LIKE PLASMID AND SPECIFICALLY THE ENTRY EXCLUSION GENE, *traS*.

By

MOPELOLA OLAMIDE OLUWADARE

B.S., University of Georgia, 2009

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTERS IN VETERINARY AND BIOMEDICAL SCIENCES

ATHENS, GA

2013

SALMONELLA ARE LESS LIKELY TO DEVELOP PLASMID MEDIATED DRUG RESISTANCE DUE TO ITS RESIDENT F-LIKE PLASMID AND SPECIFICALLY THE ENTRY EXCLUSION GENE, *traS*.

By

MOPELOLA OLAMIDE OLUWADARE

Major Professor: John Maurer

Committee: John Maurer
Margie Lee
Susan Sanchez

Electronic Version Submitted:

Maureen Grasso
The University of Georgia
May 2013

DEDICATION

This thesis is dedicated to my parents and sister for their continuous encouragement which always reminds me that even the largest task can be accomplished if it is done one step at a time

ACKNOWLEDGMENTS

I would like to thank Dr. John Maurer for giving me this opportunity. His immense knowledge and expertise in all things *Salmonella* has helped me with various challenging aspects of my research.

I would like to express my sincere appreciation to Dr. Margie Lee and Dr. Susan Sanchez for continuous guidance throughout my Master's program.

I would also like to thank Dr. Ying Cheng for her assistance and patience with my questions regarding routine experiments.

Special thanks to my undergraduate research students: Erin Nawrocki, Natasha Patel, Chandni Ranani, Raja Atchutuni, and Jose Portillo. It has been a pleasure working with these individuals and seeing their talents emerge in different aspects of the experiments.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

Infections with *Salmonella enterica* are believed to account for 20 million cases of disease and 200,000 deaths per year, worldwide (9). In the developed world, *Salmonella* is mainly encountered as a foodborne pathogen, present in fecally-contaminated poultry, meat, eggs, dairy products, fruits and vegetables (74, 75, 105). According to a recent Center for Disease Control (CDC) estimates, non-typhoidal *Salmonella* causes over a million illnesses each year and it accounts for 35% of foodborne illnesses resulting in hospitalization in the United States alone. Although most *Salmonella* infections are largely treatable with antibiotics, a disturbing trend is the rise of multi-drug resistant (MDR) *Salmonella* (1, 44, 61), especially to fluoroquinolones, β-lactams, and cephalosporins; antibiotics frequently used to treat these infections (159). According to the National Antimicrobial Resistance Monitoring Service's (NARMS) 2009 Annual Report, 9.5% of non-typhoidal *Salmonella* isolates tested were resistant to three or more classes of antibiotics (10). However, some *Salmonella* serovars (ex. *S.* Enteritidis) remain susceptible to all antimicrobials tested in the NARMS panel (160).

Resistance to antimicrobial agents can be conferred by the transfer of mobile genetic elements, such as plasmids, transposons and integrons (81). Integrons are genetic elements that capture antibiotic resistance genes; building a tandem array of distinct antibiotic resistance genes into single multi-drug resistance locus (69). Class 1 integrons are frequently found in bacteria that colonize food animals (62), and abundant in some farm environments (120). However, a few *Salmonella* serovars such as *S.* Enteritidis are slow to develop antimicrobial resistance despite their presence in environments rich in class 1 integrons and antibiotic resistance genes.

The question that arises is why are some *Salmonella* serovars slow to develop antibiotic resistance compared to others?

Some *Salmonella* serovars contain a large molecular weight plasmid that enables it to proliferate in the reticuloendothlelial system and cause systemic infection (66). This plasmid is present in the non-typhoidal *Salmonella* serotypes *S.* Choleraesuis, *S.* Derby, *S.* Dublin, *S.* Enteritidis, *S.* Pullorum, and *S.* Typhimurium (21). The key virulence factor associated with these plasmids is SpvB, an ADP-ribosylating toxin that interferes with phagocyte function in the host (101). The *spvB-v*irulence plasmid is a conjugative, FII-like plasmid (8, 154). Genomic comparisons of the *S.* Typhimurium *spvB*-virulence plasmids to similar plasmids in *S.* Choleraesuis, *S.* Dublin, and *S.* Enteritidis, has revealed large deletions within the *tra* operon of *S.* Choleraesuis and *S.* Dublin virulence plasmids, including *traS* which is responsible for entry exclusion of plasmids (78, 169). In addition, the *S.* Dublin *spvB*-virulence plasmid has acquired a unique 11kb segment in place of the *pef* (plasmid encoded fimbriae) operon that contains *fae* fimbrial operon *(31),* and it has also lost *oriT,* FIB *repA*, and serum-resistance factor *rsk* (31). The genetic organization of *S*. Typhimurium and *S*. Enteritidis *spvB*-virulence plasmids are similar, except for a single base pair substitution that inactivates *traY* in *Salmonella* Enteritidis and a 22 kb deletion in a region that contains *traT* (surface exclusion) and *traX-finO* (78). *Salmonella* Choleraesuis virulence plasmid varies in size between ~50kb to 110kb. These plasmids are neither mobilizable nor self-transmissible because they are missing *oriT* and all of the *tra* genes except for *finO, traX, traH, traT*, and *traD* (78, 169).

Like lysogenic bacteriophages, plasmids have mechanisms for preventing "super infection" of its bacterial host with similar plasmids. One mechanism, incompatibility, involves

interference with initiation of plasmid replication (125) or partitioning of competing plasmids to the dividing daughter cells (20). The second mechanism, exclusion involves interference with plasmid transfer. Exclusion is generally observed for plasmids belonging to the same incompatibility group. For plasmids belonging to incF incompatibility group, *traS* and *traT* are primarily responsible for plasmid exclusion (57). We believe that the *spvB*-virulence plasmid *traS* is an important genetic barrier to development of plasmid-mediated antimicrobial resistance. The purpose of this study is to determine whether the virulence plasmid can actively exclude other large molecular weight plasmids from entering the cell by the means of *traS.* If this is the case, it stands to reason that natural *Salmonella* isolates possessing a virulence plasmid are less likely to acquire other large molecular weight plasmids including those that mediate plasmid-mediated antibiotic resistance.

LITERATURE REVIEW

Salmonella Pathogenesis and the Virulence Plasmid

Salmonella was first described in 1885 as a gram-negative, motile, rod-shaped facultative anaerobe. There are two species within the genus *Salmonella*: *S. bongori and S. enterica* and 7 subspecies (I-VII). *S. enterica sp. Enterica* accounts for 99% of *Salmonella* infections (21). *Salmonella* is responsible for a diverse range of diseases: from life-threatening typhoid fever to gastroenteritis and septicemia. *Salmonella enterica* accounts for 20 million cases of disease and 200,000 deaths per year, worldwide (9). In the United States, *Salmonella* infection is the leading cause of hospitalization and death associated with foodborne illnesses, with reported 1.2 million illnesses each year (136). In the developed world, *Salmonella* is mainly encountered as a foodborne pathogen, present in fecally-contaminated poultry, meat, eggs, dairy products, fruits and vegetables (17, 73, 105). Manifestation of disease such as gastroenteritis, caused by non-typhoidal *Salmonella* serovars, and enteric fevers, caused by typhoidal *Salmonella* serovars in immune-competent individuals have been the subject of current research. Many *Salmonella* virulence genes are clustered as genomic islands referred to as pathogenicity islands (SPI) (135). The *Salmonella* SPI1 encodes a type III secretion system responsible for injecting *Salmonella* effector proteins directly into the cytosol of the host cell, which, in turn, manipulates the host cell response (170). In both the M cells and enterocytes, *Salmonella* effector proteins, SipA and SipC, induce actin reorganization which results in ruffling formation at the surface of the epithelial layer and uptake of the bacterial cell (113). In addition to actin reorganization, effector proteins also trigger nuclear responses that results in increased expression of chemotactic factors

such as interleukin-8 (112) which leads to infiltration of neutrophils in the lamina propria. As the inflammatory reaction progresses, neutrophils are directed into the intestinal lumen leading to the characteristic symptom of diarrhea following nontyphoidal *Salmonella* infection in humans (100).

The virulence of various *Salmonella* serovars, such as *S.* Typhimurium (66), *S.* Enteritidis (27), *S.* Choleraesuis, and *S,* Dublin partly depends on the presence of large molecular weight plasmids (65-100kb) (77). These plasmids contain a 7.8 kb region, *spv* (*Salmonella* plasmid virulence) responsible for proliferation of bacteria within the reticuloendothelial system (65, 147). The *spv* locus contains five genes identified as *spv*A-D and *spv*R (67). The divergently transcribed gene *spv*R (3) has been shown to be a positive regulator, in combination with the alternate sigma factor RpoS (99), of *spvA-D* expression through its binding to the promoter region upstream of *spv*A (47). *spv*A is induced in the exponential phase of *Salmonella* growth in a medium designed to mimic the intracellular environment of mammalian cells (166). The expression of *spvB* in *Salmonella* occurs *in vivo* only after the invasion of epithelial cells or phagocytosis by macrophages (111). SpvB is an ADP-ribosylating toxin that inactivates F-actin in macrophages (101), resulting in cytopathic effects such as the rounding up and detachment of the cells (149). This toxin is delivered into the macrophage through SPI-2 type III secretion system (23).

However, the virulence plasmid does not appear to be essential to *Salmonella* pathogenesis as *Salmonella* can survive in macrophages without this virulence plasmid (48) and this plasmid is absent in several *Salmonella* serovars associated with enteric fevers (106). The *spv* operon provides a small, but crucial growth advantage in bovine and human monocyte-

derived macrophages (102). The spv operon contains an internal promoter which leads to the regulation of individual Spv proteins. The Spv proteins may be required at different time points during infection with evidence that showed that SpvA and SpvB levels declined after reaching early-stationary phase while SpvC and SpvB levels continued to increase (166). Other virulence loci present on the virulence plasmid are the *pef* genes (plasmid-encoded fimbriae) which mediate adhesion to the small intestine and contributes to fluid accumulation in infant mouse model (16) but it is not essential for pathogenesis in other animal models (80). The *spvB* virulence plasmid also contains *rck* which encodes a 17-KD outer membrane protein that confers complement resistance and control of serum resistance genes (153). This protein also has a binding site for the C3 and C5 membrane attack complex (76). The *rck* locus in *S*. Typhimurium alone does not confer serum resistance but instead enhances the ability of "smooth" *S*. Typhimurium to grow in serum. In short, it has been shown that the *rck* genes provide protection against complement in both *Escherichia coli* (33) and *S*. Typhimurium.

Genetic Exchange in Bacteria

In bacterial populations, exchange of DNA from one bacterium to another occurs in several ways. Many bacteriologists in the 1940s believed that bacteria could adapt to their environments by a direct change and could then pass on the change to the offspring. In 1943, Salvador Luria and Max Delbruck (103) demonstrated that bacterial cultures will retain resistance to the action of a virus even if it is sub-cultured for many generations in the absence of the virus. This demonstration follows the principle outlined by Darwinian molecular basis of heredity, which dictates that properties of organisms are determined by their DNA sequences and as organisms multiply, changes in the DNA sequence could occur randomly.

Fred Griffith in 1928 made the first demonstration of genetic exchange known as transformation. Transformation is gene transfer resulting from the uptake of naked DNA and the subsequence, stable inheritance of a trait acquired by the "transformed" cell from the DNA of a donor cell. Another form of genetic inheritance in bacteria is transduction. Transduction was discovered by Zinder and Lederberg in 1953 when they demonstrated that a *S.* Typhimurium phage P22 could transmit genetic information one cell to another.

Finally, bacteria can exchange genetic information through a process known as conjugation. This mechanism of genetic exchange was first discovered by Lederberg and Tatum in 1946. Conjugation is the transfer of DNA that is mediated through the physical contact between donor and recipient cells. Generally, this genetic exchange involves an extra-chromosomal, autonomously replicating genetic element referred to as a plasmid. It is the plasmid that mediates genetic exchange between bacterial cells. The plasmid is capable of mobilizing itself, other plasmids, and the chromosome.

Why it is important for bacteria cells to conjugate? One advantage of conjugation comes from the recombination of competing beneficial mutations into one lineage (35, 51). Conjugation also facilitates natural selection by generating useful variation (158), and by breaking down negative associations (15).

Plasmid Biology

Bacterial plasmids are self-replicating, extra-chromosomal DNA that carry genetic information that is useful in stress conditions and sometimes lost in non-selective environments. Plasmids usually carry information in genes encoding for carriage and spread of antibiotic

resistance, metal ions, ultraviolet light, virulence, bacteriocin-production, degradation of organic toxic compound, and finally, the ability to transfer from one bacteria cell to another (36). Naturally occurring plasmids are self-transmissible (conjugative), mobilizable, or non-conjugative. Conjugative plasmids encode all the functions needed to move the plasmid among cells and mobilize other plasmids incapable of transmission (142).

Plasmids can exist either as a circular or linear forms and range in size from one kilobase to more than a megabase. *Borrelia burgdorferi* for instance, contains both linear and circular plasmids (144). Circular plasmids are supercoiled when extracted and can be easily isolated and distinguished from linear fragmented chromosomal DNA. Plasmids are classified into two groups on the basis of whether more than one plasmid can co-exist within a bacteria cell (compatible) or not (incompatible). Plasmids of the same group are considered incompatible with each other within the same bacterial cell if similar *rep* (replication) and/or *par* (partitioning) proteins are shared (36). The term '*inc*' and '*rep*' are used to describe these plasmid types.

