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Use of whole-genome sequencing for *Campylobacter* surveillance from NARMS retail poultry in the United States in 2015

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ABSTRACT

Whole genome sequencing (WGS) has become a rapid and affordable tool for public health surveillance and outbreak detection. In this study, we used the Illuminia MiSeq® to sequence 589 *Campylobacter* isolates obtained in 2015 from retail poultry meats as part of the National Antimicrobial Resistance Monitoring System (NARMS). WGS data were used to identify the *Campylobacter* species and to compare the concordance between resistance genotypes and phenotypes. WGS accurately identified *386 C. jejuni* and 203 *C. coli* using *gyrA* sequence information. Ten resistance genes, including *tetO*, bla_{OXA-61}, *aph(2″)-Ic, aph(2″)-If, aph(2″)-Ig, aph(3')-III, ant(6)-1a, aadE, aph(3")-VIIa,* and *Inu*(C), plus mutations in housekeeping genes (*gyrA* at position 86, 23S rRNA at position 2074 and 2075), were identified by WGS analysis. Overall, there was a high concordance between phenotypic resistance to a given drug and the presence of known resistance genes. Concordance between both resistance and susceptible phenotypes and genotype was 100% for ciprofloxacin, nalidixic acid, gentamicin, azithromycin, and florfenicol. A few discrepancies were observed for tetracycline, clindamycin, and telithromycin. The concordance between resistance phenotype and genotype ranged from 67.9% to 100%; whereas, the concordance between susceptible phenotype and genotype ranged from 98.0% to 99.6%. Our study demonstrates that WGS can correctly identify *Campylobacter* species and predict antimicrobial resistance with a high degree of accuracy.

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1. Introduction

Campylobacter species have been associated with a wide range of gastrointestinal conditions in humans, including acute watery or bloody diarrhea, fever, weight loss, and cramps (Kaakoush et al., 2015). In particular, *C. jejuni* is a major cause of foodborne diarrheal disease worldwide, with nearly 1 million cases each year in the United States alone (Scallan et al., 2011). Another species, *C. coli*, while less prevalent, causes an indistinguishable diarrheal illness (Kaakoush et al., 2015). Rarely, some cases can result in sequelae, such as Guillain-Barré syndrome or the bacteria may be a predisposing agent for other gastrointestinal manifestations including inflammatory bowel disease, celiac disease, or colorectal cancer (Kaakoush et al., 2015).

The high carriage rate of *Campylobacter* within broiler chickens has led to them being considered a primary source of food-related transmission of the bacteria to humans (Skarp et al., 2016). Commercial turkeys and ducks also can serve as reservoirs of *C. jejuni* and *C. coli* (Colles et al., 2011; Giacomelli et al., 2012; Gu et al., 2009). Other sources such as beef, pork, lamb, unpasteurized milk, water and seafood also have been associated with *Campylobacter* infections (Friedman et al., 2000; Jacobs-Reitsma, 2000; Levesque et al., 2007; Lindmark et al., 2009).

Campylobacter enteritis is usually self-limiting and typically does not require antimicrobial therapy. However, in severe and prolonged cases of enteritis, or cases of bacteremia, or other extraintestinal infection, prompt antimicrobial treatment is indicated. In these cases, macrolides, primarily erythromycin, or alternatively clarithromycin or azithromycin, are the drugs of choice (Blaser and Engberg, 2008). Fluoroquinolones, such as ciprofloxacin, are also commonly used because they are among the drugs of choice for empirical treatment of undiagnosed diarrheal illness, such as traveler's diarrhea; however, their effectiveness for treating *Campylobacter* diarrhea is limited due to expected antimicrobial

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resistance to the drug (de la Cabada Bauche and Dupont, 2011). In some regions, tetracycline or doxycycline and select beta-lactams have been used for treating intestinal infections. Gentamicin, meropenem, clindamycin, and telithromycin show potent in vitro activity, and may have potential value as alternative treatments (McDermott, 2010).

In the U.S. the National Antimicrobial Resistance Monitoring System (NARMS) monitors antimicrobial resistance in foodborne pathogens and identifies the source and magnitude of antimicrobial resistance in the food supply. In particular, NARMS monitors antimicrobial resistance in *Campylobacter* and other foodborne pathogens in human clinical specimens, retail meats, and food production animals (NARMS, 2017). Traditional methods of antimicrobial susceptibility testing for *Campylobacter* are time-consuming and laborious. The U.S. National Strategy for Combating Antibiotic Resistant Bacteria emphasizes the need for improved surveillance, highlighting the importance of rapid, reproducible, and cost-effective methods that can predict resistance phenotypes (The White House, 2014). Whole genome sequencing (WGS) technology has become a fast and affordable tool for public health surveillance and response. Recent studies have shown that WGS analysis can potentially be a single, rapid and cost-effective approach to define resistance genotypes and predict resistance phenotypes of bacteria with great sensitivity and specificity (Gordon et al., 2014; McDermott et al., 2016; Stoesser et al., 2013). We previously conducted a limited study of WGS of 114 *Campylobacter* isolates, which showed a 99.2% correlation between resistance genotypes and phenotypes (Zhao et al., 2015b). The main objective of the current study is to evaluate the implementation of WGS in our NARMS retail meat testing workflow using a larger set of 589 *Campylobacter* isolates collected in 2015 from retail poultry from 14 states in the United States. The specific objectives were 1) to evaluate *gyrA* as a target for *Campylobacter* speciation, and 2) to correlate results obtained from phenotypic in vitro antimicrobial susceptibility testing with the presence of known antimicrobial resistance genes.

