In the U.S., Salmonella Kentucky is frequently isolated from domestic chicken and dairy cattle, but incidence of S. Kentucky in humans is low. We aimed to better describe the genetic diversity, antimicrobial resistance, and virulence determinants, of S. Kentucky isolates from humans, domestic food animals, and meat products, and imported foods. We downloaded and analyzed the genomes of 773 S. Kentucky isolates from NCBI. Isolates were collected from humans, chicken, turkey, cattle, swine, and imported foods between 2002 and 2020. Isolates sequenced for this study were 752 isolates from FDA, 11 isolates from the National Antimicrobial Resistance Monitoring System (NARMS), and 9 isolates from the PubMLST database. Approximately 63% (54/86) of human isolates were ST198, 37% (32/86) were ST152, and 3 isolates were ST134. The majority (60%, 57/95) of animal and food animal isolates were ST32 or ST12-like (one allelic difference), and 9.2% (9/92) were ST68. Isolates from imported food were mostly ST198 (66%, 26/37) and ST152 (33%, 12/37). ST32 isolates from dairy cattle appeared to have a lineage (Clade 2) distinct from ST32 isolates from chicken (Clade 4), and half of ST152 isolates appeared to have a lineage of other clades (Clades 1 and 3), largely distinct from Clades 2 and 4. Isolates in Clades 1, 2, and 3 had significantly less antimicrobial resistance determinants, compared to isolates in Clade 4. ST198 isolates clustered into 7 different lineage SDs: one comprised mostly of isolates from humans and imported foods (Clade ST198.1) that contained triple quinolone resistance determining region (QRDR) mutations, confering resistance to fluoroquinolones; and one that was largely absent of QRDR mutations but with greater source diversity (Clade ST198.2) that contained triple quinolone resistance determining region (QRDR) mutations, confering resistance to fluoroquinolones. These included fish, spices, animal feed and pet food. These differences were based on the 26 antimicrobial resistance genes among the ST groups. Overall, 752 of 774 isolates sequenced fell among 4 predominate sequence types: ST198 (N=400), an ST32-like clade (similar to ST152 but with one ST68 allele difference), ST152 (N=228), and ST134 (N=22). ST198 isolates were separated into 4 major clades (Fig 1). Clades 1 and 2 contained only human isolates, Clade 2 contained isolates from cattle, Clade 4, the largest, contained isolates from varied source types, but predominately chicken sources. Only 5% of isolates in Clades 1, 2, and 3 had a functional resistance gene. However, 75% contained ST198 had predicted resistance to at least one antibiotic (not shown).

Introduction

Salmonella Kentucky (S. Kentucky) is a polyphagic aerovar, meaning it consists of multiple sequence types (ST) that do not share a common ancestor. Some research points to these STs having different ecological, host characteristics, and geographic distributions (1,2). In the U.S., not much is known about the ST distribution of S. Kentucky, however food sources have been studied. Most S. Kentucky isolated from domestic agricultural sources are ST198. ST198 has also been found in mostly dairy cattle sources (2). ST198 can be resistant to multiple antibiotics including ciprofloxacin, a first line therapy for treatment of complicated salmonellosis infection. In countries where ciprofloxacin resistance is common, poultry is thought to be a major source of infection (3). ClpA ST198 can be found in high levels among poultry isolates in countries where there is uncontrolled administration of fluoroquinolones in poultry production. Extensive use of fluoroquinolones may provide the selective pressure necessary for distribution and persistence of this strain. Although U.S. regulations prohibit the use of fluoroquinolones in poultry production, these strains may still reach consumers through other food sources, including imported foods. Travel has also been shown to be a risk factor for ClpA ST198 infection (3). Here, we sought to examine the phylogenetic profiles of a large number of S. Kentucky isolates from IL persons in the U.S., domestic food products, and imported foods. Using these data, we can begin to understand the relatedness of S. Kentucky derived from various sources and make inferences about potential sources of foodborne S. Kentucky infection.