Plasmids must have the ability to replicate in order to exist independent of the chromosome. There are three types of ways circular plasmids replicate: theta type, strand displacement, and rolling circle (39). Plasmid replication generally begins for most gram negative organism by the means of the theta mechanism. Rep proteins interact with adjacent AT-rich region containing sequence repeats which facilitate opening of the strands by transferring DnaB-DnaC complex to the *oriT* (22). DNA gyrase mainly participates in the opening of DNA double strand followed by the synthesis of RNA primers by primase (86). DNA synthesis of both strands is coupled by DNA polymerase II and single-strand binding proteins (54). The leading strand is synthesized continuously, while the lagging strand is

discontinuously made on the opposite side. Theta replication in gram negative bacteria is unidirectional, meaning replication terminates when the replication fork gets back to the origin. Termination occurs at *ter* regions (37) in conjunction with several host proteins (Tus) which forms a complex that inhibits helicase activity in the replisome (92). Plasmid replication is concluded by the formation of cantenates containing gaps in the daughter cell which could be resolves by type II topoisomerase (118).

To avoid being lost from dividing cells, plasmids carry partition systems to accurately move and position genetic material to daughter cells during cell division. Plasmid partition systems require three essential components. Each locus must contain a centomere-like DNA site and two proteins: a motor protein and a centromere-binding protein (137). The *par* locus consists of cis-acting *parS* and trans-acting genes often referred to as *parA* and *parB* (14). The *parS* site is where two copies of the plasmid are pulled apart during partitioning and it's usually identified by multiple DNA repeat elements (19). The first stage of plasmid partitioning occurs when ParB proteins form a dimer with ParS which leads to the formation of higher-order partition complexes (59). This partition complex has been speculated to be involved in the assembly of motor proteins which mediates plasmid separation (138). DNA partition is driven by the formation of filaments by ParM in an ATP dependent manner which is captured by ParR-centromere complex (138). There are two types of partitioning loci: Type I and Type II. The partitioning loci are differentiated by formation of the final segrosome which could be by means of actin-like protein (type II) (24) or by a Walker-type protein (type I)(131).

Among the many different plasmid types discovered to date, the F (56), R1 (123), RK2 (98), R6K (49), and RSF1010 (114, 130) of *E. coli* are the best studied and often used as model

systems for understanding plasmid replication (49, 98, 114, 124), partitioning (63, 98), maintenance (40, 152) and transfer (56). The *E. coli* F plasmid is a 100 kb plasmid belonging to the IncFI incompatibility group. This plasmid is self-transmissible and contains 40 genes clustered together in a 33.3 kb transfer region (*tra*) that is involved in physical transfer of plasmids between bacterial cells. Twenty of these 40 genes are essential for conjugation. There are five classes of *tra* genes responsible for pilus synthesis (*traA, traB, traC, traE, traF,traG, traH, traK, traL, traQ, traU, traV, traW, traX,* and *trbC*), surface exclusion (*traS*), mating pair stabilization (*traG* and *traN*), regulation (*finO* and *traJ*) and plasmid transfer (*traD, traY,* and *traI*) (56). The F plasmid transfer begins when the F pilus makes contact with one or more recipient cells which leads to the formation of a mating pair aggregate (56). The F pilus retracts either by pilin subunit rearrangement (52, 156) or depolymerization (109) to bring donor and recipient adhesion sites into opposition to form a pore. The OmpA protein along with TraG and TraN stabilize the pore which leads to the stabilization of the mating pair aggregates (41). Over time, the mating pair is stabilized and will not desegregate even with the addition of sodiun dodecyl sulfate (SDS) (7). There are two main genes reported to be involved in mating pair stabilization: *traN* in the outer-membrane and *traG* in the inner-membrane of an F plasmid (107). Genetic analysis indicates that TraN recognizes lipopolysaccharide and OmpA in the recipient cell which acts as a cell surface adhesin during mating pair stabilization (7). TraG which spans the inner membrane several times has been shown to be responsible for two functions during conjugation. The N-terminal is essential for F pilus assembly while the C-terminal participates in mating aggregate stabilization (50). It has been suggested that TraG in the donor cell might be

translocated into the recipient cell which then binds TraS whereby blocking redundant conjugative DNA synthesis and transport (13).

Two processes are involved in the prevention of conjugation among cells bearing closely related plasmids of the same exclusion group. The first is surface exclusion which is encoded by *traT* and entry exclusion encoded by *traS* (13). TraT is an outer membrane lipoprotein that confers serum resistance (116) and blocks conjugation by preventing the formation of stable mating aggregates (146). TraS, which is localized in the inner membrane, functions to inhibit DNA transfer even after the formation of stable mating aggregates (57) by recognizing its cognate TraG in the donor cell.

Within the donor cell, site and strand specific nicking of the origin of transfer by endonucleases occurs which initiates donor conjugal DNA synthesis (161). After nicking, the two DNA strands of donor plasmid are unwound by DNA helicase I which progressively displaces the bound strand into the recipient cell. Simultaneously during F plasmid transfer, donor cell replacement strand synthesis is catalyzed by DNA polymerase III using un-translated RNA as primer (94). Conjugative DNA complementary strand synthesis in the recipient cell involves synthesis of DNA complementary to the transferred strand and circularization of plasmid DNA. The transferred DNA is replicated to give a linear double-stranded molecule which is then converted to circular forms found in the cytoplasm (85). The *oriT* region includes four binding sites for several proteins (29) including TraI helicase I which is required for physical transfer of the F plasmid (110), an inner membrane protein, TraM (6) and finally TraY which forms a nucleo-protein complex with the *oriT* for efficient nicking needed for rolling circle replication (122). The rolling circle method of transfer begins when Rep proteins

recognize a sequence known as double strand origin (DSO) on the DNA and makes a single stranded break in the sequence (97). Rep protein nicks the DSO and recruits DNA helicase and other replication proteins such as single-stranded DNA binding proteins and DNA polymerase III. As the Rep proteins remain attached to 5' phosphate end, DNA polymerase III uses the free 3' hydroxyl end at the break as a primer to replicate around the circle. The Rep protein separates the old and newly synthesized leading strand by nicking and then it's converted to dsDNA form using single-strand origin and host proteins (45, 90, 91).

In summary, conjugation begins when a pilus establish contact with a recipient cell, which leads to mating pair formation. After the mating pair formation, the pair or aggregates are stabilized. A single strand of DNA is transferred 5' to 3' direction starting at the origin of transfer. The process concludes with circularization of the transferred strand and the synthesis of complementary DNA in both the donor and recipient cells.

The IncFII plasmid is closely related to the Inc FI plasmid (95). Plasmid incompatibility refers to the inability of two plasmids to be stably inherited in the same cell line (36). Incompatibility is established due to the fact that sharing of one or more common elements of plasmid replication or partitioning systems (126). This was historically described in the early 1960s when it was noted that the F plasmid in *Escherichia coli* strains either exist autonomously in F^+ strains or integrated in Hfr strains but never in both strains simultaneously (43). The FII plasmid is approximately 94.5kb and it is self-transmissible. In contrast with FI plasmids, FII plasmids encode resistance to chloramphenicol, streptomycin, tetracycline, and sulfonamides (119, 157). FII plasmids contain two components genetically and physically that distinguish it from other plasmid types. The first component is a resistance transfer (RTF) segment that

contains genes for self-transmissibility (*tra* genes) and autonomous replication (*rep*), and the second a resistance locus (r-determinant) that contains most of the resistance genes (167). In comparison with the FI plasmid, FII plasmids contain a leading region interrupted by transposon Tn*10*, which confers resistance to tetracycline. The FI and FII plasmids share analogous replication functions but genes are located in different positions on the plasmids (167). The homologous positions shared between FI and FII plasmids are the leading region, part of *repA*, and some of the *tra* operon. The gene products of FI's *traA, B, C, D, E, F, G, H, K, L, N, T, U, V* and *W* share homology and could be functionally replaced by corresponding gene product by FII plasmid (5, 53, 115, 162, 165). The gene products of the FI's *traI, traJ, traM, traS* and *traY* however; are not replaceable by those produced by FII plasmid (5, 163, 164). The differences between the FI and FII plasmids could further be explained at the nucleotide level. For instant, *traN*, which is involved in mating pair stabilization in both FI and FII conjugation by binding to the LPS and OmpA of recipient cells (95, 96), is similar (93 % identity at nucleotide level) between FI and FII in the N and C end of the gene, but contains a central variable region (7% identity at the nucleotide level) (167). Furthermore, *traS*, which is involved in entry exclusion as discussed earlier, is almost completely non-homologous when compared with FI and FII plasmids. The TraS protein differs in molecular weight and is not interchangeable within FI and FII plasmids (71). The basic genetic organization of both FI and FII *tra* region is very similar, but differences in molecular weight and functional irreplaceability of proteins further differentiate FI from FII plasmids.

The Physical Map and Distribution of *spvB*-Virulence Plasmids in *Salmonella*

The *spvB*-virulence plasmid is not uniform in its distribution among *Salmonella* species and serovars (21). The plasmid is found in *S. enterica* subspecies I, and even within this subspecies, its distribution is non-uniform between and within serovars (21, 26, 168). The *spvB*-virulence plasmid has been reported in *Salmonella enterica* serovars Choleraesuis, Dublin, Enteritidis, Paratyphi C, Pullorum, and Typhimurium (21). *S.* Typhimurium LT2 strain(s) virulence plasmid serves as the prototype to which all other *spvB*-virulence plasmids are compared (26, 93). The virulence plasmids in other *Salmonella* serovars differ from the prototype Typhimurium virulence in the deletions spanning the *tra* region of these plasmids (32). In Fig. 1, *S*. Typhimurium *spvB*-virulence plasmid is aligned with *S*. Enteritidis, *S*. Dublin, and S. Choleraesuis *spvB*-virulence plasmid to highlight deletions in each *Salmonella* serovar. Genomic comparisons of the *S.* Typhimurium *spvB*-virulence plasmids to similar plasmids in *S.* Choleraesuis, *S.* Dublin, and *S.* Enteritidis, has revealed large deletions within the *tra* operon of *S.* Choleraesuis and *S.* Dublin virulence plasmids, including *traS* which is responsible for entry exclusion of plasmids. In addition, the *S.* Dublin *spvB*-virulence plasmid has acquired a unique 11kb segment in place of the *pef* (plasmid encoded fimbriae) operon, and lost *oriT,* FIB *repA*, and *rsk* (155). The genetic organization of *S*. Enteritidis *spvB*-virulence plasmid is similar to the prototype *S.* Typhimurium LT2 virulence plasmid (132), except for a single base pair substitution that inactivates *traY* and a 22 kb deletion in a region that contains *traT* (surface exclusion) and *traX-finO* in *S.* Enteritidis (117). *Salmonella* Choleraesuis virulence plasmid varies in size between ~50kb to 110kb. These plasmids are neither mobilizable nor self-

transmissible because they are missing *oriT* and all of the *tra* genes except for five (*finO, traX, traH, traT*, and *traD*) (79), respectively.

The three *spvB*-virulence plasmid of *S*. Enteritidis, *S*. Dublin, and *S*. Choleraesuis are incompatible with *S*. Typhimurium *spvB*-virulence plasmid which means they belong to the same incompatibility group (127). It has been proposed that the *spvB*-virulence plasmid of *S*. Enteritidis, *S*. Typhimurium, and *S*. Choleraesuis is an ancestor of IncFI plasmid while *S*. Dublin emerged from the IncFII plasmid (31). Despite the common properties shared among *spvB*-virulence plasmids, each serovars contain unique sizes specific to a *Salmonella* serovar which is due to *tra* deletions and additional genes encoding drug resistance.

Multidrug Resistance in *Salmonella*

Multidrug resistance in *Salmonella* enterica serovars is a major problem worldwide. According to the National Antimicrobial Resistance monitoring system in 2003, 40% of *Salmonella* isolates in ground beef are resistant to at least 6 antimicrobial classes tested. The consequence of the use of antimicrobial drugs in food animals is the increase in antibiotic resistance in the enteric bacteria that reside in these animals (150). Recent studies showed that environmental *Salmonella* isolates had lower levels of resistance than fecal isolates of calves and poultry (89). In addition, an antimicrobial susceptibility analysis revealed that human strains were susceptible to all the antimicrobials in the NARMS panel while animal isolates showed a wide range of resistance to two or more antimicrobials (143). This provides compelling evidence that antimicrobials used to boost food animal's growth and health significantly contributes to enteric bacteria resistance to multiple antibiotics. According to the Food and Drug Administration 2009 report, 29 million pounds of antimicrobial drugs are used annually in food

animals. It is not surprising that enteric bacteria could then acquire resistance to commonly used antimicrobials (18, 60) and humans could in turn acquire multidrug resistant bacteria through consumption of undercooked contaminated food. Previous reports showed that human and food animal isolates carried class I integrons (58, 128) which indicates a link of the movement of antibiotic genes from food animals to humans.

How do enteric bacteria, specifically *Salmonella* acquire these resistance genes? Mentioned often is the appearance of mobile elements which contain one or more antibiotic resistance genes. Class 1 integrons are notable by the presence of one or more antibiotic resistance genes (145), an integrase (IntI) responsible for site-specific recombination (108), an adjacent *attI* integration site recognized by the integrase and also serves as the receptor site for the cassette integration and finally, a promoter for the expression of the inserted gene cassettes. The most notable feature of integrons is its ability to recognize and capture antibiotic resistance genes. (34, 68).

