Genomic screening of antimicrobial resistance markers in UK and US

Campylobacter isolates highlights stability of resistance over an 18 year period

Arnoud H.M. van Vliet ^{1,*}, Siddhartha Thakur ³, Joaquin M. Prada ²,

Jai W. Mehat ¹, Roberto M. La Ragione ¹

1,2 Departments of Pathology and Infectious Diseases ¹ and Veterinary Epidemiology and Public Health ², School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7AL, United Kingdom;

3 Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607, United States

* Corresponding author. E-mail: a.vanvliet@surrey.ac.uk

Running title: Stability *Campylobacter* AMR over 18 yr period

Keywords: antimicrobial resistance, antibiotic stewardship, *Campylobacter*, surveillance, quinolones, tetracycline, aminoglycosides, macrolides, whole genome sequencing

ABSTRACT

Campylobacter jejuni and *Campylobacter coli* are important bacterial sources of human foodborne illness. Despite several years of reduced antibiotics usage in livestock production in the UK and US, high prevalence of antimicrobial resistance (AMR) persists in *Campylobacter*. Both countries have instigated genome sequencing-based surveillance programs for *Campylobacter*, and here we have identified AMR genes in 32,256 *C. jejuni* and 8,776 *C. coli* publicly available genome sequences to compare the prevalence and trends of AMR in *Campylobacter* isolated in the UK and US between 2001-2018. AMR markers were detected in 68% of *C. coli* and 53% of *C. jejuni*, with 15% of *C. coli* being multi-drug resistant (MDR) compared to only 2% of *C. jejuni*. The prevalence of aminoglycoside, macrolide, quinolone and tetracycline resistance remained fairly stable from 2001-2018 in both *C. jejuni* and *C. coli*, but statistically significant differences were observed between the UK and US. There was a statistically significant higher prevalence of aminoglycoside and tetracycline resistance for US *C. coli* and *C. jejuni*, and macrolide resistance for US *C. coli*. In contrast, UK *C. coli* and *C. jejuni* showed a significantly higher prevalence of quinolone resistance. Specific MLST clonal complexes (e.g. ST-353/464) showed >95% quinolone resistance. This large-scale comparison of AMR prevalence has shown that the prevalence of AMR remains stable for *Campylobacter* in the UK and the US. This suggests that antimicrobial stewardship and restricted antibiotic usage may help contain further expansion of AMR prevalence in *Campylobacter*, but are unlikely to reduce it in the short term.

INTRODUCTION

Antibiotics are one of the great success stories of the 20th century, changing medicine and agricultural practices (1). Unfortunately, the fairytale did not last, and over the last decades, antimicrobial resistance (AMR) has become a significant problem (2). The excessive and inappropriate use of antibiotics in human and veterinary medicine and their widespread use as growth promoters in agriculture has led to a rise of resistance to many classes of antibiotics, including last-resort, critically important antimicrobials for human medicine (3). This is now recognised as a serious and global crisis, especially given the lack of progress in developing novel antibiotics (4). The World Health Organisation has declared AMR a severe threat to global public health in 2014 (5), and called upon the urgent introduction of multi-sectorial measures to prevent further development of AMR. This includes responsible use, which falls under antimicrobial stewardship (6, 7). One of the aspirations of reduced use of antimicrobials is that in the absence of antibiotic selection, susceptible isolates should have a fitness advantage over resistant isolates (8, 9), thereby restricting expansion of drug-resistant populations.

While phenotypic testing in the laboratory is still regarded as the gold standard, molecular tools such as those based on whole genome sequencing (WGS) are strong contenders to supersede phenotypic testing (10, 11), and have been successfully used in foodborne pathogens such as *Salmonella* and *Campylobacter* (12-15). However, harmonisation of databases and software tools is still required to avoid inter-laboratory reproducibility issues (16). Tools such as the NCBI AMRfinder software and curated Bacterial Antimicrobial Resistance Reference Gene Database at NCBI will certainly assist with such standardisation (17).

The bacterial pathogen *Campylobacter* is one of the leading causes of human bacterial diarrhoeal illness worldwide (18), and the European Food Safety Authority (EFSA) and the Centers for Disease Control (CDC) have reported similar levels of campylobacteriosis in the European Union and United States (19, 20). The causative agents *C. jejuni* and *C. coli* are

commonly associated with poultry, wild birds, ruminants and pigs, with undercooked meat and cross-contamination seen as common causes of infection (21). The major categories of antibiotics used to treat *Campylobacter* are macrolides, such as erythromycin in humans. In contrast, aminoglycosides such as gentamicin and streptomycin, macrolides, quinolones such as ciprofloxacin and nalidixic acid, and tetracycline have been commonly used in agricultural and veterinary settings (22-24). The WHO has listed *Campylobacter* as a high-priority antibiotic-resistant pathogen due to the rapid rise in quinolone resistance in *Campylobacter* (2).

