Testing AMRFinder With NARMS Isolates

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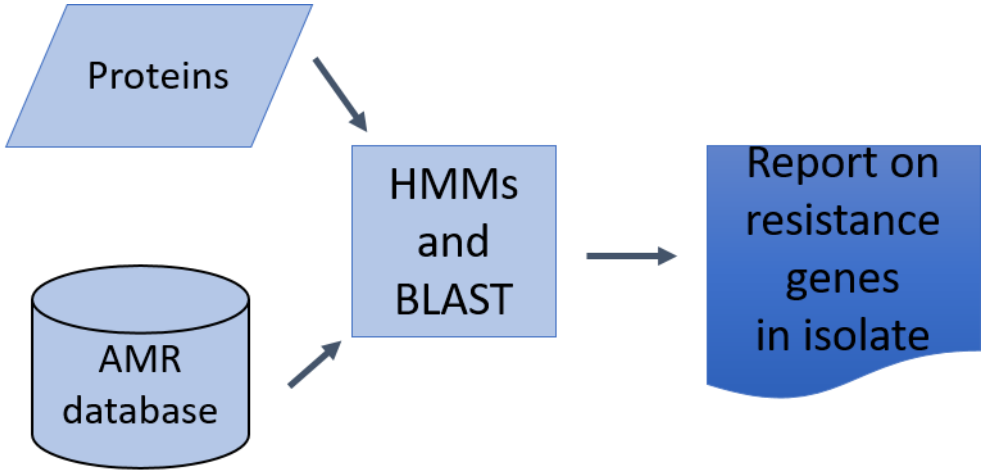
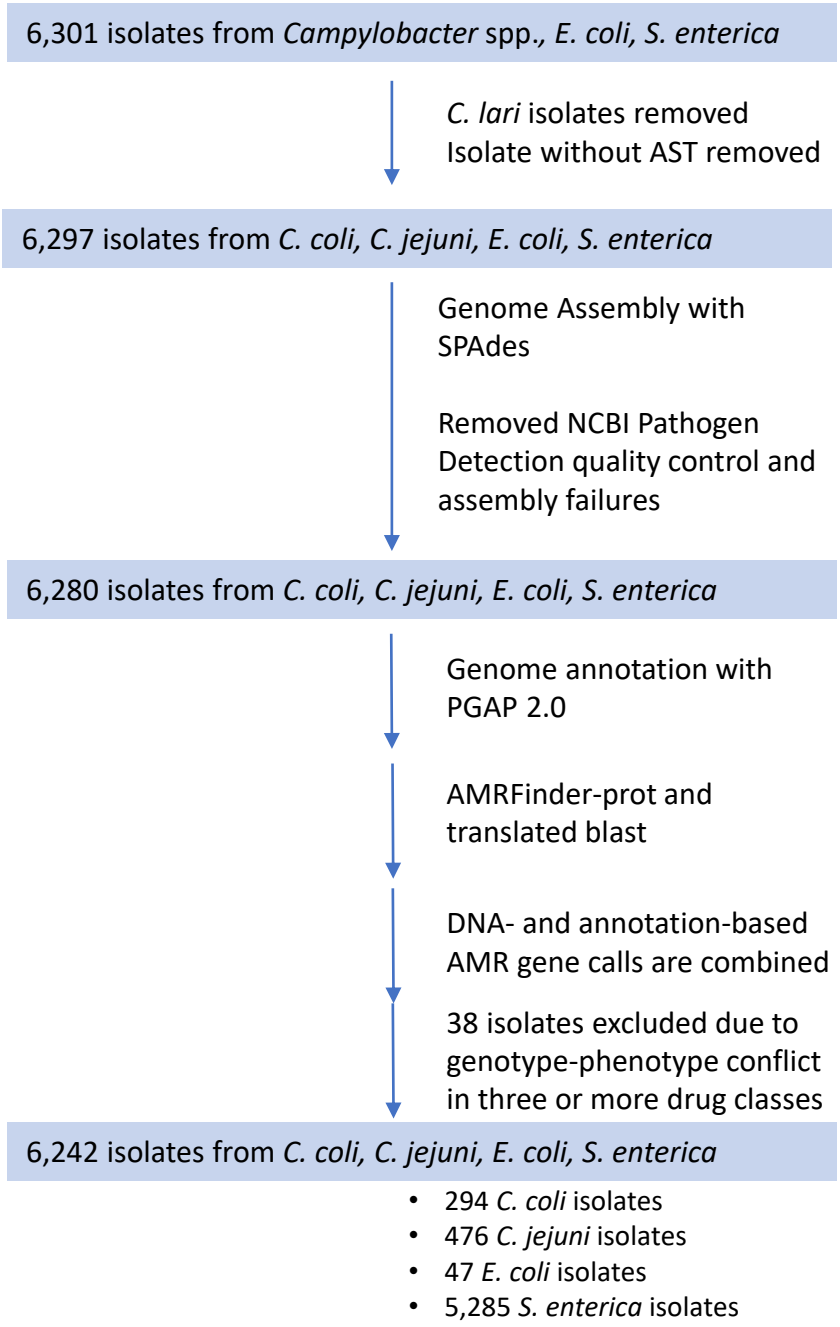
AMRFinder overview



4,322 resistance proteins
851 resistance gene families
34 drug classes resisted
~50% beta-lactamases

