Novel gentamicin resistance genes in *Campylobacter* isolated from humans and retail meats in the USA

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Received 18 September 2014; returned 26 November 2014; revised 9 December 2014; accepted 28 December 2014

**Objectives:** To understand the molecular epidemiology of gentamicin-resistant *Campylobacter* and investigate aminoglycoside resistance mechanisms.

**Methods:** One-hundred-and-fifty-one gentamicin-resistant *Campylobacter* isolates from humans (*n* = 38 *Campylobacter jejuni*, *n* = 41, *Campylobacter coli*) and retail chickens (*n* = 72 *C. coli*), were screened for the presence of gentamicin resistance genes by PCR and subtyped using PFGE. A subset of the isolates (*n* = 41) was analysed using WGS.

**Results:** Nine variants of gentamicin resistance genes were identified: *aph(2″-Ib, Ic, Ig, If1, If3, Ih, aac(6′)-le/aph(2″-Ia and aac(6′)-le/aph(2″)-Ig, The *aph(2″)-Ib, Ic, If1, If3, Ih and aac(6′)-le/aph(2″)-Ig variants were identified for the first time in *Campylobacter*. Human isolates showed more diverse aminoglycoside resistance genes than did retail chicken isolates, in which only *aph(2″)-Ic and -Ig were identified. The *aph(2″)-Ig gene was only gene shared by *C. coli* isolates from human (*n* = 27) and retail chicken (*n* = 69). These isolates displayed the same resistance profile and similar PFGE patterns, suggesting that contaminated retail chicken was probably the source of human *C. coli* infections. Human isolates were genetically diverse and generally more resistant than the retail chicken isolates. The most frequent co-resistance was to tetracycline (78/79, 98.7%), followed by ciprofloxacin/nalidixic acid (46/79, 58.2%), erythromycin and azithromycin (36/79, 45.6%), telithromycin (32/79, 40.5%) and clindamycin (18/79, 22.8%). All human and retail meat isolates were susceptible to florfenicol.

**Conclusions:** This study demonstrated that several new aminoglycoside resistance genes underlie the recent emergence of gentamicin-resistant *Campylobacter*, and that, in addition to contaminated retail chicken, other sources have also contributed to gentamicin-resistant *Campylobacter* infections in humans.

**Keywords:** *Campylobacter*, gentamicin resistance, NARMS, PCR, WGS

**Introduction**

*Campylobacter* is a leading cause of foodborne diarrheal illness worldwide, with more than one million cases each year in the USA alone.1 Human illnesses are primarily associated with *Campylobacter jejuni* and *Campylobacter coli*. Raw or undercooked poultry has long been recognized as a major source of human campylobacteriosis. *Campylobacter* enteritis is usually self-limiting and does not require antimicrobial therapy. In severe and prolonged cases of enteritis or bacteremia, septic arthritis and other extra-intestinal infections, erythromycin or a fluoroquinolone, such as ciprofloxacin, is the drug of choice.2,3 Based on *in vitro* activity, other antimicrobials such as gentamicin, meropenem, clindamycin, telithromycin and azithromycin may be viable alternative therapies.4 The National Antimicrobial Resistance Monitoring System (NARMS) is a national public health surveillance system that monitors the trends in antimicrobial resistance in foodborne pathogens from human patients, retail meats and food animals in the USA. Based on NARMS reporting, gentamicin resistance in *Campylobacter* was rare in the USA. It was first detected in a single human isolate of *C. coli* in 2000 and in a retail chicken isolate in 2007.5,6 Since then, resistance has increased rapidly, detected in 12.2% of human isolates and 18.1% of retail isolates in 2011.5,6 Although gentamicin-resistant *Campylobacter* was found in human clinical isolates of both *C. jejuni* and *C. coli*, only gentamicin-resistant *C. coli*, most of which were...
isolated from western states of the USA, were recovered in retail chicken meats. PFGE analysis showed that almost all gentamicin-resistant *C. coli* from retail chicken displayed very similar PFGE profiles, suggesting a recent clonal expansion of the organism.6