One of the requirements for unbiased monitoring of antimicrobial resistance levels in *Campylobacter* is the existence of large-scale surveillance studies with genome sequences and matching metadata deposited in public repositories such as NCBI and EMBL. Historical isolates and collections may suffer from biases introduced by a focus on isolates with 'interesting' phenotypes such as multiple antibiotic resistances. Only two countries have instigated large-scale genome sequencing-based surveillance of *Campylobacter*: the United Kingdom (UK) and United States of America (US). The UK surveillance has focused on clinical isolates (25, 26), whereas the US surveillance combines isolates from clinical, food and animal sources (27, 28).

In this study we have collected the available whole genome sequences from UK and US *C. jejuni* and *C. coli* isolates between 2001-2018, and have assessed the presence of genes conferring resistance to aminoglycosides, macrolides, quinolones and tetracyclines. We highlight the trends over time, differences between the two countries, and show the stability of the frequency of resistance to four antibiotic categories in *Campylobacter* in the UK and US which could raise concerns about future developments.

RESULTS

Comparison of genome assembly- and sequence read-based AMR marker detection

To assess the efficacy of the NCBI AMRfinder software tool with *Campylobacter* genome sequences, we compared it with a recent AMR analysis of 381 *C. jejuni* and *C. coli* isolates (13). In that study, resistance detection was done directly on the sequencing reads using in-house scripts, whereas the AMRfinder software tool requires genomes to be assembled first. There were five genome assemblies that contained an *aph*(3')-IIIa aminoglycoside resistance gene, of which two were aminoglycoside-resistant phenotypically; no other possible false-positives were detected (Table 1) and there was 100% agreement for detection of quinolone resistance (GyrA D87 mutation) without false positives. Screening for tetracycline resistance resulted in 2/161 missed *tetO*-positive isolates. Further investigation of these two samples showed the *tetO* gene to be split over two contigs, which was detectable by secondary screening with BLAST using the Abricate software tool, and this was subsequently done as standard. Finally, macrolide resistance based on 23S rRNA gene mutations resulted in 3 negative samples previously reported positive (13). However, all three samples contained both the wild-type and mutated 23S rRNA alleles, suggesting only one or two of the three *C. jejuni* 23S rRNA genes mutated. Genome assembly algorithms are likely to ignore the minority gene variants, leading to these samples being tested negative. Overall, there was very good concordance between the two tests, and we considered this a validation for testing *Campylobacter* genome assemblies with the NCBI AMRfinder software tool.

Characteristics of a 2001-2018 *C. jejuni* and *C. coli* genome assembly database from public sources

A total of 32,256 *C. jejuni* and 8,776 *C. coli* genomes were included in our study. The

distribution over the year categories is shown in Figure 1A; for *C. coli*, approximately 400 UK samples were added for each of the 2015-2018 years, whereas the number of US *C. coli* samples increased from approximately 500 samples in 2015 to just over 2,000 in 2018. Similar trends were observed for *C. jejuni*, where the number of UK samples varies between approximately 2,000 and 4,000 per year, while the number of US samples increased from approximately 1,000 in 2015 to just under 4,000 in 2018. The samples from 2001-2014 were combined, as these did not have consistent availability of samples, with yearly numbers varying between 0-350 (*C. coli*, UK), 7-704 (*C. coli*, US), 10-1,881 (*C. jejuni*, UK) and 0-277 (*C. jejuni*, US) (Supplementary Figures S1, S2).

The *C. jejuni* and *C. coli* samples were split into several groups: UK and US-derived samples, and within each historical samples from 2001-2014 versus the 2015-2018 individual years, and the three source categories (farm animals, human, poultry). Comparison of these categories (Figure 1B) showed that UK samples were dominated by human isolates (approximately 70% for *C. coli* vs 5% for *C. coli* from the US), with farm animal samples poorly represented. In contrast, US samples showed a better representation of farm animal samples for both *C. jejuni* and *C. coli*, especially in samples derived from 2001-2014.

Distribution of antimicrobial resistance markers in *C. jejuni* and *C. coli*

The antimicrobial resistance profiles were determined for all the *C. jejuni* and *C. coli* genomes using NCBI AMRfinder. The presence of any of the 44 resistance markers was translated into aminoglycoside resistance (20 markers), macrolide resistance (9 markers), quinolone resistance (10 markers) or tetracycline resistance (5 markers). Table 2 shows the breakdown of the number of isolates predicted to be resistant for both *C. coli* and *C. jejuni*. In general, there was a higher number of AMR markers in *C. coli* than in *C. jejuni*, resulting in a larger proportion of multi-drug resistant (MDR) isolates (resistant to 3 or more classes of

antibiotics). In *C. coli*, 15.1% of samples were predicted to be MDR, whereas only 2.5% of *C. jejuni* were predicted to be MDR. Similarly, almost half of *C. jejuni* samples (46.6%) did not have any resistance marker compared to one-third of *C. coli* samples (32.2%). The frequency of resistance to tetracycline was high in both *C. coli* and *C. jejuni* (55.1% and 44.3%, respectively), with quinolone resistance at similar levels in both *C. coli* and *C. jejuni* (28.7% and 31.9%, respectively). Notable differences were noted in aminoglycoside resistance, which was sixfold higher in *C. coli* (35.4% vs 5.5%), and macrolide resistance which was twelvefold higher in *C. coli* (9.6% vs 0.8%) (Table 2).