Hierarchical structure

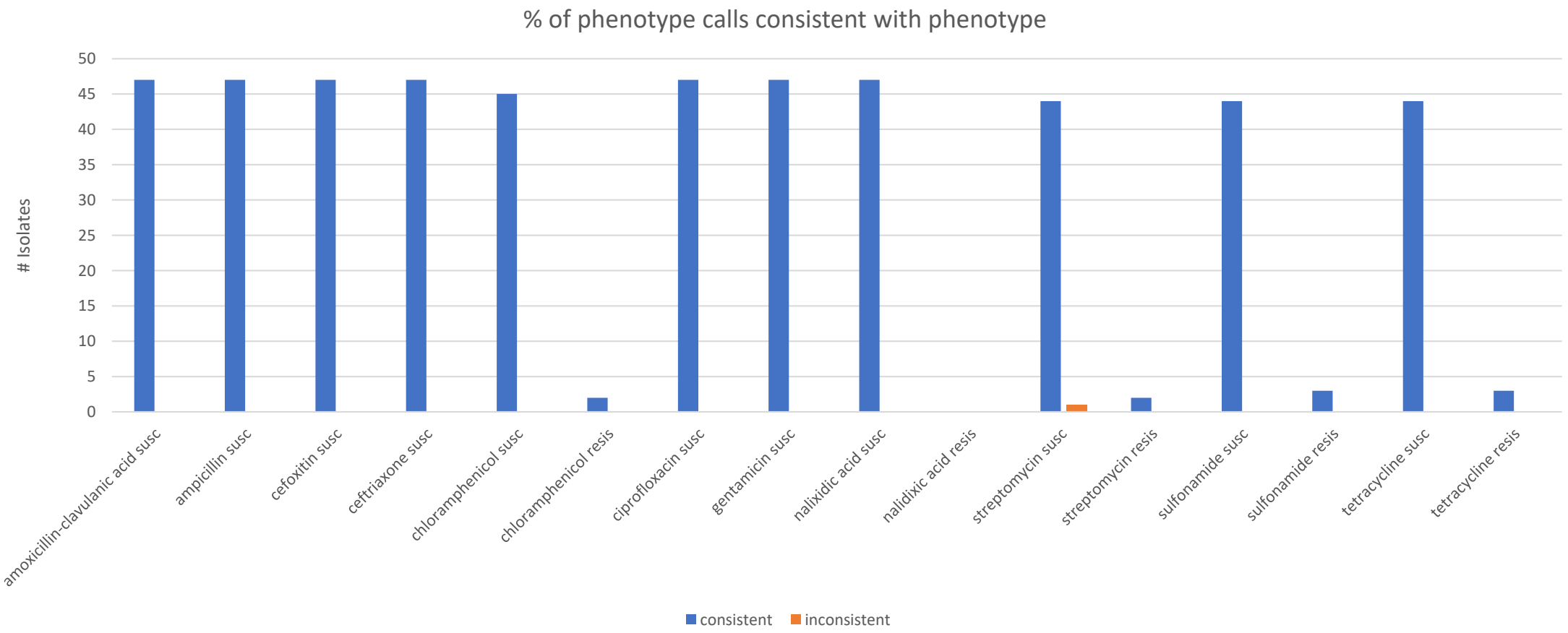
	Protein name	Functional determination
Exact match	KPC-2	*Resistance to carbapenems and other beta-lactam antibiotics.*
HMM score > cutoff of KPC family	KPC family	*Likely resistance to carbapenems and other beta-lactam antibiotics.*
HMM score > cutoff	class A β-lactamase	*Class A beta-lactamase of unknown specificity.*
HMM score > cutoff	**not** beta-lactamase	Prevents false-positive identification as a beta-lactamase. *Not reported.*


6,301 isolates from *Campylobacter* spp., *E. coli, S. enterica*
C. lari isolates removed
Isolate without AST removed
6,297 isolates from *C. coli, C. jejuni, E. coli, S. enterica*
Genome Assembly with SPAdes
Removed NCBI Pathogen Detection quality control and assembly failures
6,280 isolates from *C. coli, C. jejuni, E. coli, S. enterica*
Genome annotation with PGAP 2.0
AMRFinder-prot and translated blast
DNA- and annotation-based AMR gene calls are combined
38 isolates excluded due to genotype-phenotype conflict in three or more drug classes
6,242 isolates from *C. coli, C. jejuni, E. coli, S. enterica*
294 *C. coli* isolates
476 *C. jejuni* isolates
47 *E. coli* isolates
5,285 *S. enterica* isolates

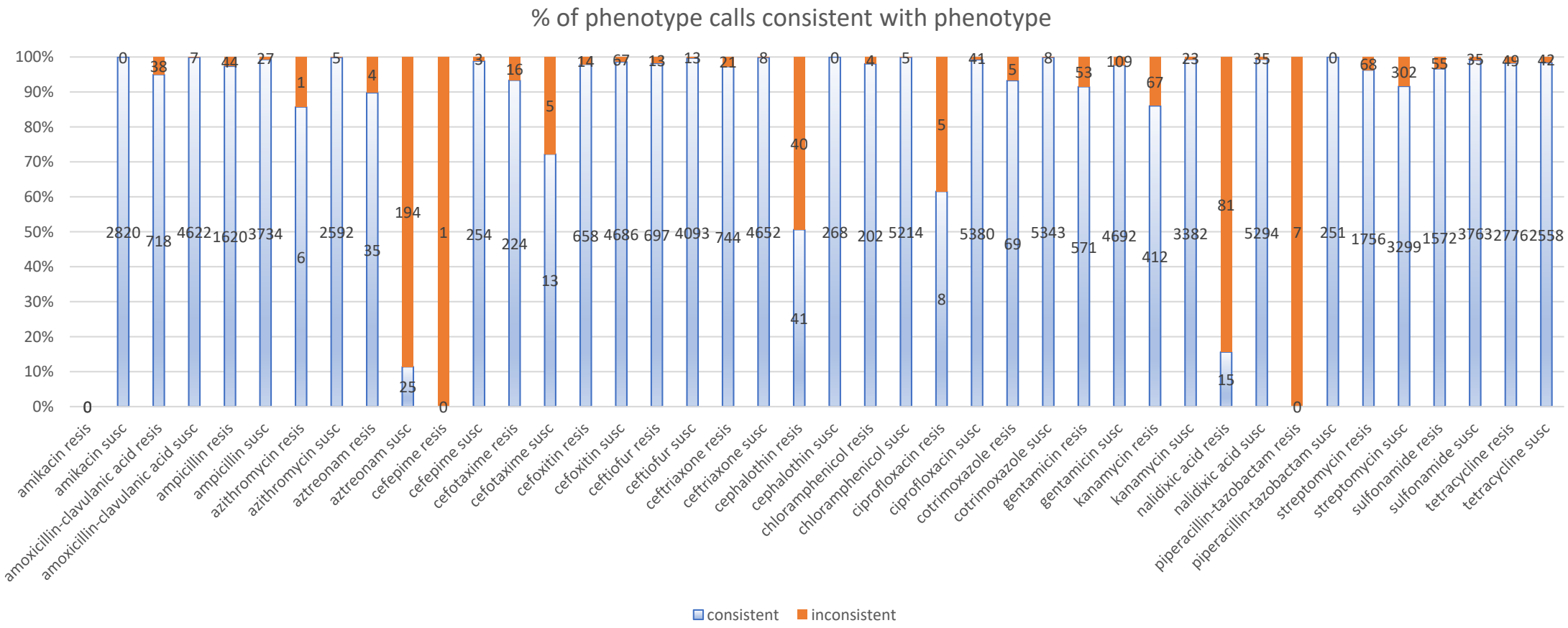
Summary of genotype-phenotype predictions

