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

Shaohua Zhao[1,*], **Sampa Mukherjee**[1], **Yuansha Chen**[1], **Cong Li**[1], **Shenia Young**[1], **Melissa Warren**[1], **Jason Abbott**[1], **Sharon Friedman**[1], **Claudine Kabera**[1], **Maria Karlsson**[2], **Patrick F. McDermott**[1]

[1]Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, US Food and Drug Administration, Laurel, Maryland, USA

[2]Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Abstract

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*, *If*, *If1*, *If3*, *Ih*, *aac(6′)-Ie/aph(2″)-Ia* and *aac(6′)-Ie/aph(2″)-If2*. The *aph(2″)-Ib*, *Ic*, *If1*, *If3*, *Ih* and *aac(6′)-Ie/aph(2″)-If2* 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.

[*]Corresponding author. Fax: +1-301-210-4685; shaohua.zhao@fda.hhs.gov.

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/).

Keywords

Campylobacter; gentamicin resistance; NARMS; PCR; WGS

Introduction

Campylobacter is a leading cause of foodborne diarrhoeal 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 under-cooked 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 bacteraemia, 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*-adenyltransferases 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/aphD* 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 pTet 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, IIa, 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 $MgCl_2$, 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.

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 Nextara 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).[18,19] MEGA software, version 5.0 (www.megasoftware.net)[20] 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.[21] In brief, agarose-embedded cells were lysed and DNA was digested with 40 U of *SmaI* and *KpnI* (Boehringer Mannhein, 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 aminoglyco-side 2″-phosphotransferase genes [*aph(2″)-Ib*, *Ic*, *Ig*, *If*, *If1*, *If3 and Ih*] and two bifunctional aminoglycoside 2″-phosphotransferase genes [*aac(6′)-Ie/aph(2″)-Ia* and *aac(6′)-Ie/aph*(2″)-*If2*]. The *aph(2″)-Ib*, *Ic*, *If1*, *If3*, *Ih* and *aac(6′)-Ie/aph(2″)-If2* were identified for the first time in *Campylobacter*. The *aph(2′)-If1*, *If3*, *Ih* and *aac(6′)-Ie/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″)-If* available in GenBank, we divided the *aph(2″)-If* genes among our isolates into variants *aph(2″)-If1*, *If2* and *If3*, based on >90% amino acid sequence identity. Among 23 *aph(2″)-If*-positive isolates selected for WGS analysis, 11 had 88.2% amino acid sequence identity with the APH(2″)-If control from GenBank; therefore, we propose naming it *aph(2″)-Ih* (Figure S1). APH(2″)-Ig shared 29.7%, 52.2%, 34.6% and 25.9% amino acid identity with subfamilies

APH(2″)-Ia, APH(2″)-IIa (Ib), APH(2″)-IVa (Id/Ie) and APH(2″)-IIIa (Ic), respectively. We propose classifying the APH(2″)-Ig as a new subfamily, APH(2″)-Va (Figure S1).

