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# *mcr*-Positive *Escherichia coli* ST131-H22 from Poultry in Brazil

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*Escherichia coli* sequence type (ST) 131 is of concern because it can acquire antimicrobial resistance and cause extraintestinal infections. *E. coli* ST131-*H*22 sublineage appears capable of being transmitted to humans through poultry. We report on multidrug-resistant ST131-*H*22 poultry isolates in Brazil closely related to international human and poultry isolates.

The pandemic, extraintestinal, pathogenic *Escherichia coli* multilocus sequence type (MLST) 131 lineage has emerged extensively, gaining notoriety for its extensively multidrug-resistant ST131-*H*30 sublineage (*1*). Whereas ST131-*H*30 appears to be transmitted primarily from person to person, the *H*22 sublineage may be transmitted zoonotically through poultry and cause urinary tract infections and urosepsis (*2*,*3*). We report isolating ST131-*H*22 strains that are multidrug resistant (MDR), meaning that they are resistant to ≥3 classes of antimicrobials (*4*), carrying mobile colistin-resistance (*mcr*) determinants from poultry in Brazil, the largest poultry-exporting country in the world.

We collected 64 *E. coli* strains from poultry with colibacillosis cases from 2 different farms in the same geographic region of Brazil and screened them by PCR for the ST131 clonal group (*5*). PCR detected 6 ST131 isolates (2 from the first farm, 4 from the second), which we whole-genome sequenced (BioProject no. PRJNA398035). We determined phenotypic antimicrobial susceptibility with disk diffusion testing, except for isolates carrying the *mcr* gene, which we tested using broth microdilution (*6*).

We trimmed the reads and used QUAST (http://quast.sourceforge.net) to evaluate the quality of assemblies (contig lengths and expected genome sizes). We assembled DNA sequences with SPAdes (http://cab.spbu.ru/software/spades), then determined the serotype, phylogroup, MLST, *fimH* protein type, virulence gene profile, plasmid replicons, and markers of antimicrobial resistance for each isolate *in silico* using the ABRicate virulence factors database (https://github.com/tseemann/abricate) and ResFinder/PlasmidFinder tools from CGE (https://cge.cbs.dtu.dk/services). Genes were identified with a minimum of ≥95% of identity and coverage.

We identified all isolates as O25:H4-ST131-*H*22, all belonging to phylogroup B2. We generated a maximum-likelihood phylogeny tree on the basis of core-genome single-nucleotide polymorphisms, including the 6 isolates from Brazil and 140 ST131-*H*22 sequences from EnteroBase (http://enterobase.warwick.ac.uk) and a previous study (*2*), using the Northern Arizona SNP Pipeline (https://tgennorth.github.io/NASP/) aligned against *E. coli* JJ1886 ST131-*H*30 (GenBank

accession no. CP006784) (Appendix, https://wwwnc.cdc.gov/EID/article/26/8/19-1724-App1.pdf). The 6 isolates from poultry were nested within a clade of intermingled poultry and human clinical isolates within the overall international isolates (Figure, panel A). The isolates from Brazil were closely related to ST131-*H*22 avian pathogenic *E. coli* isolates from poultry in the United States and those from a human urinary tract infection in Australia (Figure, panel B). Identical virulence factors and plasmid replicons were observed among 4 β-lactamase positive isolates and between 2 isolates missing the β-lactamase genes but