- 97% of susceptibility tests were consistent with AMR genotypes
- *S. enterica* and *E. coli* had very high consistency (97%, 99%), while *C. coli* had the lowest consistency (88%); *C. jejeuni* had 95%
- 7.6% (967) of positive tests for resistance were from isolates that did not have a known acquired AMR gene
  - these represent 48% of the total inconsistencies
- 21% (1,292/6,008) isolates had one or more inconsistencies
  - quinolones (52% of total inconsistencies)
  - aminoglycosides(14% of total inconsistencies)

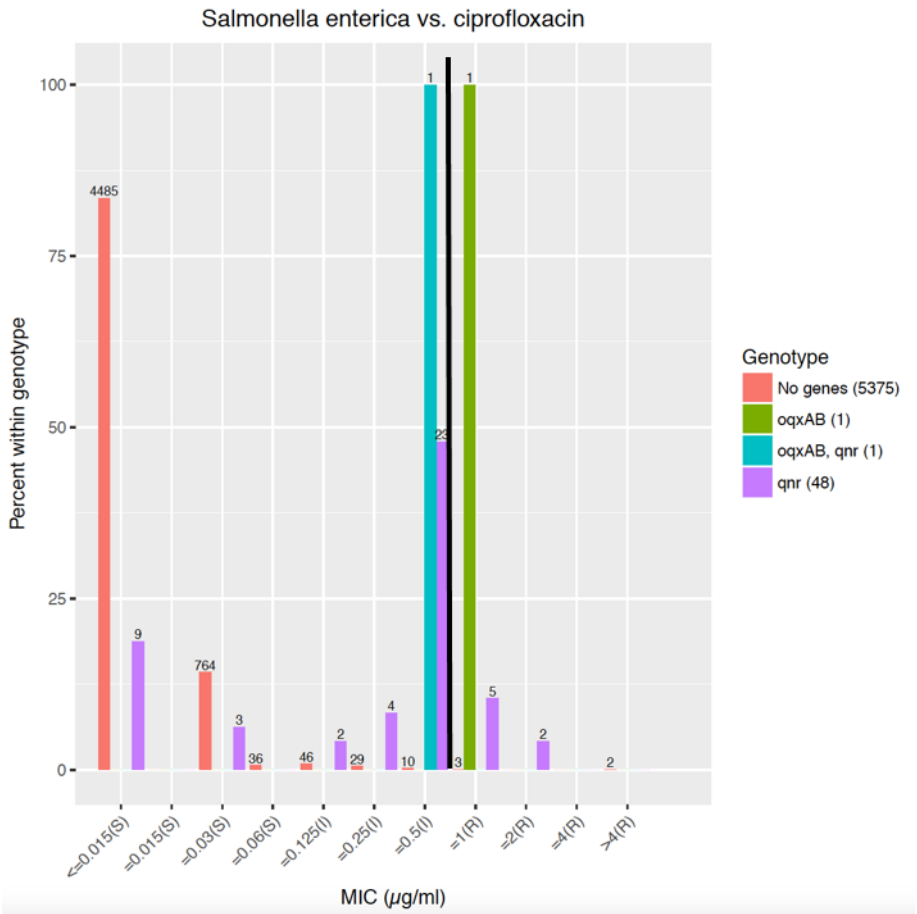
E. coli isolates are highly consistent with genotypes

