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cmv-2 Incompatibility groups in *Salmonella* multi-drug resistance plasmids

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ABSTRACT

Many Gram negative bacteria including *Salmonella enterica* species exhibit evolving multi-drug resistance coded for on their chromosome and on mobile genetic elements. *Salmonella* species carrying multi-drug resistance plasmids can potentially disperse the *cmv-2* gene conferring β -lactam resistance to new *Salmonella* species as well as to other enteric bacteria commonly found in the human and animal gut. **Objective:** This study seeks to identify the numbers and incompatibility types of plasmids carried by six different *Salmonella* clinical isolates. **Design:** Raw whole genome and DNA fragment (contig) sequence secondary data analyses were conducted using the Center for Genomic Epidemiology (Lyngby, Denmark) open source internet databases plasmid Multilocus Sequence Typing (pMLST) and Resistance Finder (ResFinder). **Method:** Nucleic acid sequences were separated into files, labeled by contig, and uploaded to the consensus databases for analysis against known *Salmonella* resistance genes and plasmid types. Results were returned in table format and identified specific resistance, susceptibility, and incompatibility groups present with greater than 90% homology between known and analyzed DNA sequences.

Results: Results of this analysis show that these bacterial strains carry plasmids which confer multi-drug resistance and that multiple incompatibility groups are present across some but not all analyzed *Salmonella* strains. **Conclusions:** The researchers continue to analyze the data and have not yet formed conclusions as to their significance. This study will advance our understanding about how bacteria develop multiple antibiotic resistances and could ultimately help design more effective drugs with greater potential to combat and prevent bacterial diseases.

MATERIALS & METHODS

The Center for Genomic Epidemiology (CGE) <http://www.genomicepidemiology.org> is a central database that continues to develop algorithms for rapid analyses of whole genome DNA-sequences, tools for analyses and extraction of information from the sequence data and internet/web-interfaces for using the tools in the global scientific and medical community. Three of their tools were used in this study.

PlasmidFinder: This database identifies plasmids including ones carrying antimicrobial resistance in total or partial sequenced isolates of bacteria (e.g. clinically important bacteria). The program can utilize raw, contig group, or completely assembled and closed plasmid sequencing data. The database currently consists of 116 replicon sequences that match with at least at 80% nucleotide identity all replicon sequences identified in the 559 fully sequenced plasmids.

Plasmid Multi-Locus Typing: Database developed to perform sequence-based subtype five plasmids very frequently isolated from clinically relevant enterobacterial strains. These plasmids are IncF, IncN, IncHI2 and IncHI1.

ResFinder data analysis: This database compares submitted whole genome sequence data to the known antibiotic resistance genes in the NCBI nucleotide database. Results are reported as % identity to known resistance genes based on the number of nucleotides that match between the sequences.

National Center for Biotechnology Information <https://www.ncbi.nlm.nih.gov> BLAST (Basic Local Alignment Search Tool) is a tool that helps in finding regions of similarity between biological sequences by comparing nucleotide/protein sequences to sequence database and calculates the statistical significance.

RESULTS

Strains with plasmids	Plasmid	%Identity	Contig	Strains with plasmids	Plasmid	%Identity	Contig
CMY-024	IncA/C2	100	10	CMY37	IncFIB	98.39	28
	Col8282	100	4		IncI2	98.1	6
CMY 30	IncA/C2	100	23		IncA/C2	100	40
	IncI2	98.1	3		IncFII(S)	100	1
	IncFIB	98.39	13	CMY 38	IncA/C2	100	17
	IncFII(S)	100	7		IncFII(S)	100	11
CMY 32	IncA/C2	100	17		IncFIB(S)	100	11
	IncFIB(S)	100	2	CMY-039	IncI1	99.3	11
	IncFII(S)	100	2				

Table 1. *Salmonella enterica* plasmid types based on incompatibility groups and their location based on whole genome analysis.

CMY-024	Locus	% Identity	Allele Length	Allele
IncI1	<i>pilL</i>	99.61	254	<i>pilL_2</i>
CMY-030	Locus	% Identity	Allele Length	Allele
IncF	<i>FII</i>	100	215	<i>FII_S1</i>
	<i>FIB</i>	100	373	<i>FIB_1</i>
CMY-032	Locus	% Identity	Allele Length	Allele
IncF	<i>FII</i>	100	215	<i>FII_S1</i>
	<i>FIB</i>	100	373	<i>FIB_17</i>
CMY-037	Locus	% Identity	Allele Length	Allele
IncF	<i>FII</i>	100	215	<i>FII_S1</i>
	<i>FIB</i>	100	373	<i>FIB_1</i>
CMY-038	Locus	% Identity	Allele Length	Allele
IncF	<i>FII</i>	100	215	<i>FII_S1</i>
	<i>FIB</i>	100	373	<i>FIB_17</i>
CMY-039	Locus	% Identity	Allele Length	Allele
IncI1	<i>ardA</i>	100	343	<i>ardA_4</i>
	<i>pilL</i>	100	254	<i>pilL_2</i>
	<i>repI1</i>	100	83	<i>repI1_2</i>
	<i>sogS</i>	100	235	<i>sogS_4</i>
	<i>trbA</i>	100	465	<i>trbA_5</i>

Table 2. *Salmonella* incompatibility groups with comparative allelic identities.