Comparing *aph(2″)* genes of *Campylobacter* from humans and retail chicken, the *aph(2″)* of human isolates were more diverse than those of chicken meat isolates (Table S2). Among the 72 chicken meat isolates, 69 carried *aph(2″)-Ig* and 2 carried *aph(2″)-Ic* (N20344 and N20402), confirmed by WGS. Even when evaluated using both PCR and WGS, one chicken isolate (N13165) did not carry any *aph(2″)* 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″)-If* 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″)-Ih*; 5 *C. jejuni* and 6 *C. coli* carried *aph(2″)-If*; and 1 *C. coli* carried *aph(2″)-If3* (41904). No *aph(2″)-Ih* was found in *C. coli* by WGS analysis. Five human isolates (four *C. coli* and one *C. jejuni*) were positive for bifunctional *aac(6″)-Ie/ahp(2″)-Ia/If* by PCR. Four of the five isolates were analysed by WGS. Two (41898 and 41971) were confirmed as *aac(6″)-Ie/ahp(2″)-Ia*, one (41912) was confirmed as *aac(6″)-Ie/ ahp(2″)-If2* and another isolate (41945) carried two copies of aminoglycoside 2″-phosphotransferase resistance genes: one bifunctional *aac(6″)-Ie/ahp(2″)-Ia* and one monofunctional *ahp(2″)-If1.* One human *C. jejuni* isolate (41921) was negative for all PCR amplifications, but was confirmed by WGS analysis to carry *ahp(2″)-Ib.* Twenty-seven *C. coli* isolated from humans were positive for *ahp(2″)-Ig* by PCR and eight were confirmed by WGS. The *ahp(2″)-Ig* was the only gene shared by *C. coli* isolated from both humans (n = 27, 34.2%) and retail chicken meats (n = 69, 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″)-If* by PCR. WGS analysis of seven isolates from this cluster confirmed the presence of *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″)-If* 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″)-Ie/ahp(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″)-If.* 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. The two human isolates that carried unique gentamicin resistance genes [*aph(2″)-If3*] (N41904) and two copies of gentamicin resistance genes: one bifunctional *aac(6′)-Ie/ahp(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*, *If1*, *If3*, *Ih* and *aac(6′)-Ie/aph(2″)-If2*] were identified for the first time in *Campylobacter*. The *aph(2″)-If1*, *If3*, *Ih* and *aac(6′)-Ie/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).[14] The plasmid backbone was similar to a previously reported pTet plasmid and shared 95% identity in the nucleotide

sequence.[15] The pN29710–1 plasmid carried additional antimicrobial resistance genes, including *aad9*, *aadE*, *sat4*, *aphA-3* and *tet*(O).[14] 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.[14]

Previous studies have shown that the APH(2″) family is widely distributed in enterococci, staphylococci and streptococci.[16,22–25] 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′)-Ie/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.[13] It has been proposed that *aac(6′)-Ie/aph(2″)-Ia* was transmitted initially from staphylococci to enterococci.[26] 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′)-Ie/aph(2″)-Ia*, a new variant of bifunctional aminoglycoside 2″-phosphotransferase genes *aac(6′)-Ie/aph(2″)-If2* was identified. To our knowledge, *aph(2″)-If* linked with *aac(6′)-Ie* as a bifunctional aminoglycoside 2″-phosphotransferase gene has not been reported.

APH(2″)-If was previously reported as a monofunctional moiety of the APH(2″)-Ia domain of the bifunctional enzyme AAC(6′)-Ie/APH(2″)-Ia, and showed similar resistance profiles.[13] The gene *aph(2″)-If* was first reported in *C. jejuni* from a US soldier deployed to Thailand; the gene was localized on an MDR pTet plasmid.[27] In our study, more than half of the human isolates carried *aph(2″)-If/h*, but no chicken meat isolates carried the gene. Based on the WGS data, APH(2″)-Ih had 88.2% amino acid similarity to APH(2″)-If, and APH(2″)-Ih 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*.[26,28,29] 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.[30–32]