The recombination machinery of integrons makes it a natural cloning and expression vectors for antibiotic resistance particularly in gram negative organisms such as *Salmonella.* Integrons are therefore one means by which some *Salmonella* serovars acquire multidrug resistance. Recent studies have shown that some *S.* Typhimurium and *S.* Choleraesuis isolates harbor a chimeric virulence/resistance plasmid which confers multidrug resistance (30, 64). In the most recent NARMS data report on *Salmonella* antimicrobial susceptibility, 93% of *S.* Enteritidis isolated from chicken breast were pan-susceptible, while only 14% of *S.* Kentucky isolates were susceptible to the panel of 15 antimicrobials (10) . This susceptibility patterns once

again draws the question why some *Salmonella* serovars remain susceptible to multiple antibiotics even though they may exist in the presence of gene elements such as integrons (121). Although, integrons are able to capture and express resistant gene cassettes, their mobility is limited between plasmids and transposons that ferry this element between cells. Cell to cell transfer is dependent on other genetic mechanisms such as conjugation.

Conjugation, as discussed earlier is the means by which a donor cell transfers copy of its plasmid to a recipient cell. The process of conjugation can be regulated by the entry exclusion gene *traS*. TraS blocks redundant transfer of plasmid DNA to a recipient that might have already contained the donor plasmid. Therefore, some *Salmonella* serovars harboring the FI virulence plasmid may remain susceptible to multiple antibiotics because TraS blocks conjugative entry of plasmid carrying integrons expressing multidrug resistance genes. This study seeks to investigate the role of TraS and the virulence plasmid in acquisition of antibiotic resistance by *Salmonella*.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains and plasmids used in this study are described in Table 1. *Escherichia coli* strains XK1200 and MC4100 served as bacterial host for F1 and FII plasmids pOX38-km and R100 respectively. Bacteria cultures were grown overnight at 37˚C in Luria-Bertani (LB) medium with appropriate antibiotic at the following concentrations: tetracycline (10μg/ml), kanamycin (30μg/ml), naladixic acid (64μg/ml), rifampicin (64μg/ml), and chloramphenicol (64μg/ml). *Salmonella* Typhimurium pSLT$^-$ strain was constructed by transducing pStLT203 Ω *parA*::Km (151) into *S.* Typhimurium strain LT2 using P22 HT *int* (129). A kanamycin-resistant, *S.* Typhimurium transductant was passaged in LB without antibiotics and subsequently screened for sensitivity to kanamycin (151). Loss of pSLT virulence plasmid was confirmed by PCR (148). Defined deletions in *traS* or *spvB* were introduced into *S.* Typhimurium virulence plasmid using λ *red* recombineering approach described by Datsenko and Wanner (38). PCR primers and conditions used to construct *red* knockouts are described in Table 2 and PCR section described below. *Escherichia coli* and *S.* Typhimurium LT2 strains were transformed using electroporation protocol described by Dower et al (42).

Mating Assays. Conjugations were performed as described by Ahmer et al (8). Bacteria were grown as standing overnight cultures at 37˚C in Luria-Bertani (LB) broth. A total of 5μL of donor strain and 50μL of recipient strain were added to 5ml of 10 mM $MgSO_4$. The cell suspension was filtered through a 0.45μm pore size cellulose filter membrane, and the filter was

placed on an M9 minimal plate containing 0.2% glucose, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 1μg/ml thiamin, and 20μg/ml leucine, cell side up. After overnight incubation at 37˚C, the filter was aseptically removed from the plate, added to 5ml of 10 mM $MgSO_4$ and vortexed to remove bacteria from the filter. 10-fold dilutions of the cell suspension were plated on LB plates containing the appropriate antibiotic for selecting recipients or transconjugants. The conjugation frequency was determined from the number of transconjugants divided by recipients. The conjugation frequency was measured by averaging the results of duplicate mating from 3 separate trials using the formula: total transconjugants/ total recipient.

PCR. Primers targeting *Salmonella* Typhimurium LT2 virulence plasmid genes *spvB*, pSLT FII *traS*, and F-plasmid FI *traS* genes were designed using the DNA software analysis program Generunner 3.1 (Hastings Software Inc.; Hastings-on-Hudson, NY). See Table 2 for description of primer sequences, PCR conditions, and expected size for PCR amplicon. Primers were prepared by the University of Georgia Molecular instrumentation Laboratory. Genomic DNA was prepared as described by Sambrook et al. (133). The PCR reaction mix contained: 2 mM $MgCl_2$, 0.1mM primer, 0.2mM nucleotide and 0.5 unit Taq DNA polymerase (Roche Molecular Biochemicals; Indianapolis, IN). PCR was performed using the Rapidcycler hot-air thermocycler (Idaho Technology; Salt Lake City, UT) with denaturation set at 93°C for 1 min; annealing as described in Table 2 for each primer set for 1sec, and primer extension at 72°C for 15sec for 30 cycles. Probes, for DNA: DNA hybridization, were prepared by PCR, substituting standard nucleotides with digoxigenin-labeled nucleotides (Roche Molecular Biochemicals) in the PCR reaction mix.

Plasmid Extraction. Bacterial isolates were revived from freezer stocks and grown on tryptic soy agar at 37°C overnight. A single colony was used to inoculate 6 mL Superbroth and incubated overnight at 37°C with aeration (235 rpm). Plasmids were extracted using the FosmidMAX DNA Purification Kit (Epicentre; Grand Island, NY). DNA samples were stored at -20°C. Plasmid DNA was separated by gel electrophoresis on a 0.5% agarose gel at 44 V in E buffer (40 mM Tris-acetate, 2 mM sodium EDTA) for 16.5 h (88). Gels were stained with 1X Sybr Gold (Invitrogen) in 1X TAE buffer while shaking for 30 min at 40 rpm. Images were acquired using a digital camera and UV transillumination (Molecular Imager Gel Doc XR System, Bio-Rad; Hercules, CA).

DNA: DNA Hybridization. Agarose gels were stored at 4°C before DNA transfer to nylon membranes. Gels were treated with HCl, followed with NaOH treatment (6), before the single stranded DNA was transferred to a nylon membrane using a vacuum blotter (Bio-Rad). Single stranded DNA was UV-cross linked onto the nylon membranes. Membranes were covered in aluminum foil and stored at -80°C. DNA: DNA hybridization was performed as described by Sambrook et al. (133) with hybridization and washes performed at 68°C. Bound probe was visualized with anti-digoxigenin alkaline phosphatase conjugate and the nitroblue tetrazolium/5-bromo-4-chloro-3indolylphosphate (BCIP) substrate as described by the manufacturer (Roche Molecular Biochemicals).

Statistical Analysis. ANOVA single factor statistical test was used to determine whether the differences observed were significant.

RESULTS

Plasmid profile and prevalence of *spvB*-virulence plasmids in *S. enterica* serovars *S.* Dublin, *S.* Enteritidis, *S.* Kentucky, and *S.* Typhimurium. We identified single to multiple, large molecular weight plasmids (>55 kb) among *S. enterica* serovars screened (Fig. 2 & 3; and summarized in Table 3). In *Salmonella* serovars *S.* Dublin, *S.* Enteritidis, and *S.* Typhimurium one of the large size plasmids was identified as the *spvB*-virulence plasmid by Southern analysis (Fig. 2). However, none of the *S.* Dublin *spvB*-virulence positive isolates contained the entry exclusion gene *traS* (Table 2). Ninety-three percent of *S.* Kentucky isolates contained one or more, large molecular weight plasmids (Table 2), but none were identified as *spvB*-virulence plasmid (Fig. 3). The prevalence of other large size plasmids (>55 kb), among *spvB*-virulence positive isolates varied from 7.7-62 % among *S. enterica* serovars screened (Table 2). There was a statistically significant difference in the distribution of these plasmids among *S.* serovars screened relative to the prevalence of *spvB*-virulence plasmid and specifically the plasmid's resident *traS* in these same isolates (Table 1; Chi-Squared test, $p<0.05$). In addition to determining the prevalence of *spvB*-virulence plasmids in *Salmonella* serovars, we also examined what role the *spvB*-virulence plasmid plays in the exclusion of F plasmids in *S.* Typhimurium.

Contribution of the *spvB*-virulence plasmid and specifically its resident FII *traS* to exclusion of F plasmids in *S.* Typhimurium. The contribution of the recipient strain's genetic background in F plasmid transfer was examined, specifically focusing on *spvB*-virulence plasmid and the plasmid's resident *traS*. Several *S.* Typhimurium LT2 strains were created that were

pSLT$^-$, or contained targeted deletions in pSLT *traS* or another plasmid gene *spvB*, unrelated to plasmid transfer, exclusion, or incompatibility and serving as a negative control. In addition to these strain constructs, *S.* Typhimurium LT2 pSLT$^-$ and *E. coli* DH5α strains with the cloned FI *traS* (pRS31) (12) served as FI plasmid exclusion control. The *spvB*-virulence plasmid significantly reduced FI and FII plasmid transfer 10 to 100-fold into *S.* Typhimurium LT2 recipient strain background from comparisons of *S.* Typhimurium LT2 wild-type strain vs. LT2 pSLT$^-$ (Table 4; $p<0.05$). Introduction of pRS31 (FI *traS*$^+$) into *S.* Typhimurium LT2 pSLT$^-$ strain restored plasmid exclusion of both F plasmids (Table 4; $p<0.05$), but exclusion was most pronounced for the FI plasmid pOX38-km from *S.* Typhimurium LT2 strain (Table 4; EI 1,699.39 vs. 3.51). Similarly, pRS31 with FI *traS*$^+$ was able to exclude F1 plasmid pOX38-km from *E. coli* DH5α and exhibited plasmid specificity in its exclusion of FI vs. FII plasmids (Table 4) (12). If pSLT *traS* is responsible for F plasmid exclusion in *S.* Typhimurium LT2, then deletion of this gene is expected to significantly increase plasmid transfer compared to wild-type or *S.* Typhimurium LT2 strain with a deletion in another, unrelated plasmid gene, *spvB.* The *traS* deletion did not significantly alter plasmid transfer frequency compared to either the wild-type or *spvB* deletion strain for F plasmids pOX38-km (FI) or R100 (FII) (Table 4). While significant plasmid transfer was not observed, further experiment was conducted to determine if the same trend will be observed in natural *Salmonella* isolates.

Contribution of the *spvB*-virulence plasmid and specifically its resident FII *traS* to exclusion of F plasmids in natural *Salmonella* isolates. We examined the ability of *spvB*-virulence plasmid to exclude F plasmids in natural *Salmonella* isolates. Critical to this study was

the identification of a natural *S.* Typhimurium clone (83) with and without the *spvB*-virulence plasmid; several *Salmonella* isolates with the virulence plasmid ± pSLT FII *traS* and an isolate negative for the virulence plasmid (Table 1). The natural isolates exhibited 10-100 fold lower conjugation frequency ($p<0.05$) for FII and FI plasmids, respectively compared to *S.* Typhimurium LT2 recipient (Table 5). Absence of either the virulence plasmid or pSLT FII *traS* in natural isolates did not correlate with an increase in conjugation frequency for either F plasmid (Table 5). *Salmonella* serovars *S.* Choleraesuis and *S.* Dublin exhibited the extremes in plasmid exclusion (EI 0.08 vs. 146.69, respectively Table 5).

F plasmid exists autonomously in *S.* Typhimurium LT2 containing IncF, *spvB*-virulence plasmid. The *spvB*-virulence plasmid contains the FIB and FII replicons of F and R100 plasmids, respectively (154). These plasmid replicons play an important role in plasmid incompatibility for IncF group of plasmids (125). Therefore, the stability of resident vs. donor plasmid is expected to be affected in wild-type *S.* Typhimurium LT2 ($pSLT^+$) transconjugants. However, we did not observe any differences in the localization of the FI plasmid in either *S.* Typhimurium LT2 vs. LT2 $pSLT^-$ transconjugants strains (Fig. 4).

DISCUSSION

Plasmids are similar to bacteriophages; "selfish DNA" that contain attributes that favors its spread and retention within its bacterial host, while excluding similar competing DNA molecules. Plasmid transmission within a bacterial population or microbial community is therefore affected by the distribution of plasmid incompatibility groups. Plasmid stability is affected by plasmids competing for initiation of DNA replication or partitioning into the dividing daughter cells (20, 125). Plasmids with similar replicons and partitioning apparatus are incompatible. This incompatibility is often tied to exclusion, mechanisms that limits plasmid transfer by disrupting mating aggregates (surface exclusion) or inhibiting DNA transfer in the presence of mating aggregates (entry exclusion) (57). Plasmids are often important vehicles for disseminating antibiotic resistance. However, some *Salmonella* serovars (ex. *S.* Enteritidis) are slower in developing antibiotic resistance compared to others. We believe that a significant genetic barrier to plasmid transmission and therefore development of antimicrobial resistance is the resident, *Salmonella spvB*-virulence plasmid. Like virulence plasmids in *E. coli* pathovars (87), the *Salmonella* virulence plasmid belongs to IncF incompatibility group , and specifically contains the FIC and FII replicons present in F and R100 plasmids, respectively (154). Similarly, newer β-lactam/cephalosporin and quinolone resistance genes reside on IncF plasmids. Those same resistance genes in *Salmonella*, however, reside on plasmid incompatibility groups other than IncF (25).