Several mechanisms of aminoglycoside resistance have been described in Gram-positive and Gram-negative bacteria. Enzymatic modification and inactivation of antibiotics are the most prevalent mechanisms of aminoglycoside resistance.7,8 Based upon the reactions that they catalyse, aminoglycoside-modifying enzymes are divided into three classes: N-acetyltransferases, O-adenylyltransferases and O-phosphotransferases. Each class of the aminoglycoside-modifying enzymes has a unique resistance profile based on the type of enzymatic modification and the site of modification.7–10 To date, a large number of aminoglycoside resistance genes have been identified, but the genetic basis for gentamicin resistance in *Campylobacter* has not been fully elucidated. Gentamicin resistance genes including aacA4, aac(6')-Ie/aph(2'')-Ia (also named aacA/apoD and encoding a bifunctional enzyme), aph(2'')-If and aph(2'')-Ig have been reported in *Campylobacter*.11–14 The aph(2'')-Ig represents the most recently identified gentamicin resistance gene; it encodes a phosphotransferase. This gene was detected in a *C. coli* isolate from retail chicken meat and was located on a 55 kb conjugative MDR plasmid that shared 95% identity of nucleotide sequence with a pETl plasmid in *Campylobacter*.15 In addition to aph(2'')-Ig, the plasmid carried additional resistance genes, including tet(O), aad9, hph, aadE, sat4 and apha-3.14 Aminoglycoside 2'-phosphotransferases [APH(2'')] are widely distributed in enterococci and staphylococci and have been recently found in *Campylobacter* isolated from broiler chicken, retail chicken and humans.12–14,16 Two nomenclature systems are currently proposed for the APH(2'') family: APH(2'')-Ia, Ib, Ic, Id, etc. and APH(2'')-Ia, Ila, IIIa and Iva. The APH(2'') family is genetically diverse, and amino acid sequence identity between subfamilies can be as low as 28%–32%.13 The recently described APH(2'')-Ig found in *C. coli* from our previous study shared 29% and 28% amino acid sequence identity with APH(2'')-Ia and APH(2'')-If, respectively.14

To understand the molecular epidemiology of gentamicin-resistant *Campylobacter* and aminoglycoside resistance mechanisms, we analysed 151 isolates of gentamicin-resistant *Campylobacter* from humans and retail meats between 2000 and 2011 from the NARMS programme. We report the identification of several new gentamicin resistance alleles. These findings offer new insights into the emergence of aminoglycoside resistance in *Campylobacter*.

**Materials and methods**

**Bacterial isolates**

*Campylobacter* isolates used in the study were from the human and retail meat components of the NARMS programme. NARMS sampling of human *Campylobacter* was based on the occurrence of laboratory-confirmed cases of infection from participating public health laboratories. The frequency of submission of the isolates to CDC was dependent upon the burden of illness in each participating state. The FDA received *Campylobacter* isolates recovered from retail meat from the participating public health laboratories. Each month, the laboratories purchased 40 meat samples, comprising 10 samples each of retail chicken, ground turkey, ground beef and pork chop, and cultured all meats for *Campylobacter*. Since 2008, only poultry meats have been cultured for the pathogen. A total of 151 gentamicin-resistant *Campylobacter* isolates collected from 2000 to 2011 by the NARMS programme were selected, including 41 *C. coli* and 38 *C. jejuni* from human clinical specimens and 72 *C. coli* from retail chicken meats (Table 1). *Campylobacter* were grown on tryptic soy agar supplemented with 5% sheep blood (Remel, Lenexa, KS, USA) under microaerophilic conditions (85% nitrogen, 10% carbon dioxide and 5% oxygen). All isolates were frozen at −80°C in Brucella broth with 20% glycerol.