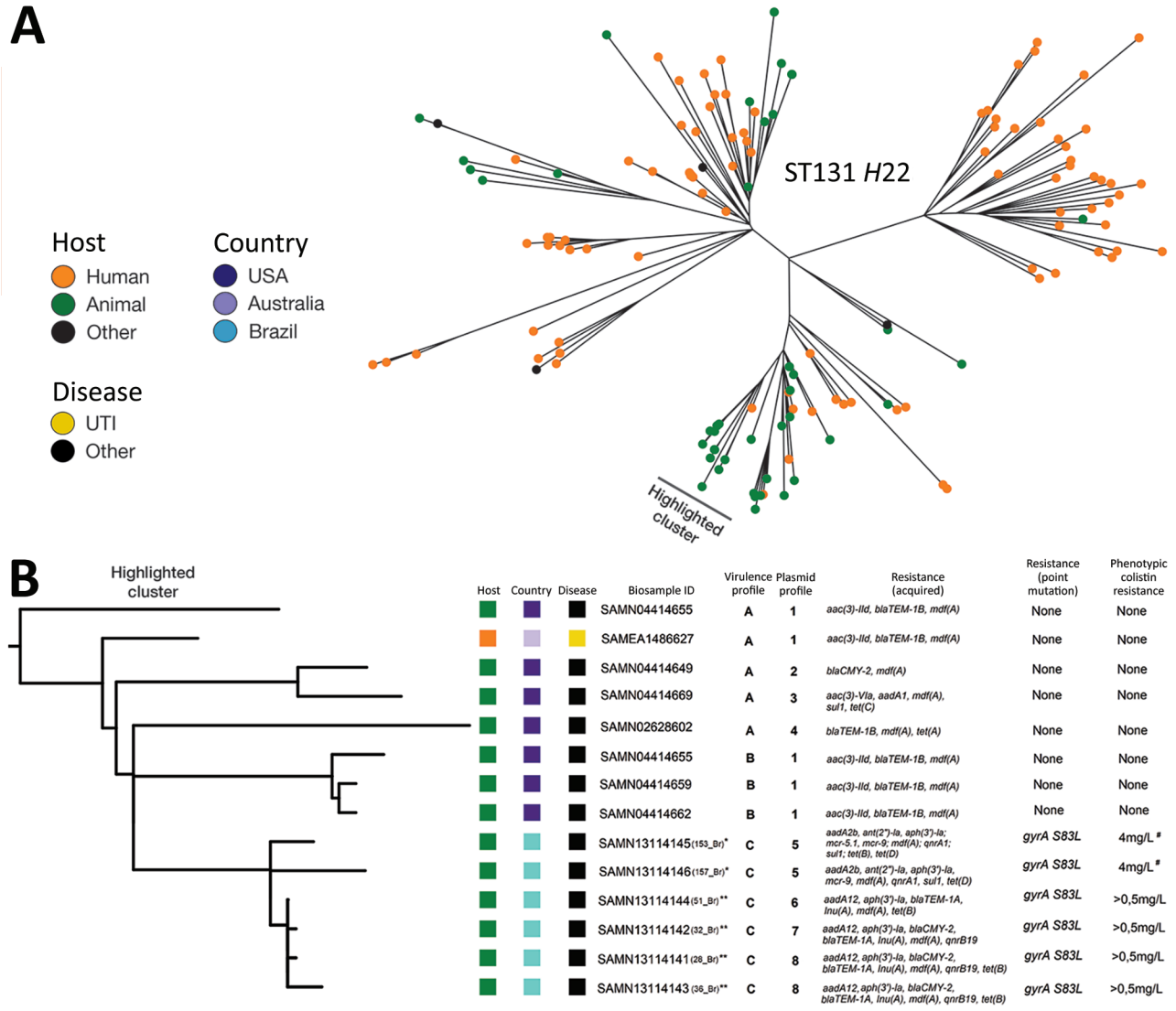


**Figure.** Phylogenetic analysis of *Escherichia coli* ST131-*H*22 isolates from poultry in Brazil and reference sequences. A) Unrooted phylogeny of 146 *E. coli* ST131-*H*22 isolates based on core genome single-nucleotide polymorphisms with the host origin outlined. The cluster containing closely related isolates to the 6 isolates from Brazil is highlighted. B) Rooted phylogeny of closely related isolates from retail meat with APEC and a human isolate with our 6 APEC isolates. The highlighted cluster includes a partial depiction of the tree including the data on host, country, and disease (urinary tract infection or other). Clusters containing the study's isolates have their individual identification in parenthesis. Asterisks indicate farm origins (*, farm 1; **, farm 2). Virulence factors profiles are identified as groups A: *cvi/cva, ent, fimA-H, ibeA, irp1/2, iroN, iucD,iss, kpsM, ompA, tsh;* B: *cvi/cva, ent, fimA-H, ibeA, irp1/2, iroN, iucD, iss, kpsM, ompA*; and C: *cvi/cva, ent, fimA-H, fyuA, ibeA, irp1/2, iroN, iucD, iss, kpsM, ompA, tsh*. Plasmid profiles are identified by group: 1: IncFIB, IncFIC(FII), IncI1; 2: IncFIB, IncFIC(FII), IncFII, IncI1; 3: IncFIB, IncFIC(FII), IncFII, IncHI2, IncHI2A, IncI1; 4: IncFIB, IncFIC(FII), IncI1, IncN; 5: IncFIB, IncFIC(FII), IncFII, IncFII(pCoo), IncHI2, IncHI2A; 6: IncFIB, IncFIC(FII), IncFII, IncHI2, IncHI2A; 7: IncFIB, IncFII, IncI1, IncX1; and 8: IncFIB, IncFIC(FII), IncFII, IncI1, IncX1. Phenotypic colistin-resistance is indicated by the symbol # for the 2 colistin-resistance *mcr* genes positive isolates, showing resistance according to 2018 Clinical Laboratory Standards Institute (https://clsi.org/) clinical breakpoints. APEC, avian pathogenic *E. coli*; ID, identification; ST, sequence type; UTI, urinary tract infection.

carrying *mcr* colistin–resistance determinants. All 6 isolates had MDR profiles, phenotypically confirmed (data not shown except for those from colistin microdilution method) (Figure, panel B).

The ST131-*H*22 lineage, while currently not as common as the *H*30 sublineage as a cause of community-acquired infections, does present a public health challenge because it colonizes poultry flocks, contaminating retail poultry products, and carries *mcr* colistin–resistance genes (*3*). The enormity and rapid growth of poultry production, in which many developing countries use antimicrobials extensively (*5*), and its zoonotic potential, make ST131-*H*22 worthy of specific attention (*2*).