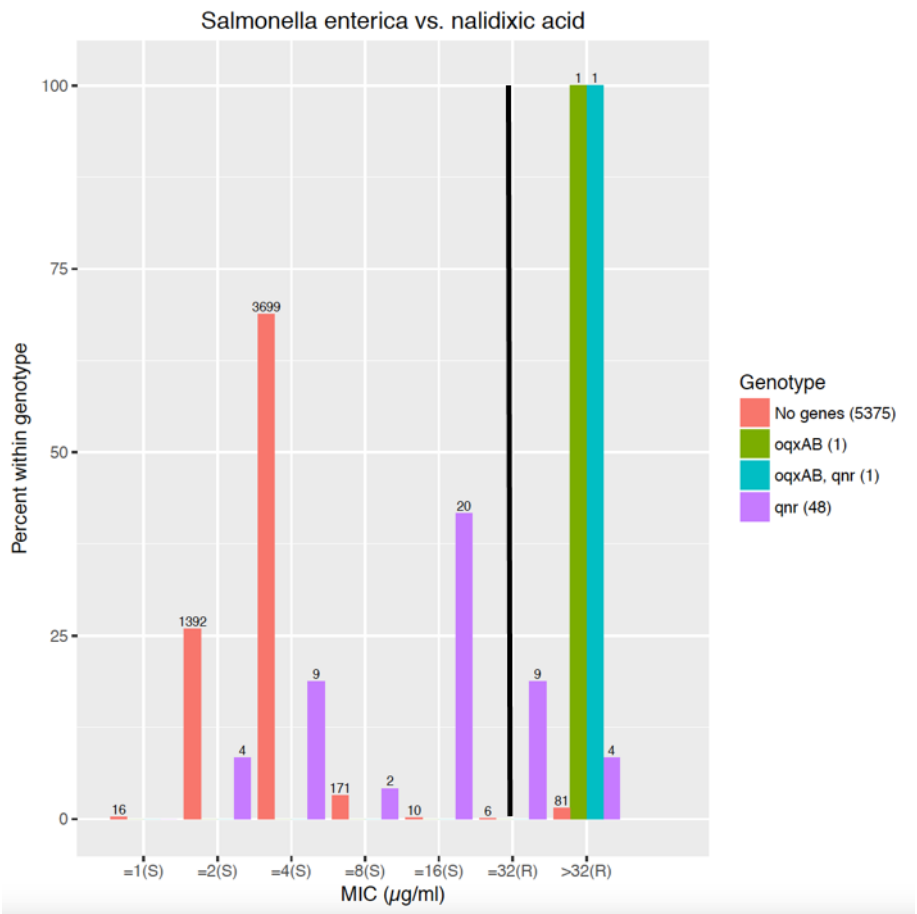


Inconsistencies in *Salmonella* are due to fluoroquinolone resistance

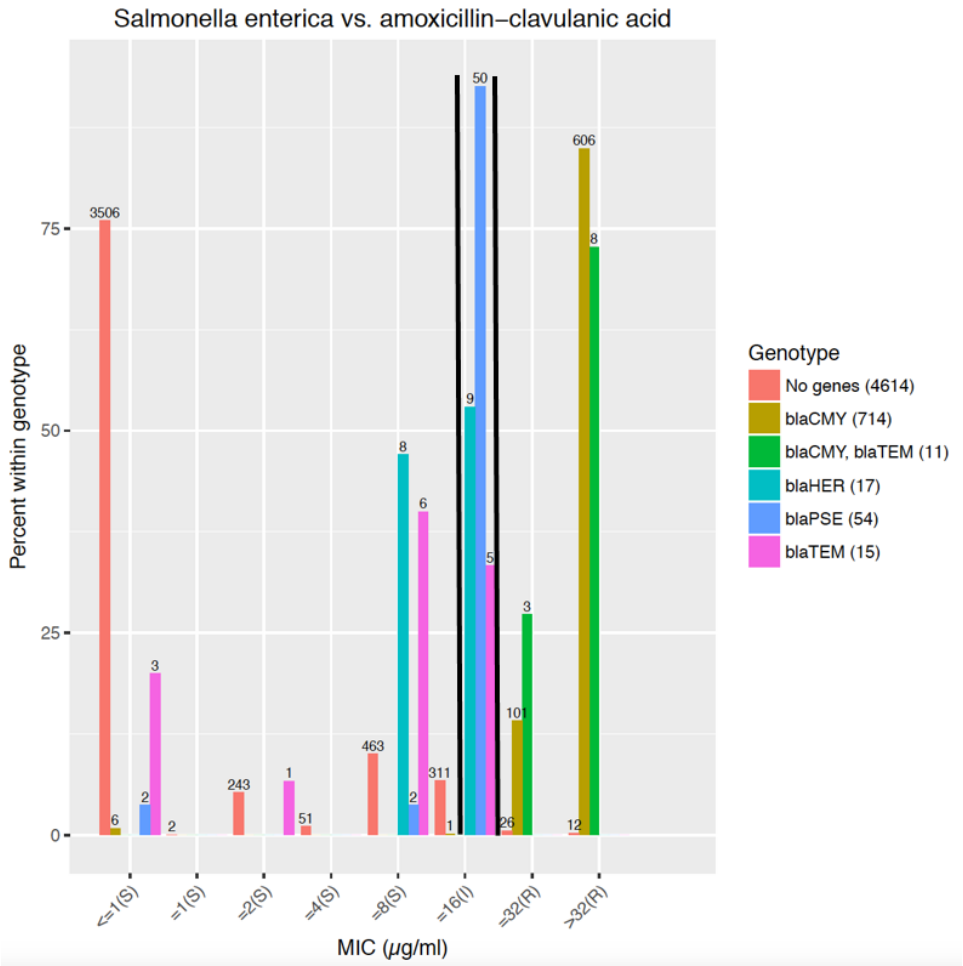


Qnr loci affect fluoroquinolone MICs in *Salmonella* (49 *qnr*+ isolates)

