# *cmy-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 *cmy-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 Genomiic 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 | *pilL* | 99.61 | 254 | *pilL_2* |
| **CMY-030** | **Locus** | **% Identity** | **Allele Length** | **Allele** |
| IncF | *FII* | 100 | 215 | *FII_S1* |
| | *FIB* | 100 | 373 | *FIB_1* |
| **CMY-032** | **Locus** | **% Identity** | **Allele Length** | **Allele** |
| IncF | *FII* | 100 | 215 | *FII_S1* |
| | *FIB* | 100 | 373 | *FIB_17* |
| **CMY-037** | **Locus** | **% Identity** | **Allele Length** | **Allele** |
| IncF | *FII* | 100 | 215 | *FII_S1* |
| | *FIB* | 100 | 373 | *FIB_1* |
| **CMY-038** | **Locus** | **% Identity** | **Allele Length** | **Allele** |
| IncF | *FII* | 100 | 215 | *FII_S1* |
| | *FIB* | 100 | 373 | *FIB_17* |
| **CMY-039** | **Locus** | **% Identity** | **Allele Length** | **Allele** |
| IncI1 | *ardA* | 100 | 343 | *ardA_4* |
| | *pilL* | 100 | 254 | *pilL_2* |
| | *repI1* | 100 | 83 | *repI1_2* |
| | *sogS* | 100 | 235 | *sogS_4* |
| | *trbA* | 100 | 465 | *trbA_5* |

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

## RESULTS CONT'D

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