Comparison of AMR levels between the UK and US (2001-2018)

The UK and US are currently the two countries which do large-scale genome sequencing-based surveillance of *Campylobacter* (14, 26), and this allows for insight in the dynamics of antibiotic resistance in these two industrialised countries over time. The years covering 2015-2018 were used individually while combining the 2001-2014 samples to function as a possible baseline for comparative purposes. The predicted resistance to aminoglycosides, macrolides, quinolones and tetracycline were plotted out for the UK and US isolates and compared for trends (Figure 2), with the individual years from 2001-2018 shown in Supplementary Figure S1 and S2.

The general trend for both *C. coli* and *C. jejuni* was that the proportion of aminoglycoside, macrolide and tetracycline-resistant isolates was significantly higher in the US than in the UK between 2015-2018, while the prevalence of quinolone resistance was significantly lower in the US (Figure 2). Around 40% of the US *C. coli* isolates were predicted to be resistant to aminoglycosides (range 39-45%), whereas in the UK *C. coli* isolates this was approximately 20% (range 12-26%). For *C. jejuni*, the prevalence of aminoglycoside resistance was between 12-14% for the US isolates compared to 1-4% for the UK isolates. Differences were less pronounced for tetracycline resistance for *C. jejuni* (range 39-46% for UK vs 48-56% for the US samples), while

in *C. coli* this varied between 36-56% for the UK isolates, and 50-65% for the US isolates. Macrolide resistance was present in much higher levels in *C. coli* in both the UK and the US isolates, ranging from 3-19% in the UK isolates and 6-14% in the US *C. coli* isolates, while it was between 0-1% in the UK and the US *C. jejuni* isolates. Finally, the trend was reversed for quinolone resistance, with a higher proportion of the UK isolates predicted to be quinolone-resistant. For *C. coli*, the proportion of quinolone-resistant UK isolates was 31-44% for UK isolates versus 25-39% for US isolates, while for *C. jejuni* the proportion was 39-44% for UK isolates and 22-23% for US isolates. For each of the *Campylobacter* species and antibiotic class, statistically significant differences were generally observed between countries and year (Supplementary Table S2).

Next to the differences between the two countries, we also observed that the only antibiotic for which there was a clear decrease in resistance from 2015 to 2018 was macrolide resistance (Figure 2), as it decreased from 15% to 6% in the US isolates, and from 19% to 5% in the UK isolates. For the other three antibiotic classes, there was little change from 2015-2018 and especially in *C. coli*, the proportion of resistant isolates remains high for aminoglycoside resistance (US), quinolone resistance (UK, US) and tetracycline resistance (UK, US). For *C. jejuni*, the levels of quinolone resistance are especially of concern in the UK.

Associations between isolation source and MLST genotypes and AMR markers in *C. coli* and *C. jejuni*

Due to the pronounced differences in isolation source between the UK and US (Figure 1B), we investigated whether there were differential contributions to the proportions of antibiotic-resistant isolates for *C. coli* and *C. jejuni* isolates from 2015-2018 (Figure 3A). There was no clear pattern of overrepresentation of any isolation source for the AMR isolates in *C. coli*. However, the low number of farm animal isolates from the UK may hide some effects. Similarly,

for *C. jejuni* there was no clear link with any of the three source categories (Figure 3A).

We also investigated whether specific multilocus sequence types (MLST) clonal complexes (CC) were associated with specific antibiotic resistances in the UK and US isolates. For *C. coli*, 97.3% of the isolates are from CC-828. For *C. jejuni*, we looked at the clonal complexes for which both for the UK and US there were at least >100 samples available for 2015-2018 (Figure 3B). For aminoglycoside resistance, there was a spread over all the major clonal complexes for US isolates, with CC-21 and CC-353 having the highest proportion of resistant isolates (17-20%). In contrast, for the UK isolates, aminoglycoside resistance was primarily found in CC-353 and CC-42. The CC-353 clonal complex was also involved in macrolide resistance, although this plays a minor role in the *C. jejuni* isolates, with CC-45 showing the highest proportion of resistant isolates in the US. For both quinolone resistance and tetracycline resistance, all major MLST genotypes contributed to resistance, but the major contribution to quinolone resistance in UK isolates came from CC-353, CC-354 and CC-464, where almost all isolates were predicted to be quinolone resistant, consistent with earlier reports (26, 29). Finally, the highest proportions of tetracycline resistance were associated with CC-354 and CC-464 for UK isolates, and CC-257 and CC-42 for US isolates. Most of the clonal complexes reported here are primarily associated with poultry and human infections, except for CC-42 associated with cattle.

208 DISCUSSION

209 In this study we have exploited the possibilities afforded by the active genome sequencing-
 210 based surveillance for *Campylobacter* in the UK and US, to determine the trends in AMR over
 211 recent years, and compare these trends within and between the two countries. Similar studies
 212 have previously been conducted in individual countries, but often at a smaller scale than
 213 employed in this study, or focusing on a shorter time period. For instance, a recent UK study
 214 investigated 528 human isolates from 2015-2016 (13), while a 2018 US-based study focused on
 215 589 isolates from 2015 (14). These, and other genome sequencing-based studies have all shown
 216 that identifying resistance markers in genome sequences matches well with phenotypic resistance.
 217 Hence we are confident that our study appropriately represents AMR trends detected in 32,256 *C.*
 218 *jejuni* and 8,876 *C. coli* samples, as listed in Supplementary Table S1.