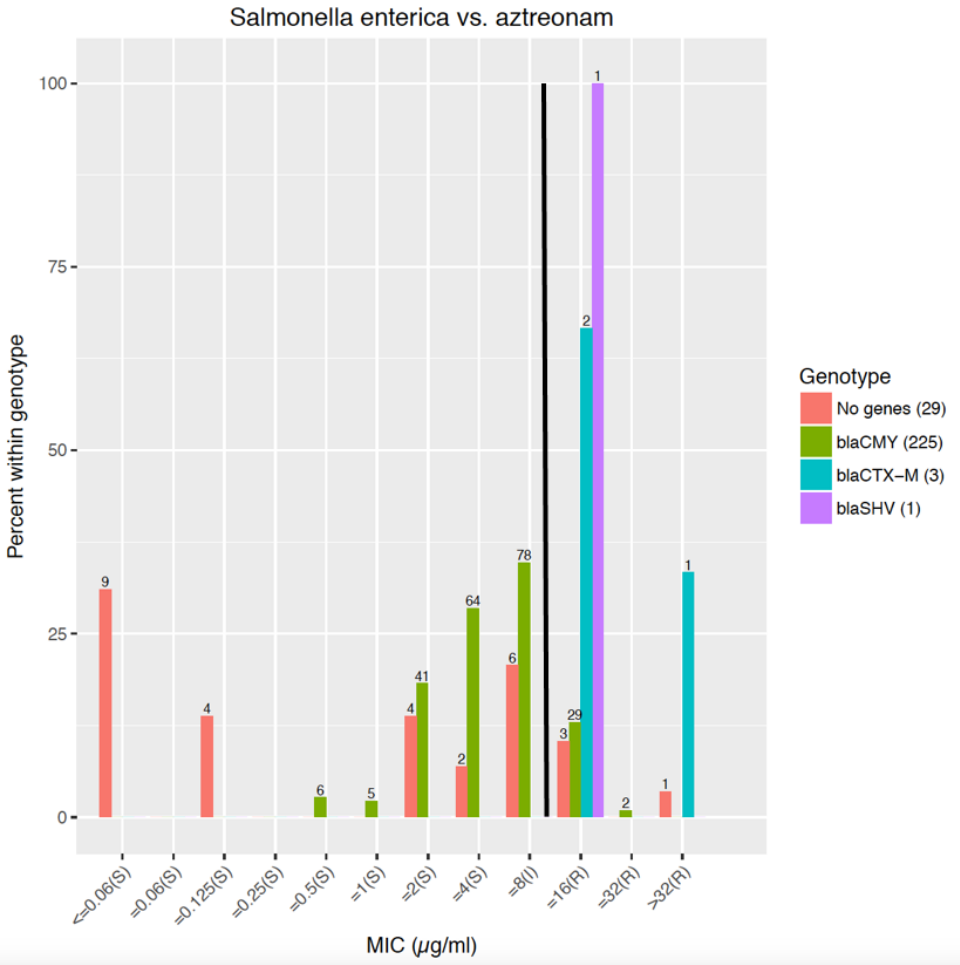




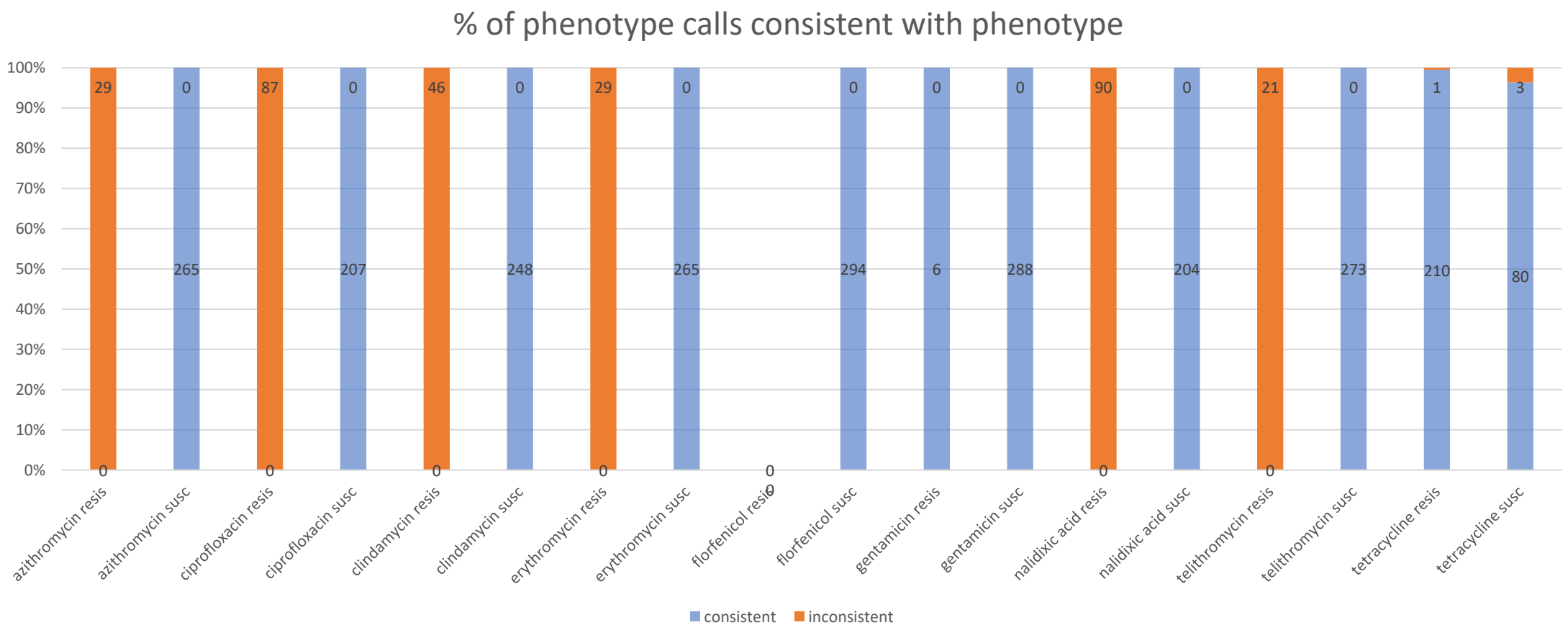
Unexpected beta-lactamases confer decreased susceptibility to amoxicillin-clavulanic acid in *S. enterica*



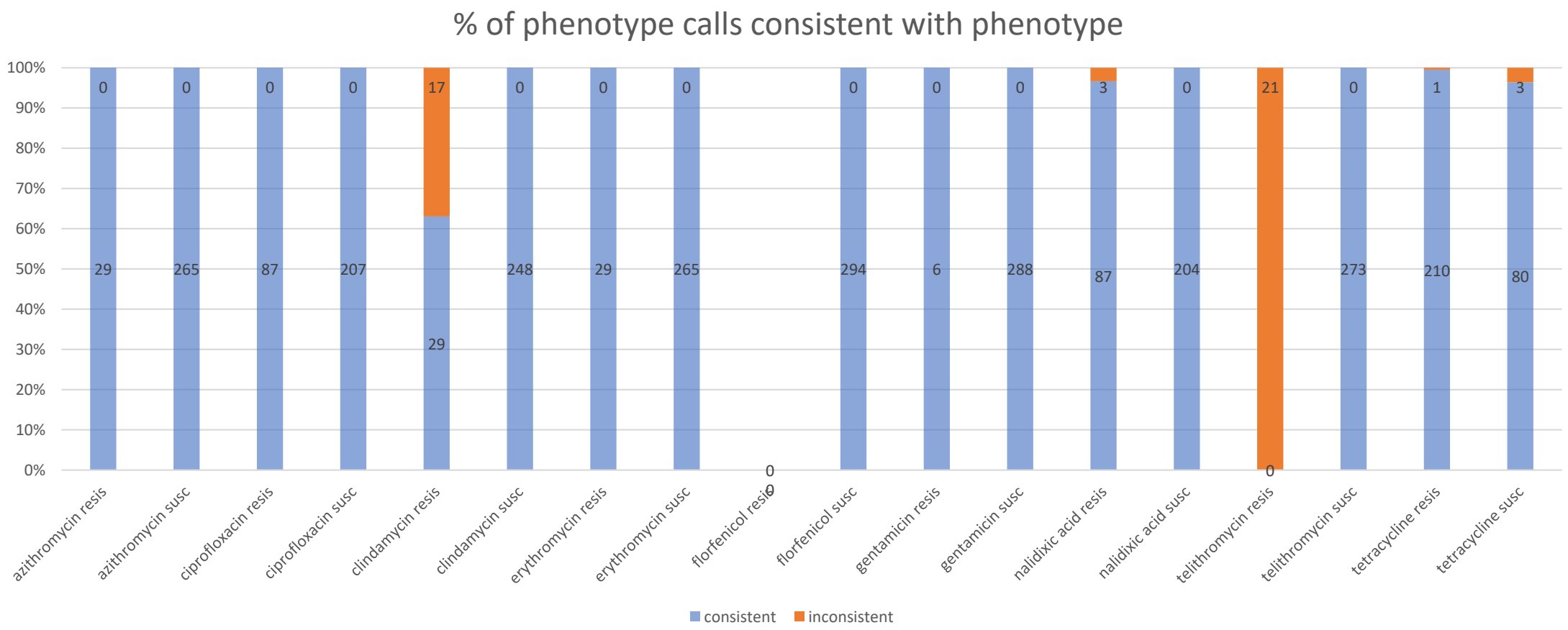
CMY beta-lactamases often do not confer clinical resistance to aztreonam in *S. enterica*