RESULTS CONT'D

Contig	Predicted phenotype	Resistance gene	%Identity
CMY-024 contig 1	Aminoglycoside resistance	<i>aph(3')-Ia</i>	99.02
CMY-024 contig 2	Phenicol resistance	<i>floR</i>	98.27
CMY-024 contig 2	Aminoglycoside resistance	<i>strA, strB, sul2</i>	100
CMY-024 contig 2	Tetracycline resistance	<i>tet(A)</i>	100
CMY-024 contig 14	Beta-lactam resistance	<i>blaCMY-2</i>	100
CMY-024 contig 102	Tetracycline resistance	<i>tet(B)</i>	100
CMY-032 contig 6	Beta-lactam resistance	<i>blaCMY-2</i>	100
CMY-032 contig 20	Aminoglycoside resistance	<i>aadA2</i>	100
CMY-032 contig 20	Trimethoprim resistance	<i>dfrA12</i>	100
CMY-032 contig 20	Sulphonamide resistance	<i>sul1</i>	100
CMY-032 contig 27	Phenicol resistance	<i>floR</i>	98.27
CMY-032 contig 27	Aminoglycoside resistance	<i>strA, strB, sul2</i>	100
CMY-032 contig 27	Tetracycline resistance	<i>tet(A)</i>	100
CMY-032 contig 56	Aminoglycoside resistance	<i>aph(3')-Ia</i>	99.4
CMY-037 contig 9	Beta-lactam resistance	<i>blaCMY-2</i>	100
CMY-037 contig 9	Aminoglycoside resistance	<i>strA, strB, sul2</i>	100
CMY-037 contig 18	Phenicol resistance	<i>floR</i>	98.19
CMY-037 contig 29	Aminoglycoside resistance	<i>aph(3')-Ic</i>	98.77
CMY-037 contig 30	Tetracycline resistance	<i>tet(A)</i>	100
CMY-037 contig 35	Beta-lactam resistance	<i>blaTEM-1B</i>	100
CMY-037 contig 36	Sulphonamide resistance	<i>sul1</i>	100
CMY-037 contig 42	Aminoglycoside resistance	<i>aadA1</i>	100
CMY-037 contig 43	Aminoglycoside resistance	<i>aadA2</i>	100
CMY-037 contig 43	Trimethoprim resistance	<i>dfrA12</i>	100
CMY-038 contig 9	Aminoglycoside resistance	<i>aadA2</i>	100
CMY-038 contig 9	Beta-lactam resistance	<i>blaCMY-2</i>	100
CMY-038 contig 9	Trimethoprim resistance	<i>dfrA12</i>	100
CMY-038 contig 9	Sulphonamide resistance	<i>sul1</i>	100
CMY-038 contig 16	Phenicol resistance	<i>floR</i>	98.27
CMY-038 contig 16	Aminoglycoside resistance	<i>strA, strB, sul2</i>	100
CMY-038 contig 16	Tetracycline resistance	<i>tet(A)</i>	100
CMY-038 contig 61	Aminoglycoside resistance	<i>aph(3')-Ia</i>	99.02
CMY-039 contig 11	Beta-lactam resistance	<i>blaTEM-1B</i>	100
CMY-039 contig 53	Aminoglycoside resistance	<i>aph(3')-Ia</i>	99.28

Table 3. *Salmonella* clinical isolate antibiotic resistances and their associated resistance genes. Note: CMY-030 displayed no antibiotic resistance genes.

CONCLUSIONS

- Plasmids belonging to four major incompatibility groups (IncA/C, IncF, IncI) were identified using the PlasmidFinder database. This database further subtyped the IncF plasmids into incFIB, IncFIB (S), IncFII (S) and the IncI plasmids into IncI1 and IncI2 (Table 1).
- pMLST database confirmed the subtyping of IncF and IncI plasmids on the basis of different alleles of select genes (Table 2).
- Several antibiotic resistance genes were identified using the ResFinder database. Location of some of these genes directly corresponded with the location of the CMY2 plasmids. For example, IncI1 plasmid and *blaTEM-18* gene are both found on contig 11 in CMY-039. Other resistance genes were often found on adjacent or nearby contigs but not on the same contig as the different plasmids (Table 3)
- Plasmid sequences were found located on multiple Contigs. BLAST search results are currently being utilized to identify and align complete sequence of each plasmid.