**Antimicrobial susceptibility testing**

MICs were determined by broth microdilution using the Sensititre™ automated antimicrobial susceptibility system in accordance with the manufacturer’s instructions (Trek Diagnostic Systems, Cleveland, OH, USA). Nine antimicrobial agents were tested, including azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin and tetracycline. *C. jejuni* ATCC 33560 was used for quality control according to guidelines of the CLSI. CLSI MIC interpretive criteria for resistance were used: ciprofloxacin (≥4 mg/L), tetracycline (≥16 mg/L) and erythromycin (≥32 mg/L). For the remaining agents, NARMS established criteria were used to define resistance: gentamicin (≥8 mg/L), azithromycin (≥8 mg/L), clindamycin (≥8 mg/L), nalidixic acid (≥64 mg/L) and tetracycline (16 mg/L).17 For florfenicol, because of the absence of a resistant population, a susceptible breakpoint was used (susceptible being defined as ≤4 mg/L).

**Detection of gentamicin resistance genes by PCR**

Genomic DNA was prepared from pure cultures using either the Ultraclean Microbial DNA Isolation kit (Mo Bio Laboratory Inc., Carlsbad, CA, USA) or the DNeasy Blood and Tissue kit (Qiagen, Gaithersburg, MD, USA). The presence of aph(2'')-Ig, aph(2'')-If, aac(6')-Ie/aph(2'')-Ia and aacA4 genes involved in gentamicin resistance in *Campylobacter* was screened for by PCR. Primers were designed based on available sequence data from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and WGS data from gentamicin-resistant *Campylobacter* generated in the current study. Amplifications were carried out in a 25 μL volume containing 50–100 ng of genomic DNA, 250 μM each of deoxynucleoside triphosphate, 2 mM MgCl2, 50 pmol of each primer and 1 U of AmpliTaq Gold Taq polymerase (Applied Biosystems, Foster City, CA, USA) for 30 cycles. The primer sequences and annealing temperatures for the different target genes are listed in Table S1, (available as Supplementary data at JAC Online). The amplified products were separated by gel electrophoresis on 1.0% agarose gels, stained with ethidium bromide and visualized under UV light.

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>Human (<em>n</em>=79)</th>
<th>Retail chicken (<em>n</em>=72)</th>
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<tbody>
<tr>
<td></td>
<td><em>C. coli</em> (<em>n</em>=41)</td>
<td><em>C. jejuni</em> (<em>n</em>=38)</td>
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<tr>
<td>2000</td>
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<tr>
<td>2011</td>
<td>19</td>
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</table>

Table 1. Gentamicin-resistant *C. jejuni/coli* isolated from humans and retail chicken, 2000–11
WGS of selected gentamicin-resistant isolates

Due to the ambiguity of PCR results from some of the isolates, 41 gentamicin-resistant Campylobacter, including 36 from humans and 5 from retail chicken meat, were selected for WGS analysis. Five gentamicin-susceptible Campylobacter (two from humans and three from retail chicken) were also included as controls for the WGS analysis. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Gaithersburg, MD, USA). Sequencing libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina Inc., San Diego, CA, USA) and sequenced on Illumina MiSeq (Illumina) with a 250 bp paired-end protocol according to the manufacturer’s instructions. The sequencing reads were de-multiplexed using MiSeq reporter software (Illumina).

Genome sequences of Campylobacter were assembled using CLC genomics workbench 6.0.2 (CLC bio, Germantown, MD, USA). Previously reported antibiotic resistance genes were downloaded from GenBank to an in-house database. The assembled WGS sequences were compared with resistance genes in GenBank using the BLAST software, version 2.2.27 (http://blast.ncbi.nlm.nih.gov/Blast.cgi).^18^ MEGA software, version 5.0 (www.megasoftware.net) was used to perform sequence alignments and calculate the percentage of amino acid identity in the APH(2′) subfamilies.