219 Monitoring of AMR on this scale requires active surveillance with sequencing data and
 220 metadata being released in the public domain. We have combined the information present in the
 221 Genbank database, which contains both US and UK samples, and the *Campylobacter* PubMLST
 222 database which includes a large number of UK isolates. Our dataset is based on a de-duplicated,
 223 quality controlled set of genome sequences with strict requirements for available metadata. The
 224 criteria for isolate source, year and country data allowed direct comparison between the UK and
 225 US *Campylobacter* samples for *C. coli* and *C. jejuni* (Figure 1B). The UK dataset leans heavily
 226 on the Oxfordshire Sentinel Surveillance (25, 26), which explains the dominance of human
 227 samples in the UK dataset in each individual year. This contrasts with the US dataset, which has
 228 an equal distribution over the three source categories. While this is not ideal for comparative
 229 purposes, we do note that for *C. jejuni* the distribution over the dominant MLST clonal
 230 complexes was very similar (Figure 3), with the major differences being that CC-257 and CC-464
 231 were prevalent in a higher proportion in UK samples (5% and 6% more of the total number of
 232 samples). In comparison, CC-353 was more prevalent in US samples (7% more). All three clonal

complexes are primarily associated with poultry, and hence for *C. jejuni*, we concluded that the datasets can still be compared. For *C. coli* this was more difficult to assess, as most samples cluster in the agricultural Clade 1A (30, 31) which only contains CC-828 and a minority of samples not part of this clonal complex. We did compare the major sequence types within the CC-828 clonal complex, and noted that there were more pronounced differences between the UK and US samples. However, these may primarily represent the ST-types prevalent in the two countries. While ST-825, ST-827, and ST-828 were more present in UK samples, ST-829 was likewise more present in US samples; all these STs contained human, poultry and farm animal samples. ST-1068 was only present in US samples, and is primarily farm animals and many isolates with multiple resistance markers, and this represents a cluster of samples missing from the UK dataset. Thus, while there are differences in the breakdown of the two datasets on isolation sources, we believe they are sufficiently similar to allow for direct comparison.

In this study we have focused on four classes of antibiotics, due to their relevance in *Campylobacter* and their usage in the categories of hosts investigated. The macrolide erythromycin and the fluoroquinolone ciprofloxacin are used to treat human infections with *Campylobacter* (22). In contrast, all four classes of antibiotics are used therapeutically in the veterinary sector for livestock (32). As highlighted earlier, the WHO has listed *Campylobacter* as a high-priority antibiotic-resistant pathogen (2), especially linked to fluoroquinolone resistance in *Campylobacter*. Antimicrobial resistance in *Campylobacter* spp. is a combination of *de novo* point mutations associated with quinolone and macrolide resistance, and genetic exchange on plasmids and other mobile elements for aminoglycoside and tetracycline resistance genes (33). In addition, the natural competence of *Campylobacter* spp. may allow further dissemination of resistance through populations. Although the usage of antibiotics has been limited in the UK and the US for a considerable period of time, there is no apparent reduction visible in the prevalence of resistance in the large numbers of *C. jejuni* and *C. coli* samples investigated here (Figure 2).

Furthermore, our statistical analysis suggest in fact possible small increases in *C. jejuni* resistance to some antibiotics in recent years compared to before 2014 levels (Supplementary Table S2). This is unlikely to be due to a sampling bias due to the different geographic locations and multiple years included. A more likely hypothesis is that resistance to especially tetracyclines, aminoglycosides and quinolones is not detrimental to *Campylobacter*, or not sufficiently detrimental to allow outcompetition by susceptible isolates. Conversely, resistance may even be associated with advantageous phenotypes, as the gyrase mutations associated with quinolone resistance gave rise to increases in virulence phenotypes in *C. jejuni* (34, 35), while *tetO*-containing plasmids are ubiquitous and stable in *C. jejuni* and *C. coli* (36). Aminoglycoside resistance genes are especially widespread in *C. coli*, and their association with mobile elements (33, 37) may assist their stability in the populations.

The similarities between the UK and US datasets suggest that the situation in other industrialised countries may be similar, but this will require more intensive surveillance programs and the need to balance clinical, food and agricultural samples, including sewage and water sources. A recent study reported that fluoroquinolone usage in pigs and poultry in France from 2011-2018 decreased by >70% in poultry, and almost 90% in pigs. However, resistance to ciprofloxacin increased in poultry *Campylobacter* isolates from 50% to approximately 60%, whereas in *Escherichia coli* prevalence of ciprofloxacin resistance was stable in broiler and pig isolates, but decreased in turkey isolates (38). Similar studies have been done in other countries, but often are based on small or limited datasets where selection bias may affect the results.