2. Materials and methods

2.1. Sample collection and bacterial isolation

As part of the NARMS retail meat surveillance program, public health laboratories in CA, CO, CT, GA, LA, MD, MN, MO, NM, NY, OR, TN, PA and WA participated in retail meat sampling and culture of *Campylobacter* in 2015. Each month, all 14 sites purchased samples of chicken and ground turkey from local grocery stores and processed them for isolation of *Campylobacter* spp. A total of 4763 retail poultry samples (2376 chicken parts and 2387 ground turkey) were collected in 2015. *Campylobacter* species isolates were grown on Campy Cefex agar plates (Hardy Diagnostics, Santa Maria, CA) followed by subculture on tryptic soy agar supplemented with 5% sheep blood (Remel, Lenexa, KS) under microaerobic conditions (85% N_2, 10% CO_2, and 5% O_2). Once bacteria were isolated by the state public health laboratories, isolates were shipped to the FDA Center for Veterinary Medicine laboratory facility for further analysis. All isolates were stored at -80 °C in *Brucella* broth with 20% glycerol (Hardy Diagnostics) until use.

2.2. In vitro antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for 9 antimicrobials, including azithromycin (AZI), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), florfenicol (FFN), gentamicin (GEN), nalidixic acid (NAL), telithromycin (TEL), and tetracycline. (TET). Minimum inhibitory concentrations (MICs) were determined by broth microdilution using the Sensititre AIM Automated Inoculation Delivery System and Sensitouch plate reader (ThermoFisher Scientific/TREK Diagnostics) in accordance with the manufacturer's instructions. We used epidemiological cutoff values (ECOFFs) as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) breakpoints to interpret MIC results. *Campylobacter jejuni* ATCC 33560 was used as a quality control strain according to the Clinical Laboratory and Standards Institute (CLSI) guidelines.

2.3. Genomic DNA extraction and whole genome sequencing

Genomic DNA was extracted using the QIAamp 96 DNA extraction kit (Qiagen, Valencia, CA) with an automated DNA extraction robot (QIAcube HT®, Qiagen) per the manufacturer's instructions. Sequencing libraries were prepared with a Nextera® XT DNA sample preparation kit (Illumina, San Diego, CA) and sequenced on the Illumina MiSeq® platform (Illumina, San Diego, CA) with a 2×300-bp paired-ended protocol according to the manufacturer's instructions. Genome sequences were *de novo* assembled using CLC Genomics Workbench, version 8.0 (CLC Bio, Aarhus, Denmark). A depth of coverage of $\geq$20 (20×) and an N50 $\geq$ 10,000 were required to ensure sequencing and assembly quality.

2.4. Antibiotic resistance gene identification

A publicly available resistance gene database ResFinder (Center for Genomic Epidemiology, Denmark Technical University) was used for identifying antibiotic resistance genes. Using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the assembled genomes were scanned for resistance genes by comparing sequences to the reference database using a cutoff set at 50% sequence length and 85% nucleic acid identity. To examine the specific genomic mutations associated with resistance to quinolones and to macrolides/lincosamides/ketolides, the *gyrA* and 23S rRNA genes were identified from the assembled sequences using BLASTX and BLASTN, respectively, and extracted using in-house PERL scripts. The genes were then aligned using ClustalW as part of the MEGA7 program (www.megasoftware.net) (Kumar et al., 2016). The amino acid in GyrA at position 86, which is associated with resistance to quinolones (Hakanen et al., 2002), and nucleotides in the 23S rRNA at positions 2074 and 2075, which are associated with resistance to macrolides/lincosamides/ketolides (Ladely et al., 2009), were then examined.

2.5. Use of gyrA sequence for the identification of campylobacter spp.

16S rRNA sequence cannot be used for the identification of *Campylobacter* spp. because of lack of discriminatory power at the species level (see below). In order to evaluate whether variations in the *gyrA* gene sequence could be used for identification of *Campylobacter* spp. a pipeline was developed using WGS data in which the species of Campylobacter was determined by performing local BLASTN with *gyrA* gene of reference strains (NCTC 11168 for *C. jejuni*; RM4661 for *C. coli*; RM2100 for *C. lari*; *Campylobacter_fetus*_subsp. *fetus* 82-40 for *C. fetus*; *Campylobacter_curvus* 525.92 for *C. curvus*) with at least 95% nucleotide identity. Hits of less than 95% identity or less than 100% sequence length were checked manually by BLAST. Strains that could not be identified by this method were re-examined by checking their assembly quality and/or BLAST against *gyrA* sequences of other likely species.