Results and Discussion

Bacterial strains and sequencing. As part of routine National Antimicrobial Resistance Monitoring System (NARMS) surveillance, S. Kentucky isolates were collected with antimicrobial resistance testing that occurred at various timepoints between 1996-2020. Imported foods were collected for various FDA sampling assignments occurring between 2002 and 2019. NARMS isolates were sequenced by short-read sequencing on the Illumina MiSeq using v2 or v3 reagent kits (Illumina, San Diego, CA, USA) with 2x150 bp paired-end reads. The Illumina paired-end reads were assembled with Newera XT kit by Illumina. Illumina sequences were submitted to the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject PRJNA292661, PRJNA322666, and PRJNA509444.

Data mining. Genomic sequences were downloaded from NCBI for all human (93%), turkey ceca (24%), retail ground turkey meat (3%), dairy cattle (3%), beef cattle (2%), retail beef (8%), non-cow (2%), and retail chicken (2%) and retail pork (1%). S. Kentucky isolates recovered during the timeframe of the study. We downloaded sequences of only 149 isolates from chicken ceca and 973 isolates from retail chicken most sampled (Table 1). After removing isolates with a relatively large number of S. Kentucky collected from these sources during the timeframe of the study. Isolates were selected to ensure that at least 1 isolate from each site, month, and year were included. We also downloaded sequences of 37 S. Kentucky recovered from FDA imported product sampling. These included 10 isolates from chicken, 17 isolates from pork, and 10 isolates from beef.

Bioinformatic analysis. The analysis of classical and multilocus sequence typing (MLST) was performed based on the whole-genome sequencing (WGS) data. S. Kentucky MLST allele profiles and sequences were downloaded from the PubMLST database (https://pubmlst.org). A total of 6,000 profiles for 7 different loci were used for the MLST analysis. The ST198 pipeline (Insyoe et al. 2014) was used to determine the MLST type for our S. Kentucky isolates. Salmonella enterica Kentucky isolates with the same ST sequence type (ST) were included into a single-nucleotide polymorphism (SNP) analysis. The SNP analysis was done by CBAN-SNP Pipeline (http://cbanpipeline.readthedocs.io/en/latest/). VarScan (Koboldt et al. 2012) was used to detect SNPs. The pipeline was executed in the analysis since they evolved through horizontal gene transfer (HGT) which will misrepresent the evolution path of the strains. Phylogenetic tree was constructed using the GARLI pipeline (Maddison et al. 2004). Antimicrobial resistance genes were identified using the ResFinder database (Breukelaar et al. 2016) downloaded from (https://www.genome.agr.ca/services/ResFinder/) in October 2020, containing 3,232 antimicrobial resistance genes. Resistance mutations were also determined using an in-house tool to determine any mutations in the QRDR gene that have been shown to cause resistance in Salmonella. An NTCP-developed Salmonella virulence factor database (4) was queried to identify putative virulence genophages. Virulence genes were extracted from the NCBI database, transformed to binary data and imported into BioNermetrics for phylogenetic analysis using Dice Coefficients and UPGMA (Applied Maths, Austin, TX, USA).

Conclusion

• In our study, the majority (67%) of clinical infections of S. Kentucky in humans were ST198.
• ClpA ST198 appears to be a major contributor to human infections in the U.S., but has low prevalence among S. Kentucky isolates from domestic food animals and meats.
• CipR ST198 infections may be linked to the consumption of imported (or foreign, if consumed while traveling) food product.
• 90% of ST152 isolates from humans clustered separately from domestic food animals and products, suggesting the majority of sporadic CipR ST198 S. Kentucky infections may be attributed to a source other than food products derived from domestic cattle, chicken, or pig.
• There are unique differences in the composition of fimbrial gene clusters and other virulence genes among the ST groups.
• The different composition of virulence gene profiles among the ST groups and AMR genes within the ST groups may provide clues to the host specificity and pathogenicity of S. Kentucky isolates.