The lack of reduction of the proportion of AMR isolates in *Campylobacter* from both countries does suggest that reduced usage of antimicrobials, such as mandated by antimicrobial stewardship, will not be sufficient to reduce the incidence of AMR in *Campylobacter*, although it may well contribute positively to reigning in further increases of AMR. For clinical purposes, macrolides such as erythromycin are still useful for treating *C. jejuni* infections, but their efficacy

may be reduced for *C. coli*. In contrast, the high level of quinolone resistance in both *C. jejuni* and *C. coli* does not bode well for the future efficacy of ciprofloxacin treatment of *Campylobacter* infections in humans. With regard to aminoglycosides and tetracyclines, the latter is not recommended due to the high level of resistance, whereas with aminoglycosides gentamicin can still be used for now. Prevalence of gentamicin resistance markers was very low in UK isolates (10/2,842 (0.3%) for *C. coli* and 11/20,659 (0.05%) for *C. jejuni*), but higher in the US isolates (203/5,934 (3.4%) for *C. coli* and 116/11,597 (1.0%) for *C. jejuni*) (Supplementary Table S1).

Taken together, the data presented here strongly suggest that reduced usage of antibiotics has not resulted in a significant reduction of antimicrobial resistance in *Campylobacter*, which is of considerable public health and economic concern. Changes in agricultural practices, slaughter and retail will be substantial to reduce the overall prevalence of *Campylobacter*. These efforts should lessen the need for antibiotic usage to achieve the goals of antimicrobial stewardship. Similar comparative studies could be done in other countries and other foodborne zoonotic infections to assess whether this situation is unique for *Campylobacter* or mirrored in other pathogenic bacteria.

MATERIALS AND METHODS

Campylobacter genome assemblies and metadata categories included in this study

A total of 44,751 *C. jejuni* and 12,709 *C. coli* genome assemblies were collected from the Genbank and *Campylobacter* PubMLST databases, and were coupled to metadata from the Genbank files and from PubMLST. Genome assemblies were obtained from the NCBI database using ncbi-genome-download version 0.2.11 (<https://github.com/kbclin/ncbi-genome-download/>), and supplemented with genome sequences from the *Campylobacter* pubMLST website (<http://pubmlst.org/campylobacter/>) (39). Metadata were extracted from Genbank flat files and the NCBI Pathogens database (<https://www.ncbi.nlm.nih.gov/pathogens>). All genome assemblies were screened for assembly statistics using Quast version 4.5 (40), and genome assemblies were excluded if failing two or more of the following criteria: number of contigs ≤ 200 , N50 ≥ 25 kb, L50 ≤ 25 contigs, largest contig ≥ 50 kb or the number of Ns per 100 kb ≥ 50 . Genomes with a total size outside 1.4 Mbp and 2.1 Mbp were automatically excluded. Duplicate entries were removed by comparing sample names, assembly statistics such as N50, L50 and genome size and metadata such as year, source and country. After deduplication, samples were subsequently only included if the following metadata were available: isolation source, year of isolation, and isolated in the UK or US. Isolation sources were combined to give three main categories: farm animals (pigs, cattle, sheep, goat, including milk and meat samples), human (clinical isolates) and poultry (chicken, turkey, including meat samples). Samples representing environmental, farm, generic "food" and wild birds were excluded, as were samples lacking other metadata. This resulted in the exclusion of 6,327 *C. jejuni* and 877 *C. coli* samples with missing information on country, year or isolation source; 1,634 *C. jejuni* and 142 *C. coli* were not from the UK or US; 3,116 *C. jejuni* and 2,086 *C. coli* were from outside the 2001-2018 period studied here, and 1,418 *C. jejuni* and 828 *C. coli* were not from animal, ruminant, poultry or human sources. We focused on 2015-2018 as

individual years as both UK and US doing surveillance projects, while historical samples were categorised as 2001-2014. Overall, we analysed a total of 41,032 genome sequences, represented by 32,256 *C. jejuni* and 8,776 *C. coli* genomes (Supplementary Table S1).

Screening for antimicrobial resistance markers

Genome assemblies were screened for AMR markers using the NCBI AMRfinder software tool version 3.1.1b (17), with the October 2019 database, and the nucleotide setting (-n) and the organism switch (-O *Campylobacter*) which includes screening for point mutation-based resistances. Genome assemblies were also screened using Abricate version 0.9.8 (<https://github.com/tseemann/abricate/>) and the NCBI database, which screens for AMR genes, but not point mutation-based resistances. Resistance genes were categorised into four types of resistance: aminoglycoside resistance [*aph*(2'')-Ia, *aph*(2'')-If, *aph*(2'')-If2, *aph*(2'')-Ig, *aph*(2'')-Ih, *aph*(2'')-IIa, *aph*(2'')-IIIa, *aph*(2'')-IVa, *apmA*, *aac*(6')-Ie, *aac*(6')-Im, *aad9*, *aadE*, *aadE*-Cc, *ant*(6)-Ia, *aph*(3')-IIa, *aph*(3')-Ib, *aph*(3')-IIIa, *aph*(3')-VIIa, *spw*], macrolide resistance [*23S*_A2074C, *23S*_A2074G, *23S*_A2074T, *23S*_A2075G, *cfr*(C), *erm*(36), *erm*(B), *erm*(C), *erm*(F)], quinolone resistance [*GyrA*_D90N, *GyrA*_D90Y, *GyrA*_P104S, *GyrA*_T86A, *GyrA*_T86I, *GyrA*_T86K, *GyrA*_T86V, *qnrB*, *qnrD*, *qnrDI*] and tetracycline resistance [*tet*(32), *tet*(L), *tet*(O), *tet*(W), *tet*(X)]. Samples were considered multi-drug resistant (MDR) when containing resistance markers for three or more classes of antibiotics.

