Comparative Analyses and Virulence Potential of Incompatibility Group FIB Plasmid Containing *Salmonella* Schwarzengrund Strains Isolated from Food and Clinical Sources

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Abstract

Introduction: Incompatibility group (Inc) FIB plasmids can contain genes that contribute to antimicrobial resistance and increased virulence. The aim of this study was to determine the genetic relatedness and virulence potential of IncFIB containing S. Schwarzengrund isolates from food and clinical sources. **Methods:** A total of 55 food and clinical S. Schwarzengrund isolates, among which 17 contained IncFIB plasmids were characterized. Food isolates were primarily collected from chicken while clinical isolates were from stool, urine, and blood. Whole genome sequencing (WGS) was performed on 25 clinical isolates using Illumina MiSeq and WGS data for the rest of the isolates were obtained from NCBI. Phylogenetic analyses were performed using single nucleotide polymorphism (SNP) and cgMLST. The virulome was analyzed by the NCTR-developed virulence database. Conjugation was carried out to determine the transferability of plasmids. Invasion and persistence assays were performed using human intestinal epithelial cells (Caco-2) at 1h and 48h post infections, respectively. **Results:** SNP-based phylogenetic analyses showed that IncFIB-containing food and clinical S. Schwarzengrund isolates clustered within the same clade, which was separated from the isolates that lacked IncFIB plasmids. IncFIB plasmids from 9 food and 3 clinical isolates were successfully transferred in to E. coli J53 by conjugation. Food and clinical isolates had nearly similar virulome profiles. All food and clinical isolates examined were able to invade the Caco-2 cells. The invasion rate was higher than the persistence irrespective to plasmid contents and sources of the isolates. **Conclusion:** Overall, IncFIB plasmids are self-conjugative and IncFIB plasmid-carrying S. Schwarzengrund core genomes of food and clinical isolates are genetically related, yet somewhat distinct from non-plasmid containing isolates. **Significance:** The study is important for better understanding the role Salmonella pathogenesis, hence significant for determination of microbiological hazards associated with food.

Introduction

Salmonellosis, the leading cause of bacterial foodborne illness in the United States, is mainly associated with the consumption of foods contaminated with *Salmonella*. Estimates from the Centers for Disease Control and Prevention indicate that in the US alone, more than 1.2 million *Salmonella* infections occur annually resulting in nearly 20,000 hospitalizations and 400 deaths. *S. enterica* has been identified as the source of multiple outbreaks associated with meat and poultry products in the US and other countries. Some *S. enterica* serovars cause more invasive infections than others; for example, a previous study demonstrated serovars Heidelberg and Typhimurium were more invasive, i.e., 13% and 6% of reported infections were invasive, respectively. Representative incompatibility group (Inc) FIB plasmids can carry both virulence factors and antimicrobial resistance genes. *Salmonella enterica* possess several iron acquisition systems, encoded on the chromosome and plasmids such as IncFIB. Recently, we demonstrated that IncFIB plasmid-encoded iron acquisition systems (Sit and aerobactin) likely play an important role in persistence of *Salmonella* in human intestinal epithelial cells. In this study, we further characterized the IncFIB plasmids containing *S.* Schwarzengrund strains isolated from food and clinical sources.

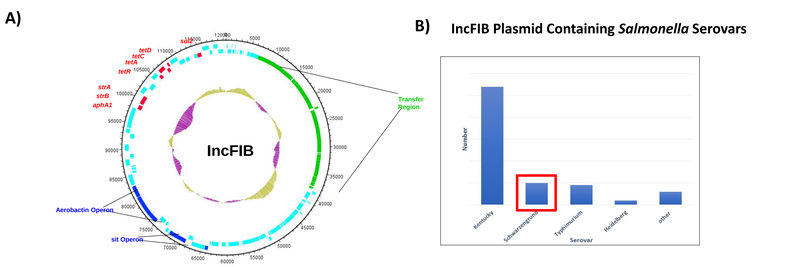


Figure 1. A) Schematic diagram of IncFIB plasmid. Blue region indicates virulence factors such as iron acquisition systems; Red region indicates multiple antibiotic resistance genes; Green region indicates plasmid transfer associated genes**. B)** Number of IncFIB plasmid containing *Salmonella* isolates from different sources based on the WGS depository at NCBI