F-plasmid exclusion is attributed to *traS* and *traT*. In genomic comparisons of *Salmonella* virulence plasmid of *Salmonella* serovars, Choleraesuis, Dublin, Enteritidis, and

Kentucky, the most notable genetic difference linking plasmid exclusion to the slow development of plasmid-mediated antibiotic resistance in *Salmonella* serovar Enteritidis vs. *S.* Choleraesuis and *S.* Dublin was *traS*. The distribution of *spvB*-virulence plasmid and specifically the pSLT FII *traS* among *Salmonella* serovars appeared to adversely affect the prevalence of other large molecular weight plasmids. The virulence plasmid also appeared to significantly exclude F plasmid transfer to *S.* Typhimurium LT2 strain. Introduction of pRS31 containing FI *traS* into "plasmidless" *S.* Typhimurium LT2 restored exclusion, with plasmid specificity exhibited by *traS* (13). However, the plasmid exclusion linked to the *Salmonella* virulence plasmid was not attributed to the plasmid's resident *traS* as deletion of this gene did not significantly decrease plasmid exclusion compared to plasmid gene deletion control, ΔspvB. It could be that *traT* plays a more significant role in plasmid exclusion. While *traT* exhibits greater conservation in its amino acid sequence compared to *traS* of F and R100 plasmids (72), like *traS* (13), *traT* exhibits plasmid specificity in its exclusion (72). There is significant sequence divergence in F plasmid replicons and *tra* genes, including *traS* and *traT*, where F plasmid evolution in *Klebsiella, Salmonella* and *Yersinia* mirrors the divergence of these genera (154). This in part explains how F plasmid pOX38-km can exist as an autonomous replicon in *Salmonella* host with the FII/FIC virulence plasmid pSLT but does not explain how this plasmid can exclude F plasmids from entry into the *Salmonella* cell. Maybe the pSLT TraT shares some amino acid sequence or motif with both FI and FII TraT or there is some other plasmid gene(s) responsible for F-plasmid exclusion.

While we observed plasmid exclusion linked to the virulence plasmid in *S.* Typhimurium laboratory strain LT2, we did not observe similar exclusion for natural *Salmonella* isolates varied in their *spvB*-virulence plasmid or FII *traS* genotype. As we did not screen these isolates for *traT*, it's possible that while negative for *traS, traT* is sufficient for excluding F plasmids in these isolates. Another possibility, is that these isolates contain other IncF plasmids (154) not recognized by our *traS* probes. Several large molecular weight plasmids have been recently characterized in *S.* Kentucky, two of which belong to IncF incompatibility group (55) and may explain plasmid exclusion by our *S.* Kentucky isolate. Still, we observed no difference in plasmid exclusion for two genetically-related *S.* Typhimurium isolates ± *spvB* virulence plasmid. No plasmids were observed in *spvB*-negative *S.* Typhimurium songbird isolate. Some other plasmid exclusion mechanism may be involved in some *Salmonella* serovars or strains. One possible candidate for plasmid exclusion is clustered regularly interspaced short palindromic repeat (CRISPR) system that functions to exclude foreign genetic elements from entering the cell by forming a perfect sequence match between the spacer in CRISPR and the spacer located in invading DNA (139). Unraveling the contribution of these candidate genes (*traT*, *cas*) to plasmid exclusion will require creating *S.* Typhimurium strains with single or multiple deletions in targeted genes, and comparative genomics of multi-drug resistant and pan-susceptible *Salmonella* isolates.

CONCLUSION

Antibiotics have been the great panacea to reducing morbidity and mortality attributed to certain bacterial pathogens that were once the scourge of mankind. Unfortunately, resistance to these "wonder" drugs often quickly followed their introduction (4). Usage of antibiotics in agriculture has long been a contentious issue; with fears that antimicrobial resistance will spill over into human pathogens through the food chain (2). However, we have observed several circumstances where there is disconnect between antibiotic usage and resistance in food animals (46, 84, 141). There is also a disparity in antimicrobial susceptibility of microbes that inhabit the same environment, high in antibiotic resistance gene load (120) and encounters the same selection pressures (ex. antibiotic usage) (140). This disparity in antimicrobial susceptibility can be observed within a species, as is the case for *S. enterica*. We believe that several genetic factors are at play that affects the speed at which antibiotic resistance develops and spreads within a bacterial population. It is only when we bring a systems-based approach that factors in genetics, physiology, and pharmacology can we better understand how and when antimicrobial resistance emerges.

TABLES

Table 1. Bacterial strains and plasmids

Bacterial strain or plasmid	Description[1]	Reference
E. coli		
XK1200	Nal^r; F$^-$ *lacΔU124* Δ(*nadA aroG gal attλ bio*) *gyrA*	(11)
MC4100	Sm^r; F$^-$ *araD139 Δ*(*argF-lacU169*) *rpsL150 relA1 flbB3501 deoC1 ptsF25 rbsR*	(11)
DH5α	Nal^r; F$^-$ *supE44 ΔlacU169* (ϕ80 *lacZΔM15*) *hsdR17, recA1 endA1 gyrA96 thi-1 relA1*	(70)
S. enterica		
LT2	*S.* Typhimurium; *spvB*-virulence plasmid$^+$ (pSLT; incFII)	(134)
LT2R	*S.* Typhimurium LT2; Rif^r, *spvB*-virulence plasmid$^+$ (pSLT)	This Study
pSLT$^-$	*S.* Typhimurium LT2; Rif^r, *spvB*-virulence plasmid$^-$	This Study
pSLT$^-$ *traS*$^+$	*S.* Typhimurium pSLT$^-$; Rif^r, Tc^r, pRS31 (FI *traS*$^+$)	This Study
LT2R Δ*traS*	*S.* Typhimurium LT2R; Rif^r, *ΔtraS*, pSLT$^+$	This Study
LT2R Δ*spvB*	*S.* Typhimurium LT2R; Rif^r, *ΔspvB*, pSLT$^+$	This Study
98A-33516R	*S.* Typhimurium 98A-33516; Rif^r, *spvB*-virulence plasmid$^{-,2}$; Songbird isolate (83)	This Study

98A-28238R	*S.* Typhimurium 98A-28238; Rif^{r}, *spvB*-virulence plasmid$^{+,3}$, pSLT FII *traS*$^{-}$; Songbird isolate (83)	This Study
108709R	*S.* Choleraesuis108709; Rif^{r}, *spvB*-virulence plasmid$^{+,4}$	This Study
564R	*S.* Dublin 564; Rif^{r}, *spvB*-virulence plasmid$^{-,2}$; Cattle isolate	This Study
413R	*S.* Enteritidis 413 phage type 4; Rif^{r}, *spvB*-virulence plasmid$^{+,4}$; human isolate (82)	This Study
415R	*S.* Enteritidis 415 phage type 4; Rif^{r}, *spvB*-virulence plasmid$^{+,3}$, pSLT FII *traS*$^{-}$; poultry isolate (82)	This Study
Plasmids		
pOX38-Km	incFI plasmid; Km^{r}, Tra^{+} Conjugative	(12)
pRS31	FI *traS*$^{+}$ in pSC101; Tc^{r}	(12)
R100-1	incFII plasmid; Cm^{r} Fa^{r} Sm^{r} Sp^{r} Su^{r} Tc^{r}, Tra^{+} conjugative	(12)
pKD3	Template plasmid for *cat* cassette used in recombineering λ red mediated insertions and subsequent "flippase" mediated excisions/deletions; Ap^{r}, Cm^{r}	(38)
pKD20	*repA101ts*, λ *γ, β, exo*; Ap^{r}	(38)
pCP20	Temperature-sensitive replicon and inducible "flippase" (Flp) for deleting *cat* and adjacent sequences to create targeted deletions; Ap^{r}, Cm^{r}	(28)

[1]Abbreviations: Ap-Ampicillin, Cm-Chloramphenicol, Fa-Fusaric acid, Nal-Nalidixic, Rif-Rifampicin, Sp-Spectinomycin, Sm-Streptomycin, Su-Sulfonamide, and Tc-Tetracycline

[2]Clinical isolate identified as *spvB*-virulence plasmid negative from PCR screens for *spvC* (83, 148) and pSLT FII *traS*.

[3]Clinical isolate identified as *spvB*-virulence plasmid positive from PCR screen for *spvC* (148). Isolate is negative for pSLT FII *traS* as determined by PCR.

[4]Clinical isolate identified as *spvB*-virulence plasmid positive from PCR screens for *spvC* (83, 148) and pSLT FII *traS*.

Table 2. PCR primers

Target	Sequence	Expected Size (bp)	Annealing Temperature (^{o}C)	Reference
spvB	F:TCATACTCCAGCAGCAGACG	587	50°C	This Study
	R:AGCAGTTTTTATCGCCTGGA			
spvB-cat	F:GTATCAGGATAAGCACAAACA	1,100	57°C	This
ins[1]	GTAAGGCGATATCCG			Study
	R:TCATCCAATTACCTTTATTTACC			
	AACCATAGTTTTCTTATTA			
spvC	F:CGGAAATACCATCTACAAATA	669	40°C	(148)
	R:CCCAAACCCATACTTACTCTG			
pSLT FII-	F:ACCTGTCATTATTATCCTGC	400	55°C	This
traS	R:ATTATCCTGTTATTTGTCCTGC			Study
traS-cat	F:CAGGAGATAGTGTATGTTGATA	1,100	54°C	This
				Study
ins[1]	CTAAATGGTTTTTCATCT			
	R:TATCGCCATATTATTAGATAT			
	AAATTCTCAG			

FI *traS*	F:TCTGCCGGAAGAATTCCTAA R:CCGTCACTAAAATTGCACCA	152	50°C	This Study

[1]Primers used to create λ *red* –targeted knockouts with *cat* gene cassette (38).

Table 3. Plasmid composition and prevalence of *spvB*-virulence plasmids and pSLT FII *traS* in *S. enterica* isolates

***Salmonella* Serovars**[4]	**Virulence Plasmid**[1] **(%)**	**pSLT FII *traS*** [2] **(%)**	**Other**[3] **Plasmids ≥ 55 kb (%)**
S. Dublin (n = 7)	100	0	62
S. Enteritidis (n = 18)	100	100	7.7
S. Typhimurium (n =18)	100	100	24
S. Kentucky (n =14)	0	0	93
Total	75	63	39[5]

Isolates were screened by PCR and DNA: DNA hybridization for *spvC*[1] (virulence plasmid marker) and pSLT FII *traS*[2].

[3]Plasmids were identified as negative for the virulence plasmid marker *spvC* as determined by gel electrophoresis and Southern analysis.

[4]Non-random distribution of *spvB*-virulence plasmid, pSLT FII *traS* or other, large molecular weight plasmids among *Salmonella* serovars (Chi-Squared Test: $p<0.05$)

[5]Non-random distribution of other plasmids among *S. enterica* isolates with or without the pSLT virulence plasmid or pSLTII FII *traS* (Chi-Squared Test: $p<0.05$)

Table 4. The contribution of the *Salmonella spvB*-virulence plasmid and specifically the resident plasmid's *traS* on exclusion of F plasmids

Plasmid	Recipient Strain	Conjugation Frequency	EI[1]	P value
FI (pOX38-km)	LT2R	$2.77x10^{-3}$		
	LT2R pSLT$^-$	$5.80x10^{-2}$	20.94	<0.05[3]
	LT2R pSLT$^-$ FI *traS*$^+$	$1.63x10^{-6}$	1,699.39	<0.05[4]
	LT2R Δ*traS*	$5.00x10^{-4}$	0.18	0.21[5]
	LT2R Δ*spvB*	$1.50x10^{-3}$	0.54	0.93[6]
	E. coli DH5α	$2.78x10^{-1}$		
	E. coli DH5α FI *traS*$^+$	$1.63x10^{-6}$	170,552.15[2]	<0.05[7]

FII (R100)	LT2R	$4.22x10^{-5}$		
	LT2R pSLT$^-$	$1.10\ x10^{-2}$	260.66	<0.05[3]
	LT2R pSLT$^-$ FI *traS*$^+$	$1.48\ x10^{-4}$	3.51	<0.05[4]
	LT2R Δ*traS*	$5.70\ x10^{-4}$	13.51	0.07[5]
	LT2R Δ*spvB*	$4.20\ x10^{-5}$	1.00	0.59[6]
	E. coli DH5α	$7.91\ x10^{-2}$		
	E. coli DH5α FI *traS*$^+$	$3.40\ x10^{-1}$	0.23[2]	0.1[8]

EI (Exclusion index): plasmid transfer frequency for *Salmonella*[1] or *E. coli*[2] recipient/plasmid transfer frequency for *S.* Typhimurium with pSLT[1] virulence plasmid or pRS31 (FI *traS*$^+$)[2].