Comparison of genome assembly-based screening for AMR genes with sequencing-read based screening

The Supplementary Data presented in (13) contained 381 accession numbers to FASTQ read files used for screening for antimicrobial resistance genes. The FASTQ files were downloaded from the Sequence Read Archive using fastq-dump from the SRA toolkit

(<https://github.com/ncbi/sra-tools>), and genomes assembled using Spades version 3.14 (41) via the Shovill version 1.0.9 tool and standard settings (<https://github.com/tseemann/shovill/>). All genomes passed the Quast QC, and were screened using the NCBI Amrfinder tool version 3.6.7 (17) with the nucleotide setting (-n) and the organism switch (-O *Campylobacter*) as described above. All genome assemblies were also screened using Abricate version 0.9.8 (<https://github.com/tseemann/abricate/>) and the NCBI database with a minimum coverage of 30% (--mincov 30) to check for the *tetO* tetracycline resistance gene being split over two or more contigs.

Statistical Analysis

The presence or absence of resistance for each of the four classes of antibiotics was assessed using a generalized linear model with a logit link function (binomial family). Country, year and source of each sample were considered as explanatory variables. Interactions between predictors were not considered. Bootstrapping with 500 repeats was carried out to estimate the 95% confidence interval (CI) of the prevalence of resistance for each country and year. All statistical analyses were carried out in R (version 4.0.3) (42).

Data description

All genome sequences used in this study are available from the Genbank/EMBL/DDBJ databases or the *Campylobacter* PubMLST website (<https://pubmlst.org/organisms/campylobacter-jejunicoli/>). The assembly accession numbers (NCBI Genome) or genome ID numbers (*Campylobacter* PubMLST) are listed in Supplementary Table S1, together with the metadata used and the AMR gene data.

375 ACKNOWLEDGMENTS

376 We gratefully acknowledge the efforts of the surveillance programs in the US and UK, and
 377 their continued contribution to data sharing by making the genome sequences and partial
 378 metadata publicly available. Part of this work was supported by funding from the European
 379 Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830:
 380 One Health European Joint Programme (WorldCOM). The US whole genome sequencing work
 381 was supported by the National Institutes of Health/Food and Drug Administration under award
 382 number 5U19FD007113-02, whereas UK *Campylobacter* surveillance was supported by the UK
 383 Food Standards Agency. This publication made use of the PubMLST website
 384 (<http://pubmlst.org/>) developed by Keith Jolley and sited at the University of Oxford. The
 385 development of that website was funded by the Wellcome Trust.

386

387 CONFLICTS OF INTEREST

388 The authors declare that there are no conflicts of interest.

389

REFERENCES

1. Gould K. 2016. Antibiotics: from prehistory to the present day. *J Antimicrob Chemother* 71:572-5.
2. World Health Organisation. 2017. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organisation, Geneva.
3. Aidara-Kane A, Angulo FJ, Conly JM, Minato Y, Silbergeld EK, McEwen SA, Collignon PJ, Group WHOGD. 2018. World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. *Antimicrob Resist Infect Control* 7:7.
4. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z. 2018. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 11:1645-1658.
5. World Health Organisation. 2014. Antimicrobial resistance: global report on surveillance 2014. World Health Organisation, Geneva.
6. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ, Srinivasan A, Dellit TH, Falck-Ytter YT, Fishman NO, Hamilton CW, Jenkins TC, Lipsett PA, Malani PN, May LS, Moran GJ, Neuhauser MM, Newland JG, Ohl CA, Samore MH, Seo SK, Trivedi KK. 2016. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clin Infect Dis* 62:e51-77.
7. Keske S, Zabun B, Aksoy K, Can F, Palaoglu E, Ergonul O. 2018. Rapid Molecular Detection of Gastrointestinal Pathogens and Its Role in Antimicrobial Stewardship. *J Clin Microbiol* 56:e00148-18.
8. Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat Rev Microbiol* 8:260-71.
9. Yu T, Jiang G, Gao R, Chen G, Ren Y, Liu J, van der Mei HC, Busscher HJ. 2020. Circumventing antimicrobial-resistance and preventing its development in novel, bacterial infection-control strategies. *Expert Opin Drug Deliv* 17:1151-1164.
10. Hendriksen RS, Bortolaia V, Tate H, Tyson GH, Aarestrup FM, McDermott PF. 2019. Using Genomics to Track Global Antimicrobial Resistance. *Front Public Health* 7:242.
11. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Koser CU, MacGowan A,

- Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen O, Woodford N. 2017. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 23:2-22.
12. Neuert S, Nair S, Day MR, Doumith M, Ashton PM, Mellor KC, Jenkins C, Hopkins KL, Woodford N, de Pinna E, Godbole G, Dallman TJ. 2018. Prediction of Phenotypic Antimicrobial Resistance Profiles From Whole Genome Sequences of Non-typhoidal *Salmonella enterica*. Front Microbiol 9:592.
13. Painset A, Day M, Doumith M, Rigby J, Jenkins C, Grant K, Dallman TJ, Godbole G, Swift C. 2020. Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* isolated from cases of diarrhoeal disease in England and Wales, 2015-16. J Antimicrob Chemother 75:883-889.
14. Whitehouse CA, Young S, Li C, Hsu CH, Martin G, Zhao S. 2018. Use of whole-genome sequencing for *Campylobacter* surveillance from NARMS retail poultry in the United States in 2015. Food Microbiol 73:122-128.
15. Pornsukarom S, van Vliet AHM, Thakur S. 2018. Whole genome sequencing analysis of multiple *Salmonella* serovars provides insights into phylogenetic relatedness, antimicrobial resistance, and virulence markers across humans, food animals and agriculture environmental sources. BMC Genomics 19:801.
16. Doyle RM, O'Sullivan DM, Aller SD, Bruchmann S, Clark T, Coello Pelegrin A, Cormican M, Diez Benavente E, Ellington MJ, McGrath E, Motro Y, Phuong Thuy Nguyen T, Phelan J, Shaw LP, Stabler RA, van Belkum A, van Dorp L, Woodford N, Moran-Gilad J, Huggett JF, Harris KA. 2020. Discordant bioinformatic predictions of antimicrobial resistance from whole-genome sequencing data of bacterial isolates: an inter-laboratory study. Microb Genom 6:e000335.
17. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu CH, McDermott PF, Tadesse DA, Morales C, Simmons M, Tillman G, Wasilenko J, Folster JP, Klimke W. 2019. Validating the AMRFinder Tool and Resistance Gene Database by Using Antimicrobial Resistance Genotype-Phenotype Correlations in a Collection of Isolates. Antimicrob Agents Chemother 63:e00483-19.
18. Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. 2015. Global Epidemiology of *Campylobacter* Infection. Clin Microbiol Rev 28:687-720.
19. European Food Safety Authority, European Centre for Disease Prevention and Control. 2021. The European Union One Health 2019 Zoonoses Report. EFSA J 19:e06406.
20. Tack DM, Ray L, Griffin PM, Cieslak PR, Dunn J, Rissman T, Jervis R, Lathrop S, Muse

- A, Duwell M, Smith K, Tobin-D'Angelo M, Vugia DJ, Zablotsky Kufel J, Wolpert BJ, Tauxe R, Payne DC. 2020. Preliminary Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016-2019. *MMWR Morb Mortal Wkly Rep* 69:509-514.
21. Nichols GL, Richardson JF, Sheppard SK, Lane C, Sarran C. 2012. *Campylobacter* epidemiology: a descriptive study reviewing 1 million cases in England and Wales between 1989 and 2011. *BMJ Open* 2:e001179.
22. Shen Z, Wang Y, Zhang Q, Shen J. 2018. Antimicrobial Resistance in *Campylobacter* spp. *Microbiol Spectr* 6:ARBA-0013-2017.
23. Yang Y, Feye KM, Shi Z, Pavlidis HO, Kogut M, A JA, Ricke SC. 2019. A Historical Review on Antibiotic Resistance of Foodborne *Campylobacter*. *Front Microbiol* 10:1509.
24. Sproston EL, Wimalaratna HML, Sheppard SK. 2018. Trends in fluoroquinolone resistance in *Campylobacter*. *Microb Genom* 4:mgen.0.000198.
25. Cody AJ, McCarthy ND, Jansen van Rensburg M, Isinkaye T, Bentley SD, Parkhill J, Dingle KE, Bowler IC, Jolley KA, Maiden MC. 2013. Real-time genomic epidemiological evaluation of human *Campylobacter* isolates by use of whole-genome multilocus sequence typing. *J Clin Microbiol* 51:2526-34.
26. Cody AJ, McCarthy NM, Wimalaratna HL, Colles FM, Clark L, Bowler IC, Maiden MC, Dingle KE. 2012. A longitudinal 6-year study of the molecular epidemiology of clinical *Campylobacter* isolates in Oxfordshire, United kingdom. *J Clin Microbiol* 50:3193-201.
27. Allard MW, Strain E, Melka D, Bunning K, Musser SM, Brown EW, Timme R. 2016. Practical Value of Food Pathogen Traceability through Building a Whole-Genome Sequencing Network and Database. *J Clin Microbiol* 54:1975-83.
28. Karp BE, Tate H, Plumblee JR, Dessai U, Whichard JM, Thacker EL, Hale KR, Wilson W, Friedman CR, Griffin PM, McDermott PF. 2017. National Antimicrobial Resistance Monitoring System: Two Decades of Advancing Public Health Through Integrated Surveillance of Antimicrobial Resistance. *Foodborne Pathog Dis* 14:545-557.
29. Cha W, Mosci R, Wengert SL, Singh P, Newton DW, Salimnia H, Lephart P, Khalife W, Mansfield LS, Rudrik JT, Manning SD. 2016. Antimicrobial Susceptibility Profiles of Human *Campylobacter jejuni* Isolates and Association with Phylogenetic Lineages. *Front Microbiol* 7:589.
30. Sheppard SK, Dallas JF, Wilson DJ, Strachan NJ, McCarthy ND, Jolley KA, Colles FM, Rotariu O, Ogden ID, Forbes KJ, Maiden MC. 2010. Evolution of an agriculture-associated disease causing *Campylobacter coli* clade: evidence from national surveillance data in

Scotland. PLoS One 5:e15708.