Findings from our phylogenetic analyses of a global collection of ST131-*H*22 isolates from humans and poultry support findings from previous studies (*2*,*3*) and underscore the zoonotic potential of this virulent sublineage. Given that Brazil annually processes 13.8 million poultry products and exports 3.8 million kilograms (*4*), these findings warrant further examination to assess potential zoonotic spillover in Brazil and poultry-importing countries. Until such studies are conducted, the zoonotic potential of ST131-*H*22 in flocks in Brazil cannot be quantified.

The discovery of *mcr* mobile colistin resistance determinants in food animals has renewed attention to the potential risks of widespread antimicrobial use in livestock. In Latin America, *mcr-5* has been found in poultry in Paraguay (9). The description of the *mcr-9* homologue from humans in the United States and horses in Sweden has raised attention to another *mcr* gene with potential for global spread (*10*). Both *mcr* variants in this study, 153_Br and 157_Br, showed phenotypic resistance (*6*) and came from the same farm (Figure, panel B). Interestingly, 153_Br carried both *mcr-5.1* and *mcr-9* variants. These isolates may portend a more widespread problem within poultry flocks in Brazil.

Isolates from this study showed resistance to all of the World Health Organization's highest priority critically important antimicrobial classes (Figure, panel B) (*8*). Analysis of the absence of tetracycline resistance (*tet*[B]/[D]) in 1 of our isolates (Figure, panel B) indicates partial plasmid loss (data not shown).

Use of colistin as a growth promoter in livestock was banned in Brazil in November 2016, although it continued being therapeutically used in poultry up to 2018 (*7*). Therefore, *mcr*-encoding *H*22 strains could be selected out of the population over time. Further restrictions will have to be implemented to combat the growing resistance of *E. coli* in poultry in Brazil to critically important antimicrobial drugs (*4*).

Our findings suggest that poultry in Brazil may serve as a reservoir for MDR extraintestinal pathogenic *E. coli* carrying mobile colistin-resistance determinants. These findings highlight the need for better antimicrobial stewardship and surveillance systems to determine the prevalence of MDR *E. coli* ST131-*H*22 in these poultry flocks and clarify the risks posed to domestic and international poultry consumers.

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# Heartland Virus in Lone Star Ticks, Alabama, USA

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We detected Heartland virus (HRTV) in lone star nymphs collected in 2018 in northern Alabama, USA. Real-time reverse transcription PCR selective for the small segment of the HRTV genome and confirmatory sequencing of positive samples showed high identity with HRTV strains sequenced from Tennessee and Missouri.

Heartland virus (HRTV) is an emerging pathogenic phlebovirus first identified in the United States in 2009 and now reported in 15 states (*1*,*2*). Nymphal lone star ticks (*Amblyomma americanum*) are considered the primary vectors of HRTV, and a variety of domestic and endemic mammalian species are potential amplification hosts of this virus (*2*,*3*). Although *A. americanum* ticks are well-established throughout the eastern, southeastern, and midwestern United States, their range is expanding northward and westward, most likely because of increased host availability and abundance, changes in environmental and climatic conditions, and adaptive genetic variation (Figure, panel A) (*4*). We tested for HRTV in *A. americanum* ticks collected in Alabama, USA, a state within the range of this vector where HRTV has not been documented previously from ticks.

From June 1, 2018, through August 31, 2018, we collected ticks as previously described (*5*) in the William B. Bankhead National Forest, Alabama (34.2270°N, 87.3461°W; Figure, panel B). In preparation for pathogen screening, we separated ticks into pools. Nymph tick pools ranged from 1 to 5 tick(s) of the same species per pool. We screened adult ticks individually (i.e., 1 adult tick per pool) (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/8/19-0494-App1.pdf). We did not include larvae in pathogen screening. We used molecular methods to extract viral RNA and detect the small (S) segment of the HRTV genome using the HRTV-4 primer and probe set (*6*) in tick pools (Appendix Table). We sequenced HRTV-4–positive samples using the Ion Torrent Personal Genomic Machine system (Life Technologies, https://www.thermofisher.com) at the Centers for Disease Control and Prevention (CDC; Fort Collins, CO, USA) as described previously (*7*). We obtained sequences of the HTRV S segment of other HRTV samples and strains from the GenBank database, and aligned sequences using the MUSCLE alignment tool (https://www.ebi.ac.uk/Tools/msa/muscle) in MEGA software (*8*). We also included a closely related severe fever with thrombocytopenia syndrome virus isolate from the GenBank database as an outgroup for this analysis. We used a maximum-likelihood tree approach with 1,000 bootstrap replications to generate the genetic relationships between the Alabama samples and the other HRTV samples available through the GenBank database.

We collected 964 ticks, of which 921 were *A. americanum* (872 nymphs, 22 adult males, and 27 adult females) and 43 were *Dermacentor variabilis* (20 adult males and 23 adult females). We tested