PFGE analysis

PFGE was performed to determine genomic DNA fingerprinting profiles of gentamicin-resistant Campylobacter according to the protocol developed by CDC. In brief, agarose-embedded cells were lysed and DNA was digested with 40 U of Smal and KpnI (Boehringer Mannheim, Indianapolis, IN, USA). The restriction fragments were separated by electrophoresis in a 0.5× TBE buffer (Invitrogen, Carlsbad, CA, USA) at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA, USA) with pulse times of 6.76–35.38 s. Salmonella Braenderup H9812 was used as the control strain. The gels were stained with ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad).

PFGE results were analysed using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium), and banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

Nucleotide sequence accession number

The sequences of the novel genes described in this paper have been submitted to GenBank under accession numbers KF652098, KF652097, KF652096, KF652095 and KF652094.

Results

Antimicrobial resistance profiles

Among the 151 gentamicin-resistant Campylobacter, including 79 from humans (C. jejuni n = 38 and C. coli n = 41) and 72 from retail chicken (all C. coli), the human isolates showed more co-resistance to other antimicrobials than did retail chicken isolates. In addition to gentamicin, human isolates were commonly resistant to tetracycline (98.7%), ciprofloxacin/ nalidixic acid (58.2%), erythromycin and azithromycin (45.6%), telithromycin (40.5%) and clindamycin (22.8%). All isolates collected from humans were susceptible to florfenicol. A higher percentage of C. jejuni than C. coli isolates was resistant to antimicrobials except tetracycline, where C. coli showed 100% resistance, but C. jejuni showed 97.4% resistance. Other antimicrobial resistance in C. jejuni and C. coli, respectively, was as follows: ciprofloxacin/nalidixic acid (84.1% and 34.1%), erythromycin/azithromycin (73.7% and 19.5%), telithromycin (65.8% and 17.1%) and clindamycin (36.8% and 9.8%) (Figure 1a and b).

Among the retail chicken isolates, 98.6% were co-resistant to tetracycline and 1.4% to ciprofloxacin/nalidixic acid. All retail chicken isolates were susceptible to the remaining antimicrobial agents tested (Figure 1b).

Presence of gentamicin resistance genes

There were nine variants of gentamicin resistance genes identified using PCR and WGS, including seven monofunctional aminoglycoside 2′-phosphotransferase genes [aph(2′)-Ib, lc, lg, if, IfI, If3 and Ih] and two bifunctional aminoglycoside 2′-phosphotransferase genes [aac(6′)-le/aph(2′)-Ia and aac(6′)-le/aph(2′)-If2]. The aph(2′)-Ib, lc, IfI, If3, Ih and aac(6′)-le/aph(2′)-If2 genes were identified for the first time in Campylobacter. The aph(2′)-Ia, IfI, If3, Ih and aac(6′)-le/aph(2′)-If2 were new variants of the aminoglycoside 2′-phosphotransferase resistance gene identified in the current study. No aacA4 was detected in the Campylobacter isolates. Compared with the sequence of aph(2′)-Ia available in GenBank, we divided the aph(2′)-Ia–If genes among our isolates into variants aph(2′)-Ia/Ia, If3 and If2, based on >90% amino acid sequence identity. Among 23 aph(2′)-Ia–If-positive isolates selected for WGS analysis, 11 had 88.2% amino acid sequence identity with the APH(2′)-Ia control from GenBank; therefore, we propose naming it aph(2′)-Ia-Jh (Figure S1). APH(2′)-Ia-Jg shared 29.7%, 52.2%, 34.6% and 25.9% amino acid identity with subfamilies APH(2′)-Ia-A, APH(2′)-Ia-B, APH(2′)-Ia-C and APH(2′)-Ia-D, respectively. We propose classifying the APH(2′)-Ia-Jg as a new subfamily, APH(2′)-Ia-A (Figure S1).