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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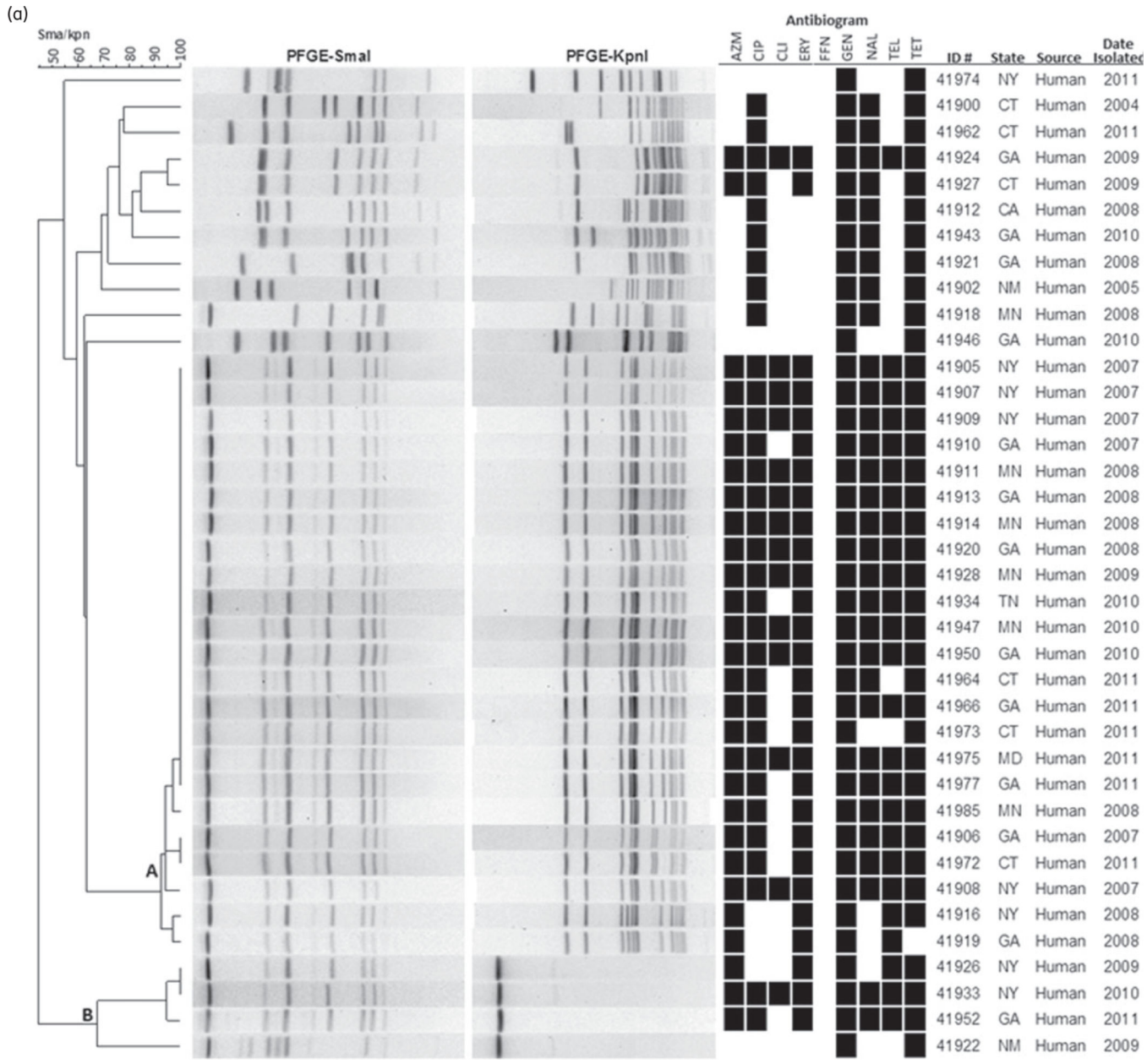