[3]Statistically significant difference between *S.* Typhimurium LT2R and *S.* Typhimurium LT2R pSLT$^-$

[4]Statistically significant difference between *S.* Typhimurium LT2R pSLT$^-$ and *S.* Typhimurium LT2R pSLT$^-$ FI *traS*$^+$

[5]No significant difference between *S.* Typhimurium LT2R and S. Typhimurium LT2R Δ*traS*

[6]No significant difference between *S.* Typhimurium LT2R and S. Typhimurium LT2R Δ*spvB*

[7]Statistically significant difference between *E. coli* DH5α (F$^-$) and *E. coli* DH5α FI *traS*$^+$

[8]No significant difference between *E. coli* DH5α (F$^-$) and *E. coli* DH5α FI *traS*$^+$

Table 5. The contribution of the *Salmonella spvB*-virulence plasmid on exclusion of F plasmids in natural *S. enterica* isolates

Plasmid	Recipient Stain	Conjugation Frequency	EI	P value
FI (pOX38-km)	*S.* Typhimurium LT2	2.75×10^{-3}		
	S. Typhimurium LT2 pSLT^{-}	4.03×10^{-2}	15.11	$<0.05^{1}$
	S. Typhimurium 98A-28238R (*spvB*-virulence plasmid^{-})	7.57×10^{-5}	0.01	
	S. Typhimurium 98A-33516R (*spvB*-virulence plasmid$^{+,}$, pSLT FII *traS*$^{-}$)	1.01×10^{-5}	0.00	$<0.05^{2}$
	S. Dublin 564R (*spvB*-virulence plasmid$^{+,}$, pSLT FII *traS*$^{-}$)	3.20×10^{-5}	0.00	$<0.05^{3}$
	S. Kentucky 114 R (*spvB*-virulence plasmid^{-})	5.70×10^{-6}	0.00	$<0.05^{3}$
	S. Choleraesuis 10708R (*spvB*-virulence plasmid^{+})	3.90×10^{-6}	0.00	$<0.05^{3}$
	E. coli DH5α	2.39×10^{-1}	64.37	$<0.05^{4}$
FII (R100)	*S.* Typhimurium LT2	1.06×10^{-5}		
	S. Typhimurium LT2 pSLT^{-}	3.83×10^{-3}	658.09	$<0.05^{1}$

S. Typhimurium 98A-28238R (*spvB*-virulence plasmid^{-})	2.47×10^{-6}	0.38	
S. Typhimurium 98A-33516R (*spvB*-virulence plasmid$^{+,}$, pSLT FII *traS*$^{-}$)	3.83×10^{-5}	7.35	<0.05[2]
S. Dublin 564R (*spvB*-virulence plasmid$^{+,}$, pSLT FII *traS*$^{-}$)	4.02×10^{-4}	146.69	0.73[5]
S. Kentucky 114 R (*spvB*-virulence plasmid^{-})	8.53×10^{-6}	1.29	0.53[5]
S. Choleraesuis 10708R (*spvB*-virulence plasmid^{+})	9.13×10^{-7}	0.08	<0.05[6]
E. coli DH5α	1.31×10^{-1}	28,308.82	<0.05[4]

[1]Statistically significant difference between *S.* Typhimurium LT2 and *S.* Typhimurium LT2 pSLT^{-}

[2]Statistically significant difference between *S.* Typhimurium 98A-28238R and *S.* Typhimurium 98A-33516R.

[3]Statistically significant difference between *S.* Typhimurium LT2 and *S.* Dublin 564R, *S.* Kentucky 114R, and *S.* Choleraesuis 10708R.

[4]Statistically significant difference between *S.* Typhimurium LT2 and *E. coli* DH5α

[5]No significant difference between *S.* Typhimurium LT2 and *S.* Dublin 564R and *S.* Kentucky 114R

[6]Statistically significant difference between *S.* Typhimurium LT2 and *S.* Choleraesuis 10708R

FIGURES

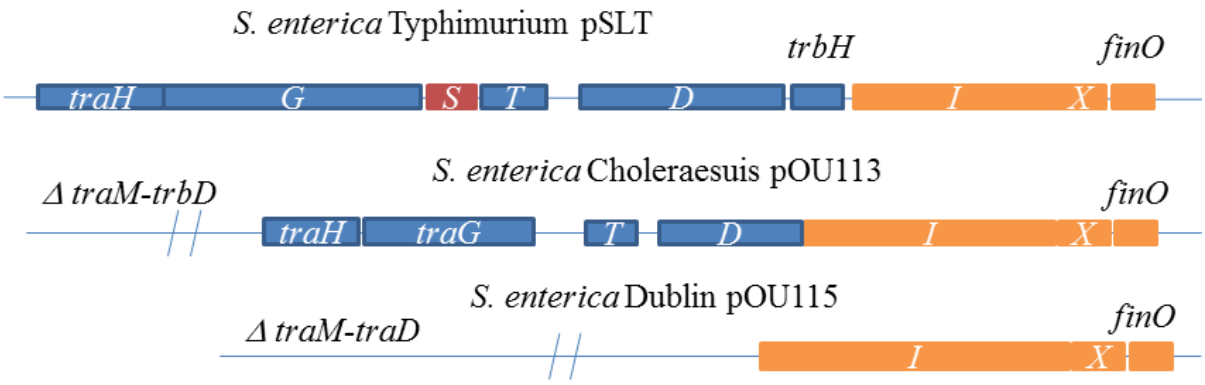

FIG 1. Genetic map of the *tra* region of *spvB*-virulence plasmids for *S. enterica* serovars Choleraesuis, Dublin and Typhimurium. The entry exclusion gene *traS*, labeled in red in the virulence plasmid of *S.* Typhimurium, is absent in the virulence plasmid of *S.* Choleraesuis and *S.* Dublin.

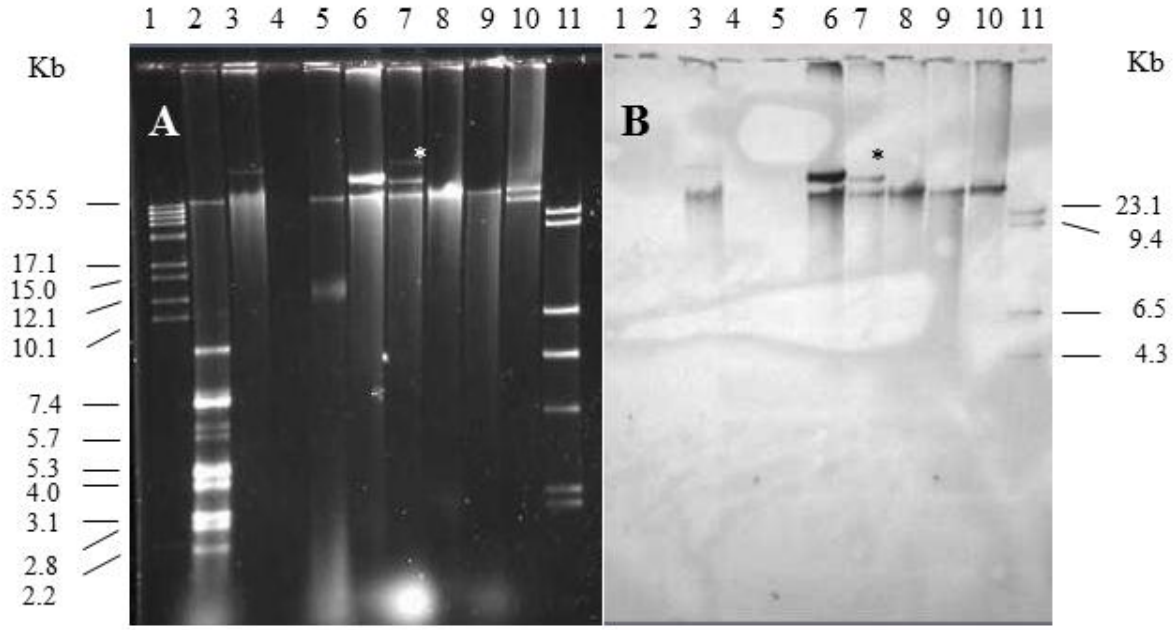

FIG. 2. Plasmid profile and identification of *spvB*-virulence plasmid in *S.* Dublin and *S. Enteritidis* isolates. Gel electrophoresis of *Salmonella* plasmids (A), DNA transfer, and hybridization with *spvC* DNA probe (B). Lane 1: supercoiled plasmid, VI molecular weight standards (Roche); lane 2: V517 plasmid, molecular weight standards (104); lane 3: *S.* Typhimurium LT2 (positive, 90kb *spvB*-virulence plasmid control); lane 4: *S.* Typhimurium LT2 pSLT$^-$ (*spvB*-virulence plasmid negative control); lane 5: *E. coli* XK1200 with pOX38-Km; lanes 6-8: *S.* Dublin isolates 564, 2078, and 2098; lanes 9,10: *S.* Enteritidis isolates 415 and 98; and lane 11: digoxigenin-labeled l *Hind* III molecular weight standards (Roche).

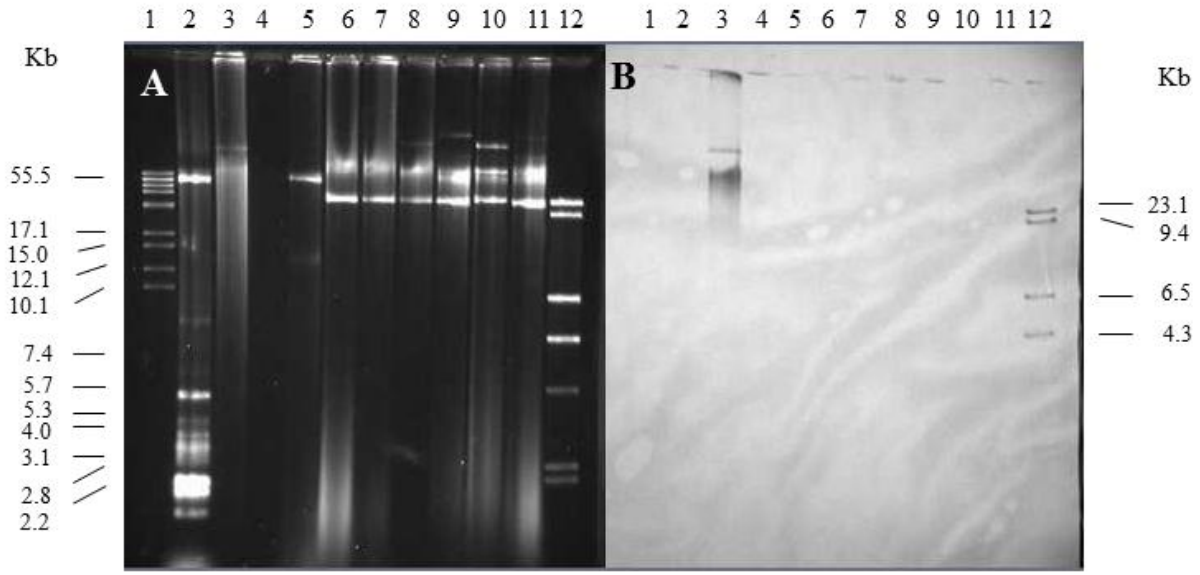

FIG. 3. Plasmid profile and identification of *spvB*-virulence plasmid in *S.* Kentucky isolates.

Gel electrophoresis of *Salmonella* plasmids (A), DNA transfer, and hybridization with *spvC* DNA probe (B). Lane 1: supercoiled plasmid, VI molecular weight standards (Roche); lane 2: V517 plasmid, molecular weight standards (104); lane 3: *S.* Typhimurium LT2 (positive, 90kb *spvB*-virulence plasmid control); lane 4: *S.* Typhimurium LT2 pSLT$^-$ (*spvB*-virulence plasmid negative control); lane 5: *E. coli* XK1200 with pOX38-Km; lanes 6-11: *S.* Kentucky isolates 102, 116, 117, 112, 118, and 105; and lane 12: digoxigenin-labeled l *Hind* III molecular weight standards (Roche).