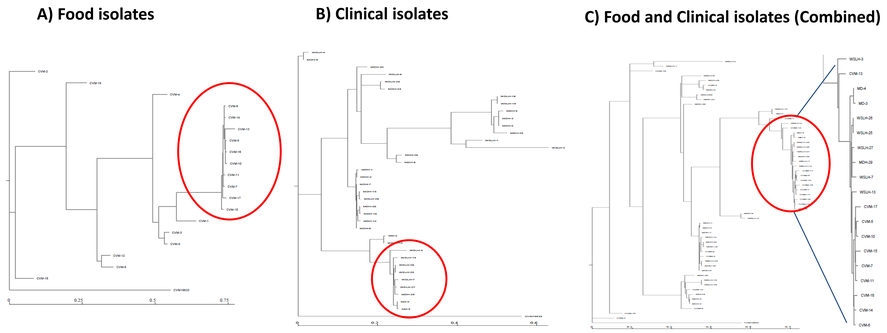


Figure 2. Single nucleotide polymorphism (SNP)-based phylogenetic analyses of *S.* Schwarzengrund isolates from food and clinical sources. A) IncFIB-containing food isolates (**red circle**) separated from the cluster of isolated that lack IncFIB, B). IncFIB-containing clinical isolates (**red circle**) separated from the cluster of isolated that lack IncFIB, C) in combine SNP analyses, IncFIB-containing food and clinical isolates clustered within the same clade (**red circle**), which was separated from the other isolates that lacked IncFIB plasmids indicating their genetic relatedness. In the original analysis number is not visible hence magnified. SNP analyses was performed using CFSAN SNP pipeline

Results and Methods

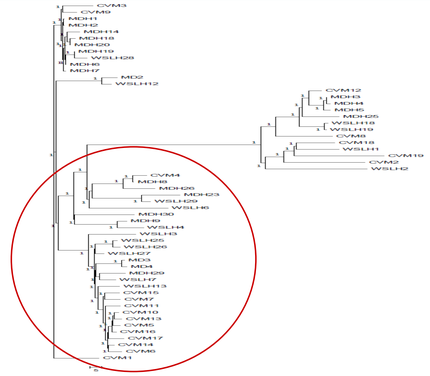
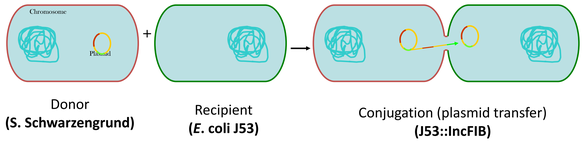


Figure 3. Core Genome multi locus typing (cgMLST) analyses of *S.* Schwarzengrund isolates from food and clinical sources. IncFIB-containing food and clinical isolates clustered within the same clade (**red circle**), which was separated from isolates that does not carry IncFIB. Outcome of phylogenetic analyses by cgMLST was similar to SNP analyses presented in the Figure 2.

Conjugative IncFIB Plasmid Transfer



Source	No. of IncFIB Isolates tested	Successfully Transferred to *E. coli*
Food	9	9
Human	8	3

Figure 4. IncFIB plasmid was transferred from *Salmonella* donors to the recipient *E. coli* J53 using plate mating approach. IncFIB plasmids from 9 food and 3 clinical isolates were successfully transferred in to *E. coli* J53 by conjugation.

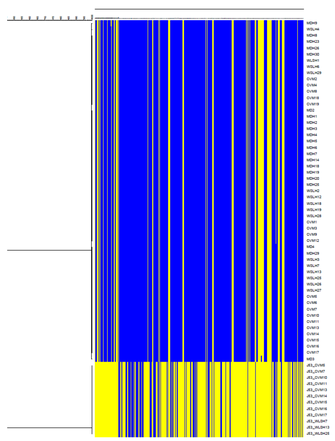


Figure 5. Virulome profiles of S. Schwarzengrund strain isolated from food and clinical sources. FASTA files of sequence assemblies from each strain were analyzed using an in-house Salmonella virulence factor database made up of 490 genes curated from PATRIC, Victor, VFDB, and the literature (NCTR Virulence Factor Database) were queried to identify putative virulence genes. Most of the Salmonella isolates had very similar virulence factor profiles, while the transconjugants separated to a distinct clade, due to them being *E. coli* and lacking a large number of the Salmonella-associated virulence genes. In the dendrogram, a blue box indicated presence of the gene and a yellow box indicated that the gene was not detected.