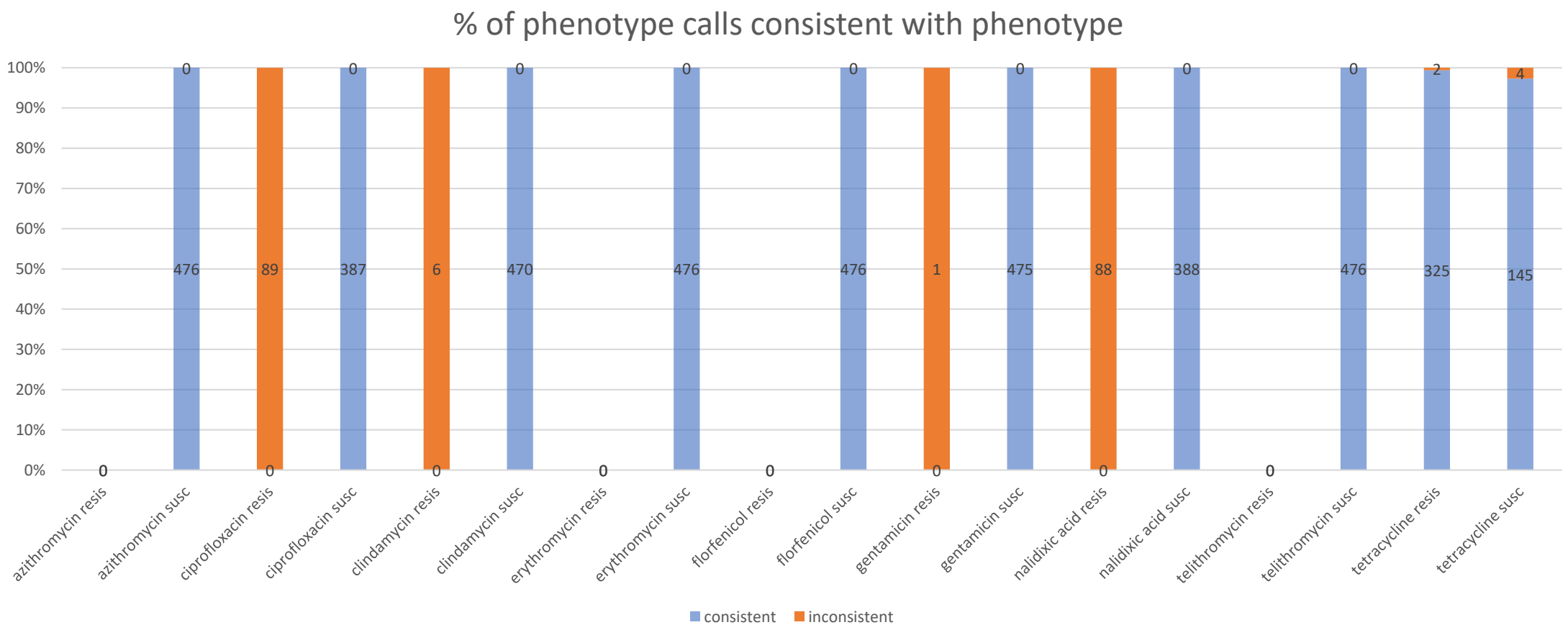
Inconsistencies in *C. coli* are mostly not due to acquired resistance mechanisms (shown without point mutations)



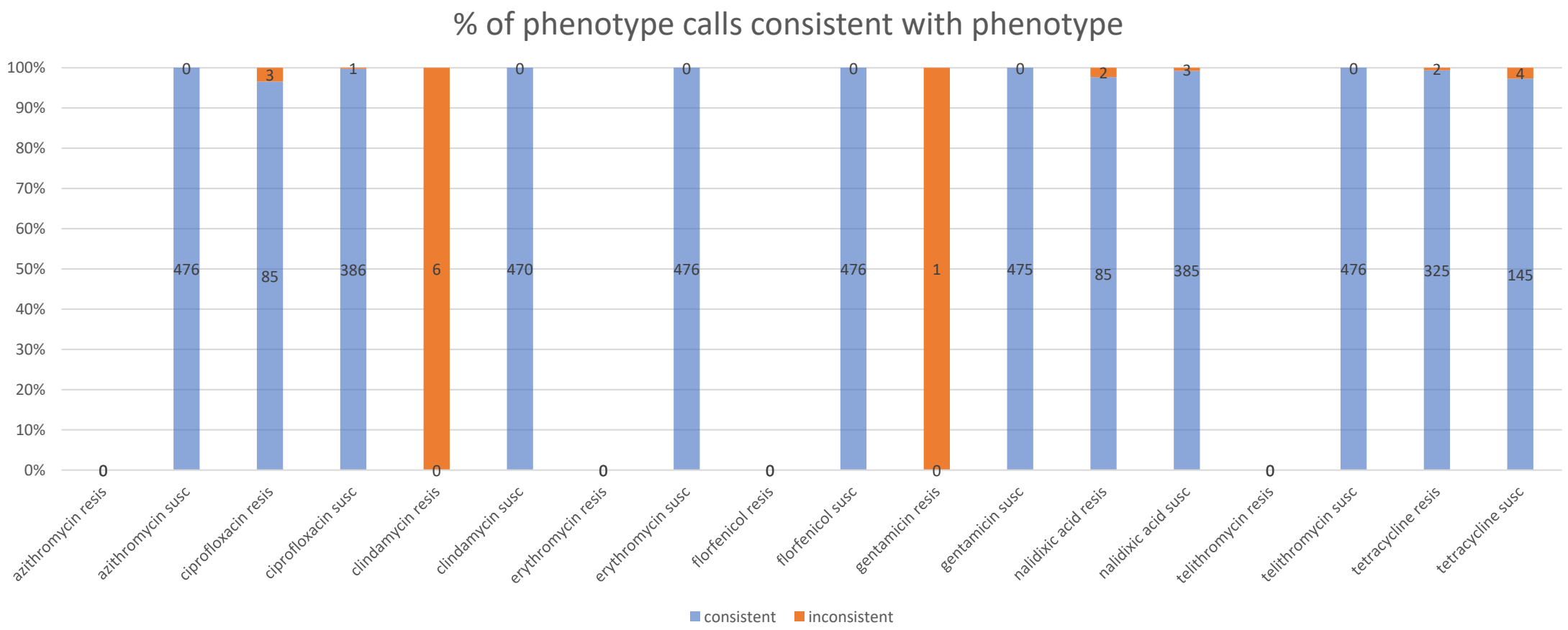
Inconsistencies in *C. coli* are mostly not due to acquired resistance mechanisms (point mutations added)