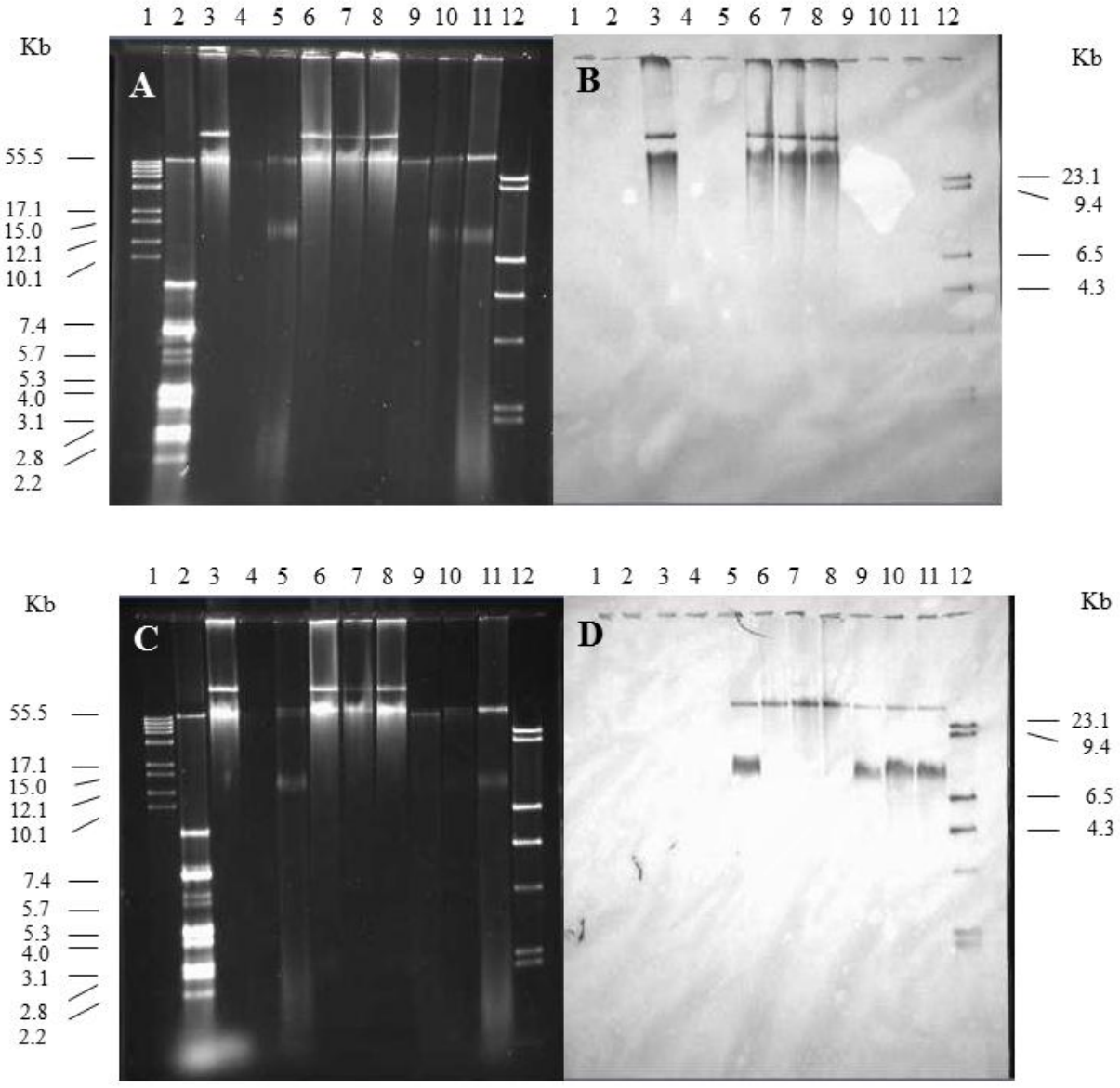

FIG. 4. The contribution of the *spvB*-virulence plasmid on localization of FI plasmids in *S.* Typhimurium transconjugants. Gel electrophoresis of *Salmonella* plasmids (A, C), DNA transfer, and hybridization with *spvC* (B) or FI *traS* (D) DNA probes. Lane 1: supercoiled plasmid, VI molecular weight standards (Roche); lane 2: V517 plasmid, molecular weight standards (104); lane 3: *S.* Typhimurium LT2 (positive, 90kb *spvB*-virulence plasmid control); lane 4: *S.* Typhimurium LT2 pSLT$^-$

(*spvB*-virulence plasmid negative control); lane 5: *E. coli* XK1200 with pOX38-Km; lanes 6-8: *S.* Typhimurium LT2R transconjugants (pOX38-Km); lanes 9-11: *S.* Typhimurium LT2 pSLT^{-} transconjugants (pOX38-Km); and lane 12: digoxigenin-labeled l *Hind* III molecular weight standards (Roche).

LITERATURE CITED

1. 2002. Outbreak of multidrug-resistant *Salmonella newport*--United States, January-April 2002. MMWR Morb Mortal Wkly Rep **51:**545-548.
2. 1975. Swann Committee recommendations. Vet Rec **97:**121-124.
3. **Abe, A., and K. Kawahara.** 1995. Transcriptional regulation and promoter sequence of the spvR gene of virulence plasmid pKDSC50 in *Salmonella choleraesuis* serovar Choleraesuis. FEMS Microbiol Lett **129:**225-230.
4. **Abraham, E. P., and E. Chain.** 1988. An enzyme from bacteria able to destroy penicillin. 1940. Rev Infect Dis **10:**677-678.
5. **Achtman, M., B. Kusecek, and K. N. Timmis.** 1978. Tra cistrons and proteins encoded by the *Escherichia coli* antibiotic resistance plasmid R6-5. Mol Gen Genet **163:**169-179.
6. **Achtman, M., P. A. Manning, C. Edelbluth, and P. Herrlich.** 1979. Export without Proteolytic processing of inner and outer-membrane proteins encoded by F-sex factor tra cistrons in *Escherichia coli* minicells. Proc Natl Acad Sci U S A **76:**4837-4841.
7. **Achtman, M., G. Morelli, and S. Schwuchow.** 1978. Cell-cell interactions in conjugating *Escherichia coli*: role of F pili and fate of mating aggregates. J Bacteriol **135:**1053-1061.
8. **Ahmer, B. M., M. Tran, and F. Heffron.** 1999. The virulence plasmid of *Salmonella typhimurium* is self-transmissible. J Bacteriol **181:**1364-1368.
9. **Andrews-Polymenis, H. L., A. J. Baumler, B. A. McCormick, and F. C. Fang.** 2010. Taming the elephant: *Salmonella* biology, pathogenesis, and prevention. Infect Immun **78:**2356-2369.
10. **Anonymous.** 2010. 2010 Retail Meat Report: National Antimicrobial Resistance Monitoring System. Food and Drug Administration's Center for Veterinary Medicine http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM293581.pdf. Accessed 03/26/2013.
11. **Anthony, K. G., P. Kathir, D. Moore, K. Ippen-Ihler, and L. S. Frost.** 1996. Analysis of the *traLEKBP* sequence and the TraP protein from three F-like plasmids: F, R100-1 and ColB2. J Bacteriol **178:**3194-3200.
12. **Anthony, K. G., W. A. Klimke, J. Manchak, and L. S. Frost.** 1999. Comparison of proteins involved in pilus synthesis and mating pair stabilization from the related plasmids F and R100-1: insights into the mechanism of conjugation. J Bacteriol **181:**5149-5159.
13. **Audette, G. F., J. Manchak, P. Beatty, W. A. Klimke, and L. S. Frost.** 2007. Entry exclusion in F-like plasmids requires intact TraG in the donor that recognizes its cognate TraS in the recipient. Microbiology **153:**442-451.
14. **Austin, S., and A. Abeles.** 1983. Partition of unit-copy miniplasmids to daughter cells. I. P1 and F miniplasmids contain discrete, interchangeable sequences sufficient to promote equipartition. J Mol Biol **169:**353-372.

15. **Barton, N. H.** 2010. Genetic linkage and natural selection. Philos Trans R Soc Lond B Biol Sci **365:**2559-2569.
16. **Baumler, A. J., R. M. Tsolis, F. A. Bowe, J. G. Kusters, S. Hoffmann, and F. Heffron.** 1996. The pef fimbrial operon of *Salmonella typhimurium* mediates adhesion to murine small intestine and is necessary for fluid accumulation in the infant mouse. Infection and immunity **64:**61-68.
17. **Bean, N. H., J. S. Goulding, C. Lao, and F. J. Angulo.** 1996. Surveillance for foodborne-disease outbreaks--United States, 1988-1992. MMWR CDC Surveill Summ **45:**1-66.
18. **Benacer, D., K. L. Thong, H. Watanabe, and S. D. Puthucheary.** 2010. Characterization of drug resistant *Salmonella enterica* serotype Typhimurium by antibiograms, plasmids, integrons, resistance genes and PFGE. J Microbiol Biotechnol **20:**1042-1052.
19. **Biek, D. P., and J. Shi.** 1994. A single 43-bp *sopC* repeat of plasmid mini-F is sufficient to allow assembly of a functional nucleoprotein partition complex. Proc Natl Acad Sci U S A **91:**8027-8031.
20. **Bouet, J. Y., K. Nordstrom, and D. Lane.** 2007. Plasmid partition and incompatibility--the focus shifts. Mol Microbiol **65:**1405-1414.
21. **Boyd, E. F., and D. L. Hartl.** 1998. *Salmonella* virulence plasmid. Modular acquisition of the *spv* virulence region by an F-plasmid in *Salmonella enterica* subspecies I and insertion into the chromosome of subspecies II, IIIa, IV and VII isolates. Genetics **149:**1183-1190.
22. **Bramhill, D., and A. Kornberg.** 1988. A model for initiation at origins of DNA replication. Cell **54:**915-918.
23. **Browne, S. H., P. Hasegawa, S. Okamoto, J. Fierer, and D. G. Guiney.** 2008. Identification of *Salmonella* SPI-2 secretion system components required for SpvB-mediated cytotoxicity in macrophages and virulence in mice. FEMS Immunol Med Microbiol **52:**194-201.
24. **Campbell, C. S., and R. D. Mullins.** 2007. In vivo visualization of type II plasmid segregation: bacterial actin filaments pushing plasmids. The Journal of cell biology **179:**1059-1066.
25. **Carattoli, A.** 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother **53:**2227-2238.
26. **Chan, K., S. Baker, C. C. Kim, C. S. Detweiler, G. Dougan, and S. Falkow.** 2003. Genomic comparison of *Salmonella enterica* serovars and *Salmonella bongori* by use of an *S. enterica* serovar typhimurium DNA microarray. J Bacteriol **185:**553-563.
27. **Chart, H., E. J. Threlfall, and B. Rowe.** 1989. Virulence of *Salmonella enteritidis* phage type 4 is related to the possession of a 38 MDa plasmid. FEMS Microbiol Lett **49:**299-303.
28. **Cherepanov, P. P., and W. Wackernagel.** 1995. Gene disruption in *Escherichia coli:* TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene **158:**9-14.

29. **Chu, C., C. H. Chiu, C. H. Chu, and J. T. Ou.** 2002. Nucleotide and amino acid sequences of *oriT-traM-traJ-traY-traA-traL* regions and mobilization of virulence plasmids of *Salmonella enterica* serovars Enteritidis, Gallinarum-Pullorum, and Typhimurium. J Bacteriol **184:**2857-2862.
30. **Chu, C., C. H. Chiu, W. Y. Wu, C. H. Chu, T. P. Liu, and J. T. Ou.** 2001. Large drug resistance virulence plasmids of clinical isolates of *Salmonella enterica* serovar Choleraesuis. Antimicrob Agents Chemother **45:**2299-2303.
31. **Chu, C., Y. Feng, A. C. Chien, S. Hu, C. H. Chu, and C. H. Chiu.** 2008. Evolution of genes on the *Salmonella* virulence plasmid phylogeny revealed from sequencing of the virulence plasmids of *S. enterica* serotype Dublin and comparative analysis. Genomics **92:**339-343.
32. **Chu, C., S. F. Hong, C. Tsai, W. S. Lin, T. P. Liu, and J. T. Ou.** 1999. Comparative physical and genetic maps of the virulence plasmids of *Salmonella enterica* serovars Typhimurium, Enteritidis, Choleraesuis, and Dublin. Infect Immun **67:**2611-2614.
33. **Cirillo, D. M., E. J. Heffernan, L. Wu, J. Harwood, J. Fierer, and D. G. Guiney.** 1996. Identification of a domain in Rck, a product of the *Salmonella typhimurium* virulence plasmid, required for both serum resistance and cell invasion. Infect Immun **64:**2019-2023.
34. **Collis, C. M., and R. M. Hall.** 1995. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob Agents Chemother **39:**155-162.
35. **Cooper, T. F.** 2007. Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. PLoS Biol **5:**e225.
36. **Couturier, M., F. Bex, P. L. Bergquist, and W. K. Maas.** 1988. Identification and classification of bacterial plasmids. Microbiol Rev **52:**375-395.
37. **Dasgupta, S., H. Masukata, and J. Tomizawa.** 1987. Multiple mechanisms for initiation of ColE1 DNA replication: DNA synthesis in the presence and absence of ribonuclease H. Cell **51:**1113-1122.
38. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A **97:**6640-6645.
39. **del Solar, G., R. Giraldo, M. J. Ruiz-Echevarria, M. Espinosa, and R. Diaz-Orejas.** 1998. Replication and control of circular bacterial plasmids. Microbiol Mol Biol Rev **62:**434-464.
40. **Diago-Navarro, E., A. M. Hernandez-Arriaga, J. Lopez-Villarejo, A. J. Munoz-Gomez, M. B. Kamphuis, R. Boelens, M. Lemonnier, and R. Diaz-Orejas.** 2010. *parD* toxin-antitoxin system of plasmid R1--basic contributions, biotechnological applications and relationships with closely-related toxin-antitoxin systems. Febs J **277:**3097-3117.
41. **DiRienzo, J. M., and B. Rosan.** 1984. Isolation of a major cell envelope protein from *Fusobacterium nucleatum*. Infect Immun **44:**386-393.
42. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res **16:**6127-6145.
43. **Dubnau, E., and W. K. Maas.** 1968. Inhibition of replication of an F'*lac* episome in Hfr cells of *Escherichia coli*. J Bacteriol **95:**531-539.