Comparing aph(2′)-Ia genes of Campylobacter from humans and retail chicken, the aph(2′)-Ia of human isolates were more diverse than those of chicken meat isolates (Table S2). Among the 72 chicken meat isolates, 69 carried aph(2′)-Ia-Jg and 2 carried aph(2′)-Ia-Jc (N20344 and N20402), confirmed by WGS. Even when evaluated using both PCR and WGS, one chicken isolate (N13165) did not carry any aph(2′)-Ia genes. Analysis by WGS, however, revealed that N13165 carried aphA3, which was previously reported to be responsible for resistance to kanamycin, but not gentamicin.

Among the 79 human isolates, 46 were positive for aph(2′)-Ia-Jg when evaluated by PCR, but some showed weakly positive results. WGS data for 16 C. jejuni and 7 C. coli isolates showed that 11 C. jejuni carried aph(2′)-Ia-Jg, 5 C. jejuni and 6 C. coli carried aph(2′)-Ia-Ic (2041904). No aph(2′)-Ia-Jh was found in C. coli by WGS analysis. Five human isolates (four C. coli and one C. jejuni) were positive for bifunctional aac(6′)-le/aph(2′)-Ia/Ia by PCR. Fourier of the five isolates were analysed by WGS. Two (41898 and 41971) were confirmed as aac(6′)-le/aph(2′)-Ia, one (41912) was confirmed as aac(6′)-le/aph(2′)-If2 and another isolate (41945) carried two copies of aminoglycoside 2′-phosphotransferase resistance genes: one bifunctional aac(6′)-le/aph(2′)-Ia and one monofunctional aph(2′)-Ia-Jf. One human C. jejuni isolate (41921) was negative for all PCR amplifications, but was confirmed by WGS analysis to carry aph(2′)-Ia-Ic. Twenty-seven C. coli isolated from humans were positive for aph(2′)-Ia-Jg by PCR and eight were confirmed by WGS. The aph(2′)-Ia-Jg was the only gene shared by C. coli isolated from both humans (n = 27, 34.2%) and retail chicken meats (n = 69, 34.1%).
95.8%). When analysed by both PCR and WGS, gentamicin resistance genes were absent from all five gentamicin susceptible isolates.

**DNA fingerprint profiles**

PFGE was used to assess the genetic relatedness of strains. Seventeen PFGE profiles were generated from 38 *C. jejuni* isolates and 25 from 113 *C. coli* isolates (Figure 1a and b). *C. coli* isolates from humans showed more diverse PFGE profiles than retail chicken isolates (Figure 1b). Some PFGE profiles showed good correlation with their antimicrobial resistance profiles and gentamicin resistance gene types. For example, cluster A (Figure 1a), containing 23 *C. jejuni* isolated from humans, had >93% PFGE pattern similarity and were MDR, mostly to azithromycin, ciprofloxacin, erythromycin, nalidixic acid, telithromycin and tetracycline. All 23 isolates in this cluster were positive for *aph(2’)-I* by PCR. WGS analysis of seven isolates from this cluster confirmed the presence of

![Figure 1](image-url)
Figure 1. Continued
aph(2′′)-Ih. Most isolates in cluster A were obtained from the eastern USA between 2007 and 2011. Cluster B, containing four C. jejuni isolated from humans, had 70%–100% PFGE pattern similarity; all were unrestricted by KpnI and all were co-resistant to tetracycline. One isolate (41933) showed resistance to additional antimicrobials (azithromycin, ciprofloxacin, clindamycin, erythromycin, nalidixic acid and telithromycin). All were positive for aph(2′)-Iif when analysed by PCR, and two carried aph(2′)-Ih as shown by WGS. The isolates that carried unique genes such as aph(2′′)-Ib (41921) and aac(6′)-le/aph(2′)-If2 (41912) had distinct PFGE profiles (Figure 1a).

