Characterization of the Resistome and Horizontal Transfer Mechanisms of C. coli in Brazil

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Campylobacter is the most commonly detected bacterial genus in foodborne illnesses in countries with active surveillance, and C. coli is the second most prevalent species, accounting for up to 10% of cases. This pathogen is part of the intestinal microbiota of various animals. Although less prevalent than *C. jejuni*, *C. coli* often harbors multiple resistance genes and can share them with *C. jejuni* when they coexist in the same environment. In Brazil, official data on this genus is scarce, and cases are underreported. The aim of this study was to evaluate horizontal transfer mechanisms and explore the resistome of *C. coli* in Brazil. To achieve this, 32 *C. coli* strains from chickens in the states of MG, PR, SC, and RS, collected between 2012 and 2018, were sequenced using the Illumina NextSeq 2000 platform. Additionally, 76 SRA reads from NCBI, pertaining to *C. coli* from Brazil, collected between 1995 and 2019, from chickens, humans, monkeys, cattle, pigs, sewage, potable water, and environmental water, were downloaded. Shovill v.1.1.0 was used to assemble contigs from paired sequences, and genome quality was assessed with QUAST v.5.0.2 and CheckM v.1.1.3. Average Nucleotide Identity (ANI) was calculated with FastANI v.1.32, comparing to the C. coli reference genome ASM973039v1. Genome annotation was performed with Bakta, and pangenome analysis with Panaroo. SNPs were extracted with SNP-sites v.2.5.1, and the phylogenetic tree was constructed with RAxML v.8.2.12. Sequence Types (STs) were determined with MLST v.2.19.0, and resistance genes were identified with CARD via ABRicate v.1.0.0 and AMRFinderPlus. Plasmids were identified with MOB-suite v.3.1.9, and bacteriophages were searched for with VirSorter2 v.2.2.4. Transposons and integrons were investigated with Bacant v.3.3.3. The results revealed a diversity of 33 STs, with no STs common to all states. Some STs, such as 829 and 860, persisted over decades. Genes related to six antibiotic classes were identified: aminoglycosides, quinolones, macrolides, beta-lactams, tetracyclines, and streptotricins, in addition to the *cmeA, cmeB*, and *cmeC* operons, which form the *cmeABC* gene conferring multidrug resistance. The number of resistance genes per genome increased over time, with chicken isolates showing the highest antimicrobial resistance. The ST with the greatest diversity of AMR genes was 5181, followed by 9694, predominantly in chicken genomes. The most prevalent gene was the mutation in *gyrA* (T86I), conferring quinolone resistance, found in chickens, humans, monkeys, and pigs. The second most prevalent gene was *tet(O),* followed by *blaOXA193* and *cmeABC*. The mutation 23SA2075G, conferring macrolide resistance, was exclusive to chickens. A total of 51 non-mobilizable plasmids, 2 mobilizable plasmids, and 44 conjugative plasmids were identified, with 2 mobilizable and 13 conjugative plasmids containing *genes tetO, tetO/M/O*, *aad9, aph3-IIIa*, and *sat4*. The

high prevalence of mutations in macrolide and quinolone resistance genes, particularly in chickens, underscores the urgent need for monitoring *C. coli* in Brazil, as these drugs are crucial for treating human diseases. The mobilome of *C. coli* in Brazil is limited, with plasmids playing a critical role in the spread of resistance, especially to aminoglycosides and tetracyclines. Genomic sequencing is essential for effective public health strategies, and has highlighted the emerging issue of the *C. coli* resistome in Brazil.

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