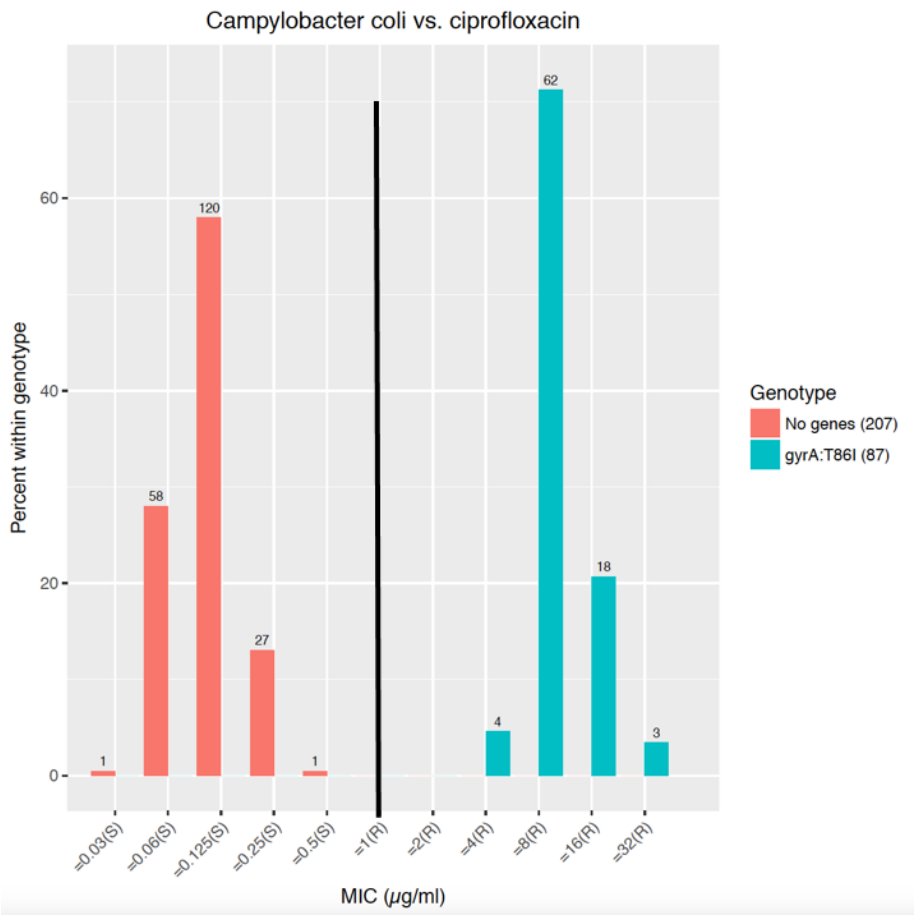
Inconsistencies in *C. jejeuni* are mostly not due to acquired resistance mechanisms (shown without point mutations)

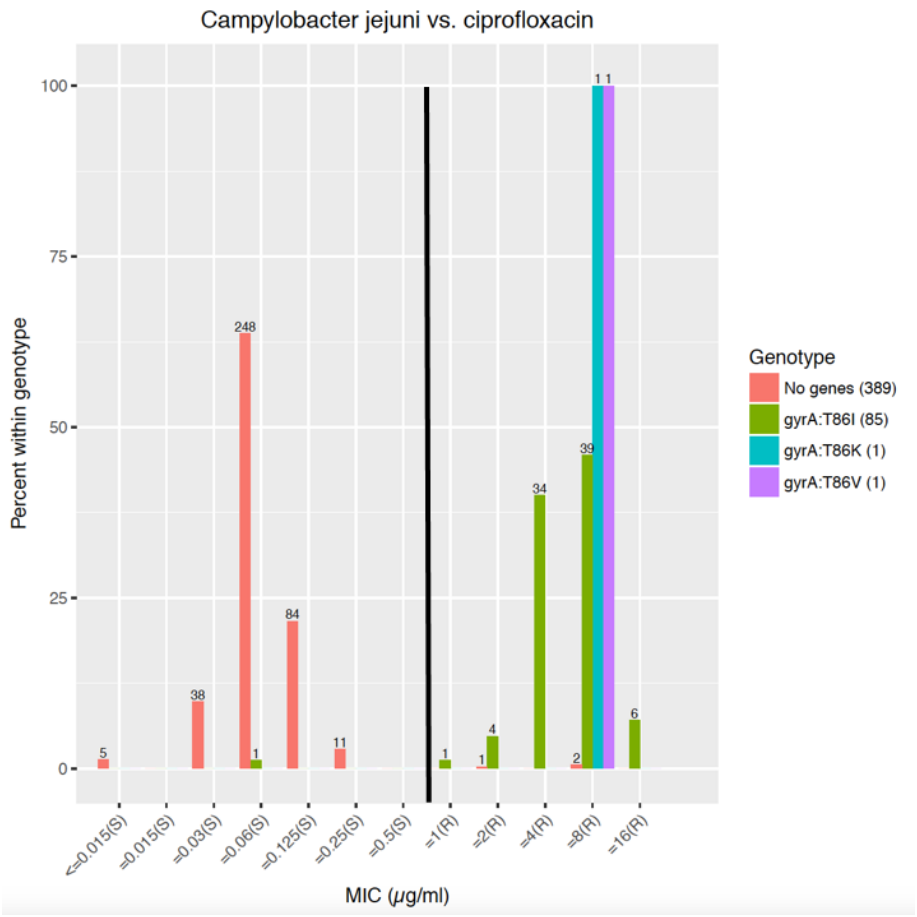


Inconsistencies in *C. jejeuni* are mostly not due to acquired resistance mechanisms (point mutations added)