Cluster C (Figure 1b), containing five C. coli isolated from humans, had >75% PFGE pattern similarity and showed co-resistance to azithromycin, ciprofloxacin, erythromycin, nalidixic acid, telithromycin and tetracycline; two were also resistant to clindamycin. PCR and WGS results showed that all five contained aph(2′′)-Iff. Cluster D, containing three C. coli isolated from humans, showed >80% PFGE pattern similarity with all co-resistant to ciprofloxacin, nalidixic acid and tetracycline; one was also resistant to azithromycin, clindamycin, erythromycin and telithromycin. PCR and WGS analysis revealed that all three contained bifunctional aminoglycoside resistance genes.

Cluster E was a major cluster containing 96 C. coli isolates: 27 from humans and 69 from retail chicken meat. The isolates shared >95% PFGE pattern similarity; all except one (N26729) were co-resistant to tetracycline; and all carried aph(2′)-Ig. The majority of isolates in this cluster, both from humans and from retail chicken meats, were obtained from the western USA during 2008–11. Of three C. coli isolated from retail chicken, two (N20344 and N20402) carrying aph(2′′)-Ic and one (N13165) carrying aphA3 displayed unique PFGE profiles. N20344 and N20402 had the same resistance profile and were co-resistant to tetracycline. N13165 was the only chicken isolate that showed co-resistance to ciprofloxacin and nalidixic acid, in addition to tetracycline. Two human isolates that carried unique gentamicin resistance genes [aph(2′′)-If3] (N41904) and two copies of gentamicin resistance genes: one bifunctional aac(6′)-le/aph(2′′)-Ia and one monofunctional ahp(2′)-If1 (41945) also had distinct PFGE profiles. Overall, the human isolates showed more resistance, had more diverse PFGE profiles and carried more diverse aminoglycoside resistance genes than those from retail chicken. The PFGE clusters showed certain degrees of correlation with resistance profiles and gentamicin resistance genotypes.

Discussion

In this study, we report the emergence of novel genes conferring gentamicin resistance in Campylobacter and their presence with other resistance phenotypes. Nine variants of aminoglycoside 2′′-phosphotransferase genes [aph(2′′)] were identified: six [aph(2′′)-Ib, Ic, Iff, If3, Iff and aac(6′)-le/aph(2′′)-If2] were identified for the first time in Campylobacter. The aph(2′′)-Iff, If3, Ih and aac(6′)-le/aph(2′′)-If2 were novel variants of aminoglycoside resistance genes. Almost all gentamicin-resistant Campylobacter (98.7%) were co-resistant to tetracycline, and 65.8% of human isolates were resistant to three or more antimicrobials.

The aph(2′′)-Ig was the only aminoglycoside 2′′-phosphotransferase gene shared in C. coli isolated from both humans and retail chicken. The first aph(2′′)-Ig-positive C. coli isolate appeared in retail chicken meat in 2008 and in humans in 2009. In 2011, the number of aph(2′′)-Ig positive C. coli isolates increased significantly in both humans and retail chicken. The majority of these isolates were isolated from the western USA and shared similar PFGE and antimicrobial resistance profiles, suggesting a possible recent clonal expansion. Our findings suggest that poultry meat contaminated with gentamicin-resistant C. coli was the likely source of the human C. coli infections. Genomic data from two gentamicin-resistant C. coli isolated from retail chicken showed that aph(2′′)-Ig was located on a 55 kbp self-transmissible MDR plasmid (pN29710-1). The plasmid backbone was similar to a previously reported pTeT plasmid and shared 95% identity in the nucleotide sequence. The pN29710-1 plasmid carried additional antimicrobial resistance genes, including aad9, aade, sat4, aphA-3 and tet(O). This explains why all isolates in the same cluster, except one, were co-resistant to tetracycline. The G+C contents of the plasmid backbone and resistance gene cluster suggest that the resistance gene cluster was from an exogenous source.