44. **Dutil, L., R. Irwin, R. Finley, L. K. Ng, B. Avery, P. Boerlin, A. M. Bourgault, L. Cole, D. Daignault, A. Desruisseau, W. Demczuk, L. Hoang, G. B. Horsman, J. Ismail, F. Jamieson, A. Maki, A. Pacagnella, and D. R. Pillai.** 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis **16:**48-54.
45. **Espinosa, M., G. del Solar, F. Rojo, and J. C. Alonso.** 1995. Plasmid rolling circle replication and its control. FEMS Microbiol Lett **130:**111-120.
46. **Fairchild, A. S., J. L. Smith, U. Idris, J. Lu, S. Sanchez, L. B. Purvis, C. Hofacre, and M. D. Lee.** 2005. Effects of orally administered tetracycline on the intestinal community structure of chickens and on tet determinant carriage by commensal bacteria and *Campylobacter jejuni*. Appl Environ Microbiol **71:**5865-5872.
47. **Fang, F. C., M. Krause, C. Roudier, J. Fierer, and D. G. Guiney.** 1991. Growth regulation of a *Salmonella* plasmid gene essential for virulence. J Bacteriol **173:**6783-6789.
48. **Fierer, J., L. Eckmann, F. Fang, C. Pfeifer, B. B. Finlay, and D. Guiney.** 1993. Expression of the *Salmonella* virulence plasmid gene *spvB* in cultured macrophages and nonphagocytic cells. Infect Immun **61:**5231-5236.
49. **Filutowicz, M., and S. A. Rakowski.** 1998. Regulatory implications of protein assemblies at the gamma origin of plasmid R6K - a review. Gene **223:**195-204.
50. **Firth, N., and R. Skurray.** 1992. Characterization of the F plasmid bifunctional conjugation gene, *traG*. Mol Gen Genet **232:**145-153.
51. **Fisher, R. A.** 1930. The genetical theory of natural selection. The Clarendon press, Oxford,.
52. **Folkhard, W., D. A. Marvin, T. H. Watts, and W. Paranchych.** 1981. Structure of polar pili from *Pseudomonas aeruginosa* strains K and O. J Mol Biol **149:**79-93.
53. **Foster, T. J., and N. S. Willetts.** 1976. Genetic analysis of deletions of R100-1 that are both transfer-deficient and tetracycline-sensitive. J Gen Microbiol **93:**133-140.
54. **Fouser, L., and R. E. Bird.** 1983. Accumulation of ColE1 early replicative intermediates catalyzed by extracts of *Escherichia coli dnaG* mutant strains. J Bacteriol **154:**1174-1183.
55. **Fricke, W. F., P. F. McDermott, M. K. Mammel, S. Zhao, T. J. Johnson, D. A. Rasko, P. J. Fedorka-Cray, A. Pedroso, J. M. Whichard, J. E. Leclerc, D. G. White, T. A. Cebula, and J. Ravel.** 2009. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic *Escherichia coli strains* in *Salmonella enterica* serovar Kentucky isolates from poultry. Appl Environ Microbiol **75:**5963-5971.
56. **Frost, L. S., K. Ippen-Ihler, and R. A. Skurray.** 1994. Analysis of the sequence and gene products of the transfer region of the F sex factor. Microbiol Rev **58:**162-210.
57. **Garcillan-Barcia, M. P., and F. de la Cruz.** 2008. Why is entry exclusion an essential feature of conjugative plasmids? Plasmid **60:**1-18.
58. **Gebreyes, W. A., and S. Thakur.** 2005. Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. Antimicrob Agents Chemother **49:**503-511.

59. **Gerdes, K., J. Moller-Jensen, and R. Bugge Jensen.** 2000. Plasmid and chromosome partitioning: surprises from phylogeny. Mol Microbiol **37:**455-466.
60. **Glenn, L. M., M. D. Englen, R. L. Lindsey, J. F. Frank, J. E. Turpin, M. E. Berrang, R. J. Meinersmann, P. J. Fedorka-Cray, and J. G. Frye.** 2012. Analysis of antimicrobial resistance genes detected in multiple-drug-resistant *Escherichia coli* isolates from broiler chicken carcasses. Microb Drug Resist.
61. **Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo.** 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. N Engl J Med **338:**1333-1338.
62. **Goldstein, C., M. D. Lee, S. Sanchez, C. Hudson, B. Phillips, B. Register, M. Grady, C. Liebert, A. O. Summers, D. G. White, and J. J. Maurer.** 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicro Agents Chemother **45:**723-726.
63. **Gordon, G. S., and A. Wright.** 2000. DNA segregation in bacteria. Annu Rev Microbiol **54:**681-708.
64. **Guerra, B., S. Soto, R. Helmuth, and M. C. Mendoza.** 2002. Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. Antimicrob Agents Chemother **46:**2977-2981.
65. **Gulig, P. A., A. L. Caldwell, and V. A. Chiodo.** 1992. Identification, genetic analysis and DNA sequence of a 7.8-kb virulence region of the *Salmonella typhimurium* virulence plasmid. Mol Microbiol **6:**1395-1411.
66. **Gulig, P. A., and R. Curtiss, 3rd.** 1987. Plasmid-associated virulence of *Salmonella typhimurium*. Infect Immun **55:**2891-2901.
67. **Gulig, P. A., H. Danbara, D. G. Guiney, A. J. Lax, F. Norel, and M. Rhen.** 1993. Molecular analysis of spv virulence genes of the *Salmonella* virulence plasmids. Mol Microbiol **7:**825-830.
68. **Hall, B. G.** 1989. Genetic mobility: mobile DNA. Science **245:**84-85.
69. **Hall, R. M., and C. M. Collis.** 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol Microbiol **15:**593-600.
70. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol **166:**557-580.
71. **Hansen, B. S., P. A. Manning, and M. Achtman.** 1982. Promoter-distal region of the *tra* operon of F-like sex factor R100 in *Escherichia coli* K-12. J Bacteriol **150:**89-99.
72. **Harrison, J. L., I. M. Taylor, K. Platt, and C. D. O'Connor.** 1992. Surface exclusion specificity of the TraT lipoprotein is determined by single alterations in a five-amino-acid region of the protein. Mol Microbiol **6:**2825-2832.
73. **Hedberg, C. W., F. J. Angulo, K. E. White, C. W. Langkop, W. L. Schell, M. G. Stobierski, A. Schuchat, J. M. Besser, S. Dietrich, L. Helsel, P. M. Griffin, J. W. McFarland, and M. T. Osterholm.** 1999. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health. The Investigation Team. Epidemiol Infect **122:**385-393.

74. **Hedberg, C. W., M. J. David, K. E. White, K. L. MacDonald, and M. T. Osterholm.** 1993. Role of egg consumption in sporadic *Salmonella enteritidis* and *Salmonella typhimurium* infections in Minnesota. J Infect Dis **167:**107-111.
75. **Hedberg, C. W., J. A. Korlath, J. Y. D'Aoust, K. E. White, W. L. Schell, M. R. Miller, D. N. Cameron, K. L. MacDonald, and M. T. Osterholm.** 1992. A multistate outbreak of *Salmonella javiana* and *Salmonella oranienburg* infections due to consumption of contaminated cheese. JAMA **268:**3203-3207.
76. **Heffernan, E. J., S. Reed, J. Hackett, J. Fierer, C. Roudier, and D. Guiney.** 1992. Mechanism of resistance to complement-mediated killing of bacteria encoded by the *Salmonella typhimurium* virulence plasmid gene *rck*. J Clin Invest **90:**953-964.
77. **Helmuth, R., R. Stephan, C. Bunge, B. Hoog, A. Steinbeck, and E. Bulling.** 1985. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect Immun **48:**175-182.
78. **Hong, S. F., C. H. Chiu, C. Chu, Y. Feng, and J. T. Ou.** 2008. Complete nucleotide sequence of a virulence plasmid of *Salmonella enterica* serovar Dublin and its phylogenetic relationship to the virulence plasmids of serovars Choleraesuis, Enteritidis and Typhimurium. FEMS Microbiol Lett **282:**39-43.
79. **Hong, S. F., C. H. Chiu, C. Chu, Y. Feng, and J. T. Ou.** 2008. Complete nucleotide sequence of a virulence plasmid of *Salmonella enterica* serovar Dublin and its phylogenetic relationship to the virulence plasmids of serovars Choleraesuis, Enteritidis and Typhimurium. FEMS Microbiol Lett **282:**39-43.
80. **Horiuchi, S., N. Goto, Y. Inagaki, and R. Nakaya.** 1991. The 106-kilobase plasmid of *Salmonella braenderup* and the 100-kilobase plasmid of *Salmonella typhimurium* are not necessary for the pathogenicity in experimental models. Microbiol Immunol **35:**187-198.
81. **Hsu, S. C., T. H. Chiu, J. C. Pang, C. H. Hsuan-Yuan, G. N. Chang, and H. Y. Tsen.** 2006. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Inter J Antimicrob Agents **27:**383-391.
82. **Hudson, C. R., M. Garcia, R. K. Gast, and J. J. Maurer.** 2001. Determination of close genetic relatedness of the major *Salmonella enteritidis* phage types by pulsed-field gel electrophoresis and DNA sequence analysis of several Salmonella virulence genes. Avian Dis **45:**875-886.
83. **Hudson, C. R., C. Quist, M. D. Lee, K. Keyes, S. V. Dodson, C. Morales, S. Sanchez, D. G. White, and J. J. Maurer.** 2000. Genetic relatedness of *Salmonella* isolates from nondomestic birds in Southeastern United States. J Clin Microbiol **38:**1860-1865.
84. **Idris, U., J. Lu, M. Maier, S. Sanchez, C. L. Hofacre, B. G. Harmon, J. J. Maurer, and M. D. Lee.** 2006. Dissemination of fluoroquinolone-resistant *Campylobacter spp.* within an integrated commercial poultry production system. Appl Environ Microbiol **72:**3441-3447.
85. **Ippen-Ihler, K. A., and E. G. Minkley, Jr.** 1986. The conjugation system of F, the fertility factor of *Escherichia coli*. Ann Rev Genet **20:**593-624.
86. **Itoh, T., and J. Tomizawa.** 1980. Formation of an RNA primer for initiation of replication of ColE1 DNA by ribonuclease H. Proc Natl Acad Sci U S A 77**:**2450-2454.

87. **Johnson, T. J., and L. K. Nolan.** 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev **73:**750-774.
88. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol **145:**1365-1373.
89. **Kaneene, J. B., R. Miller, K. May, and J. A. Hattey.** 2010. An outbreak of multidrug-resistant *Salmonella enterica* serotype Oranienburg in Michigan dairy calves. Foodborne Pathog Dis **7:**1193-1201.
90. **Khan, S. A.** 2005. Plasmid rolling-circle replication: highlights of two decades of research. Plasmid **53:**126-136.
91. **Khan, S. A.** 2000. Plasmid rolling-circle replication: recent developments. Mol Microbiol **37:**477-484.
92. **Khatri, G. S., T. MacAllister, P. R. Sista, and D. Bastia.** 1989. The replication terminator protein of *E. coli* is a DNA sequence-specific contra-helicase. Cell **59:**667-674.
93. **Kim, H. J., S. H. Park, and H. Y. Kim.** 2006. Comparison of *Salmonella enterica* serovar Typhimurium LT2 and non-LT2 salmonella genomic sequences, and genotyping of salmonellae by using PCR. Appl Environ Microbiol **72:**6142-6151.
94. **Kingsman, A., and N. Willetts.** 1978. The requirements for conjugal DNA synthesis in the donor strain during flac transfer. J Mol Biol **122:**287-300.
95. **Klimke, W. A., and L. S. Frost.** 1998. Genetic analysis of the role of the transfer gene, traN, of the F and R100-1 plasmids in mating pair stabilization during conjugation. J Bacteriol **180:**4036-4043.
96. **Klimke, W. A., C. D. Rypien, B. Klinger, R. A. Kennedy, J. M. Rodriguez-Maillard, and L. S. Frost.** 2005. The mating pair stabilization protein, TraN, of the F plasmid is an outer-membrane protein with two regions that are important for its function in conjugation. Microbiol **151:**3527-3540.
97. **Koepsel, R. R., R. W. Murray, W. D. Rosenblum, and S. A. Khan.** 1985. The replication initiator protein of plasmid pT181 has sequence-specific endonuclease and topoisomerase-like activities. Proc Natl Acad Sci U S A **82:**6845-6849.
98. **Kolatka, K., S. Kubik, M. Rajewska, and I. Konieczny.** 2010. Replication and partitioning of the broad-host-range plasmid RK2. Plasmid **64:**119-134.
99. **Krause, M., C. Roudier, J. Fierer, J. Harwood, and D. Guiney.** 1991. Molecular analysis of the virulence locus of the *Salmonella dublin* plasmid pSDL2. Mol Microbiol **5:**307-316.
100. **Lee, C. A., M. Silva, A. M. Siber, A. J. Kelly, E. Galyov, and B. A. McCormick.** 2000. A secreted *Salmonella* protein induces a proinflammatory response in epithelial cells, which promotes neutrophil migration. Proc Natl Acad Sci U S A **97:**12283-12288.
101. **Lesnick, M. L., N. E. Reiner, J. Fierer, and D. G. Guiney.** 2001. The *Salmonella spvB* virulence gene encodes an enzyme that ADP-ribosylates actin and destabilizes the cytoskeleton of eukaryotic cells. Mol Microbiol **39:**1464-1470.
102. **Libby, S. J., M. Lesnick, P. Hasegawa, E. Weidenhammer, and D. G. Guiney.** 2000. The *Salmonella* virulence plasmid spv genes are required for cytopathology in human monocyte-derived macrophages. Cell Microbiol **2:**49-58.

103. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:**491-511.
104. **Macrina, F. L., D. J. Kopecko, K. R. Jones, D. J. Ayers, and S. M. McCowen.** 1978. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid **1:**417-420.
105. **Mahon, B. E., A. Ponka, W. N. Hall, K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M. A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker.** 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. J Infect Dis **175:**876-882.
106. **Manchanda, V., P. Bhalla, M. Sethi, and V. K. Sharma.** 2006. Treatment of enteric fever in children on the basis of current trends of antimicrobial susceptibility of *Salmonella enterica* serovar Typhi and Paratyphi A. Indian J Med Microbiol **24:**101-106.
107. **Manning, P. A., G. Morelli, and M. Achtman.** 1981. *traG* protein of the F sex factor of *Escherichia coli* K-12 and its role in conjugation. Proc Natl Acad Sci U S A **78:**7487-7491.
108. **Martinez, E., and F. de la Cruz.** 1988. Transposon Tn*21* encodes a RecA-independent site-specific integration system. Mol Gen Genet **211:**320-325.
109. **Marvin, D. A., and W. Folkhard.** 1986. Structure of F-pili: reassessment of the symmetry. J Mol Biol **191:**299-300.
110. **Matson, S. W., and B. S. Morton.** 1991. *Escherichia coli* DNA helicase-I catalyzes a site-specific and strand-specific nicking reaction at the F-plasmid *oriT*. J Biol Chem **266:**16232-16237.
111. **Matsui, H., C. M. Bacot, W. A. Garlington, T. J. Doyle, S. Roberts, and P. A. Gulig.** 2001. Virulence plasmid-borne *spvB* and *spvC* genes can replace the 90-kilobase plasmid in conferring virulence to *Salmonella enterica* serovar Typhimurium in subcutaneously inoculated mice. J Bacteriol **183:**4652-4658.
112. **Mccormick, B. A., S. I. Miller, D. Carnes, and J. L. Madara.** 1995. Transepithelial signaling to neutrophils by salmonellae - a novel virulence mechanism for gastroenteritis. Infect Immun **63:**2302-2309.
113. **McGhie, E. J., R. D. Hayward, and V. Koronakis.** 2001. Cooperation between actin-binding proteins of invasive *Salmonella*: SipA potentiates SipC nucleation and bundling of actin. Embo Journal **20:**2131-2139.
114. **Meyer, R.** 2009. Replication and conjugative mobilization of broad host-range IncQ plasmids. Plasmid **62:**57-70.
115. **Miki, T., T. Horiuchi, and N. S. Willetts.** 1978. Identification and characterization of four new *tra* cistrons on the *E. coli* K12 sex factor F. Plasmid **1:**316-323.
116. **Moll, A., P. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. Infect Immun **28:**359-367.
117. **Montenegro, M. A., G. Morelli, and R. Helmuth.** 1991. Heteroduplex analysis of *Salmonella* virulence plasmids and their prevalence in isolates of defined sources. Microb Pathog **11:**391-397.

118. **Nakasu, S., and J. Tomizawa.** 1992. Structure of the ColE1 DNA molecule before segregation to daughter molecules. Proc Natl Acad Sci U S A **89:**10139-10143.
119. **Nakaya, R., A. Nakamura, and Y. Murata.** 1960. Resistance transfer agents in *Shigella*. Biochem Biophys Res Commun **3:**654-659.
120. **Nandi, S., J. J. Maurer, C. Hofacre, and A. O. Summers.** 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proc Natl Acad Sci U S A **101:**7118-7122.
121. **Nandi, S., J. J. Maurer, C. Hofacre, and A. O. Summers.** 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proceedings of the National Academy of Sciences of the United States of America **101:**7118-7122.
122. **Nelson, W. C., M. T. Howard, J. A. Sherman, and S. W. Matson.** 1995. The *traY* gene-product and integration host factor stimulate Escherichia-Coli DNA helicase I-catalyzed nicking at the F-plasmid *oriT*. J Biol Chem **270:**28374-28380.
123. **Nordstrom, K.** 2006. Plasmid R1--replication and its control. Plasmid **55:**1-26.
124. **Nordstrom, K., and S. J. Austin.** 1993. Cell-cycle-specific initiation of replication. Mol Microbiol **10:**457-463.
125. **Novick, R. P.** 1987. Plasmid incompatibility. Microbiol Rev **51:**381-395.
126. **Novick, R. P., and F. C. Hoppensteadt.** 1978. On plasmid incompatibility. Plasmid **1:**421-434.
127. **Ou, J. T., L. S. Baron, X. Y. Dai, and C. A. Life.** 1990. The virulence plasmids of *Salmonella* serovars Typhimurium, Choleraesuis, Dublin, and Enteritidis, and the cryptic plasmids of *Salmonella* serovars Copenhagen and Sendai belong to the same incompatibility group, but not those of Salmonella serovars Durban, Gallinarum, Give, Infantis and Pullorum. Microb Pathog **8:**101-107.
128. **Patchanee, P., B. M. Zewde, D. A. Tadesse, A. Hoet, and W. A. Gebreyes.** 2008. Characterization of multidrug-resistant *Salmonella enterica* serovar Heidelberg isolated from humans and animals. Foodborne Pathog Dis **5:**839-851.
129. **Provence, D. L., and R. Curtiss III.** 1994. Gene transfer in gram-negative bacteria. p. 317-364. In P. Gerhardt, R. G. E. Murray, W. A. Wood, N. R. Krieg Eds., Methods for General and Molecular Bacteriology **ASM Press. Washington, D.C.**
130. **Rawlings, D. E., and E. Tietze.** 2001. Comparative biology of IncQ and IncQ-like plasmids. Microbiology and molecular biology reviews. Microbiol Mol Biol Rev **65:**481-496.
131. **Ringgaard, S., J. Lowe, and K. Gerdes.** 2007. Centromere pairing by a plasmid-encoded type I ParB protein. J Biol Chem **282:**28216-28225.
132. **Rodriguez-Pena, J. M., I. Alvarez, M. Ibanez, and R. Rotger.** 1997. Homologous regions of the Salmonella enteritidis virulence plasmid and the chromosome of Salmonella typhi encode thiol: disulphide oxidoreductases belonging to the DsbA thioredoxin family. Microbiology **143 (Pt 4):**1405-1413.
133. **Sambrook, J., and D. W. Russell.** 2006. The condensed protocols from Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

134. **Sanderson, K. E., and J. R. Roth.** 1988. Linkage map of *Salmonella typhimurium*, edition VII. Microbiol Rev **52:**485-532.
135. **Santos, R. L., R. M. Tsolis, A. J. Baumler, and L. G. Adams.** 2003. Pathogenesis of *Salmonella*-induced enteritis. Braz J Med Biol Res **36:**3-12.
136. **Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin.** 2011. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis **17:**7-15.
137. **Schumacher, M. A.** 2008. Structural biology of plasmid partition: uncovering the molecular mechanisms of DNA segregation. Biochem J **412:**1-18.
138. **Schumacher, M. A., T. C. Glover, A. J. Brzoska, S. O. Jensen, T. D. Dunham, R. A. Skurray, and N. Firth.** 2007. Segrosome structure revealed by a complex of ParR with centromere DNA. Nature **450:**1268-1271.
139. **Semenova, E., M. Nagornykh, M. Pyatnitskiy, Artamonova, II, and K. Severinov.** 2009. Analysis of CRISPR system function in plant pathogen *Xanthomonas oryzae*. FEMS Microbiol Lett **296:**110-116.
140. **Simjee, S., P. F. McDermott, D. G. White, C. Hofacre, R. D. Berghaus, P. J. Carter, L. Stewart, T. Liu, M. Maier, and J. J. Maurer.** 2007. Antimicrobial susceptibility and distribution of antimicrobial-resistance genes among *Enterococcus* and coagulase-negative *Staphylococcus* isolates recovered from poultry litter. Avian Dis **51:**884-892.
141. **Smith, J. L., D. J. Drum, Y. Dai, J. M. Kim, S. Sanchez, J. J. Maurer, C. L. Hofacre, and M. D. Lee.** 2007. Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. Appl Environ Microbiol **73:**1404-1414.
142. **Snyder, L., and W. Champness.** 2007. Molecular genetics of bacteria, 3rd ed. ASM Press, Washington, D.C.
143. **Stepan, R. M., J. S. Sherwood, S. R. Petermann, and C. M. Logue.** 2011. Molecular and comparative analysis of *Salmonella enterica* Senftenberg from humans and animals using PFGE, MLST and NARMS. BMC Microbiol **11:**153.
144. **Stewart, P. E., R. Byram, D. Grimm, K. Tilly, and P. A. Rosa.** 2005. The plasmids of *Borrelia burgdorferi*: essential genetic elements of a pathogen. Plasmid **53:**1-13.
145. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol **3:**1669-1683.
146. **Sukupolvi, S., and C. D. O'Connor.** 1990. TraT lipoprotein, a plasmid-specified mediator of interactions between gram-negative bacteria and their environment. Microbiol Rev **54:**331-341.
147. **Suzuki, S., K. Komase, H. Matsui, A. Abe, K. Kawahara, Y. Tamura, M. Kijima, H. Danbara, M. Nakamura, and S. Sato.** 1994. Virulence region of plasmid pNL2001 of *Salmonella enteritidis*. Microbiol **140 (Pt 6):**1307-1318.
148. **Swamy, S. C., H. M. Barnhart, M. D. Lee, and D. W. Dreesen.** 1996. Virulence determinants *invA* and *spvC* in salmonellae isolated from poultry products, wastewater, and human sources. Appl Environ Microbiol **62:**3768-3771.

149. **Tezcan-Merdol, D., T. Nyman, U. Lindberg, F. Haag, F. Koch-Nolte, and M. Rhen.** 2001. Actin is ADP-ribosylated by the *Salmonella enterica* virulence-associated protein SpvB. Mol Microbiol **39:**606-619.
150. **Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw.** 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. Int J Food Microbiol **62:**1-5.
151. **Tinge, S. A., and R. Curtiss, 3rd.** 1990. Conservation of *Salmonella typhimurium* virulence plasmid maintenance regions among *Salmonella* serovars as a basis for plasmid curing. Infect Immun **58:**3084-3092.
152. **Van Melderen, L.** 2002. Molecular interactions of the CcdB poison with its bacterial target, the DNA gyrase. Int J Med Microbiol **291:**537-544.
153. **Vandenbosch, J. L., D. K. Rabert, D. R. Kurlandsky, and G. W. Jones.** 1989. Sequence analysis of *rsk*, a portion of the 95-kilobase plasmid of *Salmonella typhimurium* associated with resistance to the bactericidal activity of serum. Infect Immun **57:**850-857.
154. **Villa, L., A. Garcia-Fernandez, D. Fortini, and A. Carattoli.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother **65:**2518-2529.
155. **Wallis, T. S., S. M. Paulin, J. S. Plested, P. R. Watson, and P. W. Jones.** 1995. The *Salmonella dublin* virulence plasmid mediates systemic but not enteric phases of salmonellosis in cattle. Infect Immun **63:**2755-2761.
156. **Wang, Y. A., X. Yu, P. M. Silverman, R. L. Harris, and E. H. Egelman.** 2009. The structure of F-pili. J Mol Biol **385:**22-29.
157. **Watanabe, T.** 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol Rev **27:**87-115.
158. **Weismann, A., E. B. Poulton, S. Schönland, and A. E. Shipley.** 1891. Essays upon heredity and kindred biological problems, 2d ed. Clarendon press, Oxford,.
159. **Whichard, J. M., K. Gay, J. E. Stevenson, K. J. Joyce, K. L. Cooper, M. Omondi, F. Medalla, G. A. Jacoby, and T. J. Barrett.** 2007. Human *Salmonella* and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. Emerg Infect Dis **13:**1681-1688.
160. **White, P. L., A. L. Naugle, C. R. Jackson, P. J. Fedorka-Cray, B. E. Rose, K. M. Pritchard, P. Levine, P. K. Saini, C. M. Schroeder, M. S. Dreyfuss, R. Tan, K. G. Holt, J. Harman, and S. Buchanan.** 2007. *Salmonella Enteritidis* in meat, poultry, and pasteurized egg products regulated by the U.S. Food Safety and Inspection Service, 1998 through 2003. J Food Prot **70:**582-591.
161. **Willetts, N.** 1972. The genetics of transmissible plasmids. Ann Rev Genet **6:**257-268.
162. **Willetts, N., and J. Maule.** 1974. Interactions between the surface exclusion systems of some F-like plasmids. Genet Res **24:**81-89.
163. **Willetts, N., and J. Maule.** 1979. Investigations of the F conjugation gene *traI:traI* mutants and lambdatraI transducing phages. Mol Gen Genet **169:**325-336.
164. **Willetts, N. S.** 1973. Characterization of the F transfer cistron, *traL*. Genet Res **21:**205-213.

165. **Willetts, N. S.** 1971. Plasmid specificity of two proteins required for conjugation in *E. coli* K12. Nature: New biology **230:**183-185.
166. **Wilson, J. A., T. J. Doyle, and P. A. Gulig.** 1997. Exponential-phase expression of *spvA* of the *Salmonella typhimurium* virulence plasmid: induction in intracellular salts medium and intracellularly in mice and cultured mammalian cells. Microbiol **143 (Pt 12):**3827-3839.
167. **Womble, D. D., and R. H. Rownd.** 1988. Genetic and physical map of plasmid NR1: comparison with other IncFII antibiotic resistance plasmids. Microbiol Rev **52:**433-451.
168. **Woodward, M. J., I. McLaren, and C. Wray.** 1989. Distribution of virulence plasmids within salmonellae. J Gen Microbiol **135:**503-511.
169. **Yu, H., J. Wang, J. Ye, P. Tang, C. Chu, S. Hu, and C. H. Chiu.** 2006. Complete nucleotide sequence of pSCV50, the virulence plasmid of *Salmonella enterica* serovar Choleraesuis SC-B67. Plasmid **55:**145-151.
170. **Zhou, D.** 2001. Collective efforts to modulate the host actin cytoskeleton by *Salmonella* type III-secreted effector proteins. Trends Microbiol **9:**567-569; discussion 569-570.