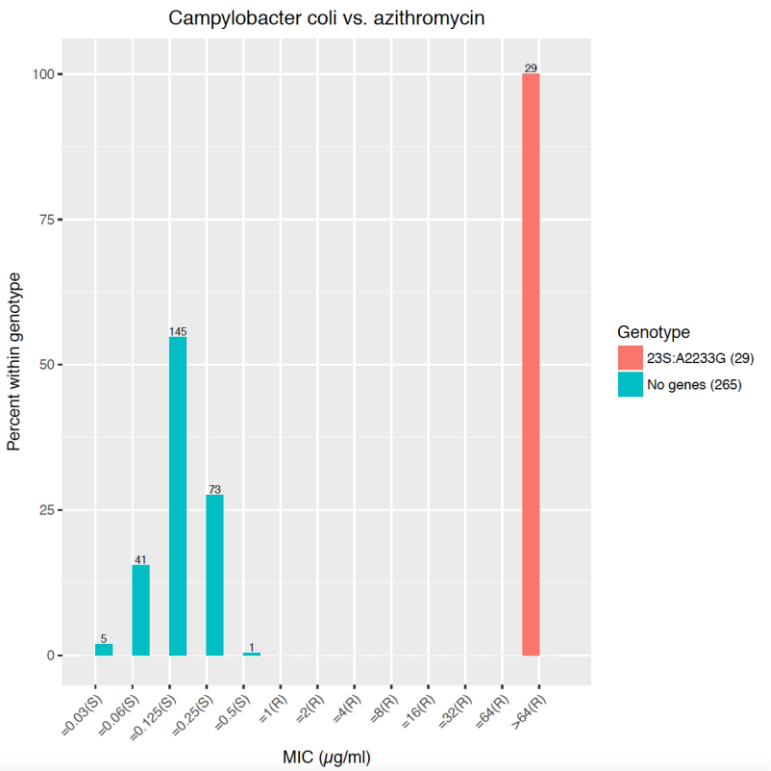


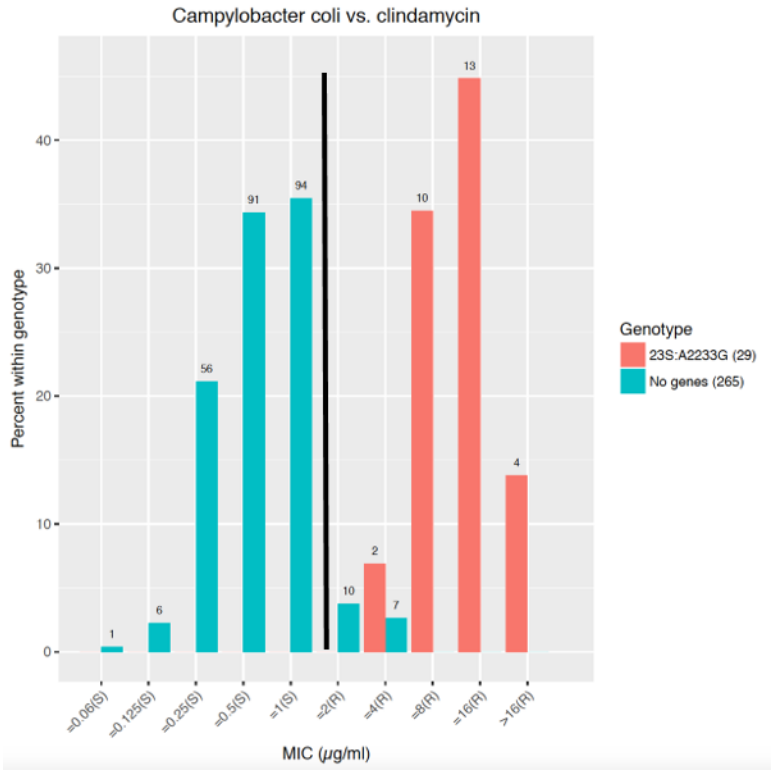
Point mutations account for fluoroquinolone resistance in *Campylobacter*

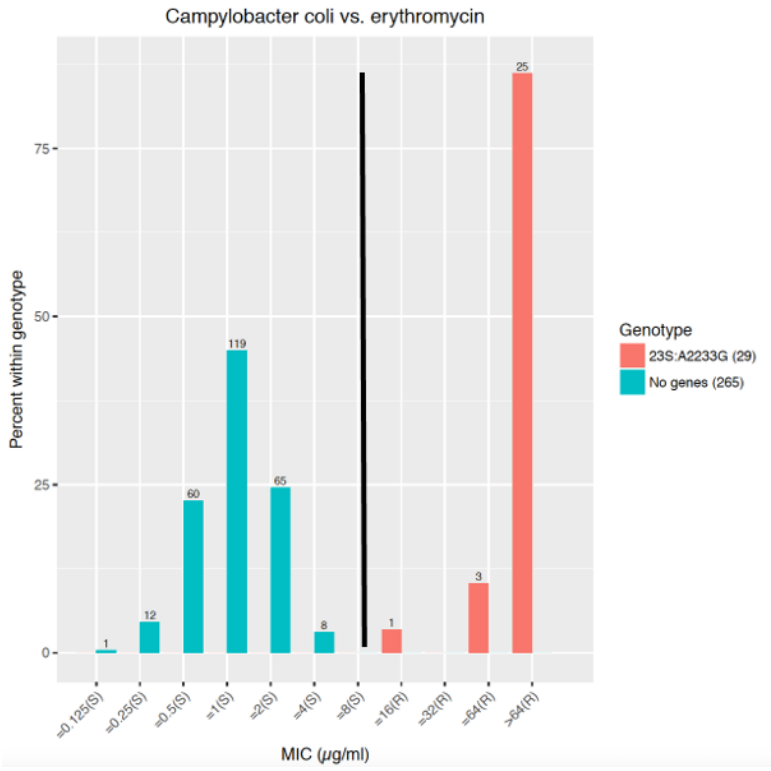




Point mutations account for macrolide resistance in *C. coli*







AMRFinder-Resfinder comparison

- Determined if the two methods called genes at near identical coordinates in genome (±40 bp)
- Compared gene symbols produced by each method
- Four classes of discrepancies:
  - *Synonyms*: exact same gene call, but different gene symbol (common in aminoglycoside modifying enzymes)
  - *Overspecification*: different gene symbol, where the symbol is too specific (often refers to specific allele incorrectly); *blaTEM-1* instead of *blaTEM*
  - *Underspecification*: different gene symbol, where the symbol lacks specificity (e.g., *fosA* when 100% identity match to *fosA7*)
  - *Incorrect*: wrong gene symbol is assigned to known sequence (*blaOXA-61* instead of *blaOXA-193*)
- **Unlike AMRFinder, Resfinder has no gene hierarchy, so it will never underspecify genes, only overspecify**

AMRFinder-Resfinder comparison

	AMRFinder	Resfinder
misclassification	0	248
underspecification	5	0
overspecification	0	979

- Earlier analysis with AMRFinder underspecified calls:
  - *fosA7* called as *fosA* due to lockdown of paper database before discovery of *fosA7*
  - *blaCMY-2* called as *blaCMY* due to annotation/gene calling issue (since fixed)
- Overspecifications were either due to novel or partial sequences
- Incorrect assignments are important:
  - Aminoglycoside modifying enzymes were miscalled:
    - 32 instances where *aac(6')-Ib** was miscalled as *aac(6')-Ib-cr*
    - *aac(6')-Ib-cr* confers amikacin, tobramycin, and ciprofloxacin resistance*, while *aac(6')-Ib** do not confer resistance to one or more of these drugs

Summary

- Overall, high consistency based on acquired genes
- Inconsistencies in *S. enterica* were mostly due to decreased susceptibility that fell below resistance thresholds
- Inconsistences in *Campylobacter* spp. were largely due to point mutations
- Importance of MICs, not just clinical breakpoints
- Resfinder had a high number of incorrect gene symbol assignments relative to AMRFinder
- A hierarchical gene ontology is important

- Questions, concerns: contact **pd-help@ncbi.nlm.nih.gov**
- **ncbi.nlm.nih.gov/pathogens/**

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