Previous studies have shown that the APH(2′′) family is widely distributed in enterococci, staphylococci and streptococci. All APH(2′′) are monofunctional enzymes, with the exception of APH(2′′)-Ia, which is expressed as the C-terminal domain of the bifunctional enzyme AAC(6′)-le/APH(2′′)-Ia and confers resistance to all aminoglycosides except streptomycin. This is the most important enzyme associated with high-level gentamicin resistance in enterococci isolated in clinical settings. It has been proposed that aac(6′)-le/aph(2′′)-Ia was transmitted initially from staphylococci to enterococci. In the present study, we identified five isolates (four C. coli and one C. jejuni) from humans that carried the bifunctional aminoglycoside 2′′-phosphotransferase genes. In addition to aac(6′)-le/aph(2′′)-Ia, a new variant of bifunctional aminoglycoside 2′′-phosphotransferase genes aac(6′)-le/aph(2′′)-Iff2 was identified. To our knowledge, aph(2′′)-Iff linked with aac(6′)-le as a bifunctional aminoglycoside 2′′-phosphotransferase gene has not been reported. APH(2′)-Iff was previously reported as a monofunctional moiety of the APH(2′)-Ia domain of the bifunctional enzyme AAC(6′)-le/APH(2′)-Ia, and showed similar resistance profiles. The gene aph(2′)-Iff was first reported in C. jejuni from a US soldier deployed to Thailand; the gene was localized on an MDR pTeT plasmid. In our study, more than half of the human isolates carried aph(2′)-Iff/h, but no chicken meat isolates carried the gene. Based on the WGS data, APH(2′)-Iff had 88.2% amino acid identity similarity to APH(2′)-Iff, and APH(2′)-If2 was found only in C. jejuni, not C. coli. One human C. jejuni isolate carried aph(2′)-Ib and two C. coli from retail chicken carried aph(2′)-Ic. Both aph(2′)-Ib and aph(2′)-Ic were associated with high-level gentamicin resistance in Enterococcus. Given the similarity of genes in Enterococcus and Campylobacter, and since Campylobacter species are naturally competent and transformable, the aph(2′)-Ib and aph(2′)-Ic in Campylobacter probably originated from Enterococcus. Enterococcus has become one of the top drug-resistant microbes that are capable of rapid acquisition and dissemination of resistance genes.

Our study demonstrates that WGS provides an excellent tool for detecting newly emerging resistance genes as well as resistance gene families with high sequence variations that prove difficult to detect using PCR. WGS of one gentamicin-resistant isolate (N13165), on the other hand, did not show any genes associated
with gentamicin resistance. This indicates a possible limitation of WGS, where presumably the sequence gaps from draft genomes cause WGS to overlook underlying resistance genes. Alternatively, the isolate might carry a unique resistance mechanism. Further studies are necessary to explore the mechanism of gentamicin resistance in this isolate.

In summary, this study shows that retail chicken is a potential source for human gentamicin-resistant C. coli infections. Although the rapid rise in gentamicin-resistant Campylobacter is mirrored in human and chicken meat isolates, many isolates from clinical cases carried diverse resistance genes that were not detected in the food isolates tested. More sampling of meats, examination of other foods and epidemiological studies are needed in determining the source attribution for Campylobacter infections. Our findings also indicate that the emerged gentamicin resistance genes in Campylobacter probably have resulted from horizontal transfer from other microorganisms. The clonal expansion and horizontal transfer of aminoglycoside resistance genes in Campylobacter highlight the need to sustain and enhance monitoring for antimicrobial resistance of foodborne pathogens from humans, food animals and retail meats in order to better track infections by resistant pathogens and protect public health.

Acknowledgements
We are grateful to Drs Jean Whichard and Maureen Davidson for helpful comments and manuscript review.

Funding
The study was conducted as part of our routine work.

Transparency declarations
None to declare.

Supplementary data
Tables S1 and S2 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