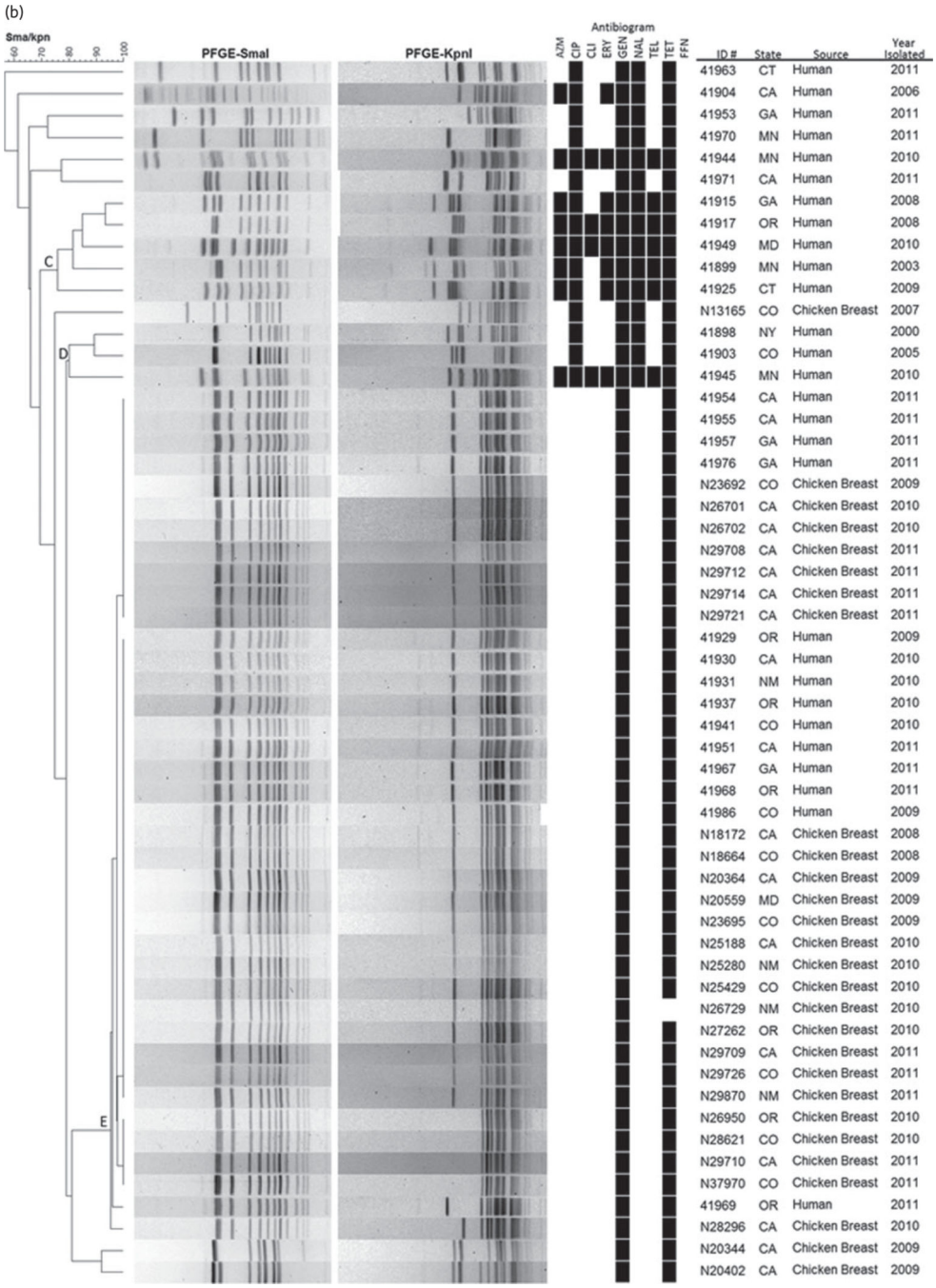

Figure 1.

(a) PFGE and antimicrobial resistance profiles of gentamicin-resistant *C. jejuni* isolated from humans from 2004 to 2011. For the susceptibility results, a black box indicates resistance to a particular antimicrobial (all isolates were susceptible to florfenicol). (b) PFGE and antimicrobial resistance profiles of selected gentamicin-resistant *C. coli* isolates from humans and retail chicken from 2000 to 2011. Cluster E was a major cluster containing 96 *C. coli* isolates: 27 from humans and 69 from retail chicken meat. Here, only 14 from humans and 25 from retail chicken meat were selected to represent this cluster

in the dendrogram. For the susceptibility results, a black box indicates resistance to a particular antimicrobial. AZM, azithromycin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FFN, florfenicol; GEN, gentamicin; NAL, nalidixic acid; TEL, telithromycin; TET, tetracycline.

Table 1.

Gentamicin-resistant *C. jejuni/coli* isolated from humans and retail chicken, 2000–11

	Human (*n* = 79)		Retail chicken (*n* = 72)	
Year of isolation	***C. coli* (*n*=41)**	***C. jejuni* (*n*=38)**	***C. coli* (*n* = 72)**	***C. jejuni* (*n*=0)**
2000	1	0	0	0
2003	1	0	0	0
2004	0	1	0	0
2005	1	1	0	0
2006	1	0	0	0
2007	0	6	1	0
2008	2	9	3	0
2009	2	5	11	0
2010	14	6	19	0
2011	19	10	38	0