Invasion and Persistence Assays using Human Intestinal Epithelial Cells (CaCo-2)

Number	Sample	Source	PlasmidFinder	ResFinder
1	CVM N38851	Ground Turkey	Col156	no acquired resistance gene
2	CVM N38910	Ground Turkey	IncY	tet(C)
3	CVM N39866	Ground Turkey	IncI1, Col156	aph(3')-Ia, blaCMY-2
4	CVM N41900	Ground Turkey	IncHI2A, IncHI2	aph(6)-Id, strA, tet(B)
5	CVM N43459	Chicken Wings	FIB, FIC (FII)	strA, strB
6	CVM N43479	Chicken Breast	IncFIC(FII)	no acquired resistance gene
7	CVM N44700	Chicken Breast	FIB, FIC (FII)	strA, strB
8	CVM N45932	Ground Turkey	IncFII(PCTU2), pESA2	strA, blaTEM-1B
9	CVM N45952	Ground Turkey	IncX4	no acquired resistance gene
10	CVM N47711	Chicken Breast	FIB, FIC (FII)	strA, strB
11	CVM N47712	Chicken Breast	FIB, FIC (FII)	strA, strB
12	CVM N48682	Ground Turkey	No Rep Plasmid	no acquired resistance gene
13	CVM N50434	Chicken Breast	FIB, FIC (FII)	strA, strB
14	CVM N51257	Chicken Breast	FIB, FIC (FII)	strA, strB
15	CVM N51259	Chicken Breast	FIB, FIC (FII)	strA, strB
16	CVM N51267	Chicken Breast	FIB, FIC (FII)	strA, strB
17	CVM N51289	Chicken Breast	FIB, FIC (FII)	strA, strB
18	CVM N51311	Chicken Breast	No Rep Plasmid	no acquired resistance gene
19	CVM N57032	Animal-Swine-Sow	Rep(pKPC-2), IncQ1	aph(3'')-Ib, sul2, tet(A)

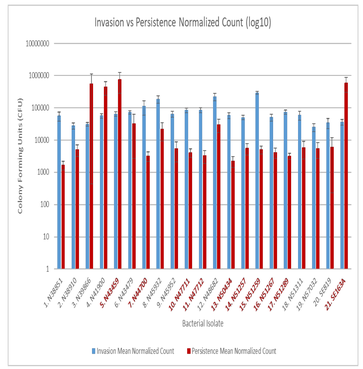
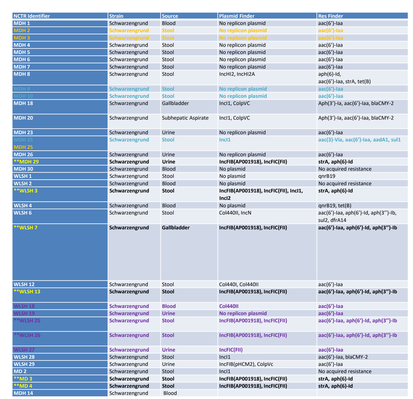


Figure 6. Invasion and persistence of 19 wildtype *S.* Schwarzengrund food isolates. Nine food isolates contained IncFIB indicating on the table as Red. The general trend was that the amount of surviving colony forming units were lower after 48 hrs (persistence) as compared to 1 hr (Invasion). X-axis indicate the number of isolates (number 1 to 19 corresponds to the strains number and designation as listed in table 1, 20= SE819 (less virulent strain that lacked IncFIB), 21=SE163A (virulent strain that contains IncFIB along with other virulence associated plasmids),



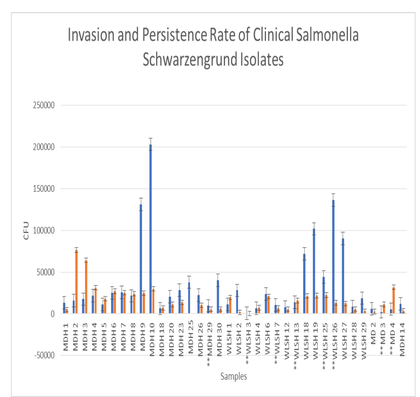


Figure 7. Invasion and persistence of 36 wildtype *S.* Schwarzengrund clinical isolates. Similar to food isolates, The general trend was that the amount of surviving colony forming units were lower in persistence as compared to Invasion in clinical isolates. X-axis indicate the number of isolates with sample ID and Y-axis represents the CFU/ml.

Key Points

- SNP and cgMLST showed that IncFIB containing food and clinical isolates clustered within the same clade indicating their genetic relatedness.
- All the IncFIB plasmids of *S.* Schwarzengrund food isolates (n=9) and 3 out of 8 tested clinical isolates were self conjugative as we successfully transferred them into *E. coli* J53.
- Food and clinical isolates had nearly similar virulome profiles.
- All food and clinical *S.* Schwarzengrund isolates examined were able to invade and persist into Caco-2 cells at variable rate.
- The number of surviving colony forming units were lower in persistence as compared to invasion.