31. Sheppard SK, Didelot X, Jolley KA, Darling AE, Pascoe B, Meric G, Kelly DJ, Cody A, Colles FM, Strachan NJ, Ogden ID, Forbes K, French NP, Carter P, Miller WG, McCarthy ND, Owen R, Litrup E, Egholm M, Affourtit JP, Bentley SD, Parkhill J, Maiden MC, Falush D. 2013. Progressive genome-wide introgression in agricultural *Campylobacter coli*. Mol Ecol 22:1051-64.
32. Lhermie G, La Ragione RM, Weese JS, Olsen JE, Christensen JP, Guardabassi L. 2020. Indications for the use of highest priority critically important antimicrobials in the veterinary sector. J Antimicrob Chemother 75:1671-1680.
33. Mourkas E, Florez-Cuadrado D, Pascoe B, Calland JK, Bayliss SC, Mageiros L, Meric G, Hitchings MD, Quesada A, Porrero C, Ugarte-Ruiz M, Gutierrez-Fernandez J, Dominguez L, Sheppard SK. 2019. Gene pool transmission of multidrug resistance among *Campylobacter* from livestock, sewage and human disease. Environ Microbiol 21:4597-4613.
34. Scanlan E, Ardill L, Whelan MV, Shortt C, Nally JE, Bourke B, T OC. 2017. Relaxation of DNA supercoiling leads to increased invasion of epithelial cells and protein secretion by *Campylobacter jejuni*. Mol Microbiol 104:92-104.
35. Whelan MVX, Ardill L, Koide K, Nakajima C, Suzuki Y, Simpson JC, T OC. 2019. Acquisition of fluoroquinolone resistance leads to increased biofilm formation and pathogenicity in *Campylobacter jejuni*. Sci Rep 9:18216.
36. Batchelor RA, Pearson BM, Friis LM, Guerry P, Wells JM. 2004. Nucleotide sequences and comparison of two large conjugative plasmids from different *Campylobacter* species. Microbiology 150:3507-17.
37. Cobo-Diaz JF, Gonzalez Del Rio P, Alvarez-Ordóñez A. 2021. Whole Resistome Analysis in *Campylobacter jejuni* and *C. coli* Genomes Available in Public Repositories. Front Microbiol 12:662144.
38. Perrin-Guyomard A, Jouy E, Urban D, Chauvin C, Granier SA, Mourand G, Chevance A, Adam C, Moulin G, Kempf I. 2020. Decrease in fluoroquinolone use in French poultry and pig production and changes in resistance among *E. coli* and *Campylobacter*. Vet Microbiol 243:108637.
39. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124.
40. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for

genome assemblies. *Bioinformatics* 29:1072-5.

41. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455-77.
42. R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria: <https://www.R-project.org/>.

Table 1. Comparison of AMR marker detection directly on sequencing reads (13) and detection using assembled genomes.

Antibiotic category	positive	negative	Percentage agreement
Aminoglycosides	14 / 14 + [2 / 5*]	362 / 362	100% [74%], 100%
Macrolides	28 / 31**	350 / 350	90.3%, 100%
Quinolones	172 / 172	209 / 209	100%, 100%
Tetracycline	159 / 161***	220 / 220	98.6%, 100%

* Five samples contained an *aph(3')-IIIa* aminoglycoside resistance gene not reported in (13). Two of these samples were phenotypically resistant to aminoglycosides (13).

** Two of the three negative samples were susceptible in resistance testing, and all three showed inconclusive mutation detection in (13).

*** The two negative samples had the *tetO* resistance gene divided over two contigs. Additional screening with Abricate with the "--mincov 30" allowed the detection of this AMR marker.

Table 2. Distribution of antibiotic resistance markers in *C. coli* and *C. jejuni* genome sequences (2001-2018 combined).

Antibiotic resistance markers	<i>C. coli</i> (N=8,776)	<i>C. jejuni</i> (N=32,256)
no antibiotic resistance markers	2,828 (32.2%)	15,044 (46.6%)
1 Antibiotic resistance marker*	2,079 (23.7%)	8,749 (27.1%)
2 Antibiotic resistance markers†	2,551 (29.1%)	7,647 (23.7%)
3 Antibiotic resistance markers‡ (MDR)	1,146 (13.1%)	718 (2.2%)
4 Antibiotic resistance markers (MDR)	172 (2.0%)	98 (0.3%)
Aminoglycoside resistance	3,105 (35.4%)	1,770 (5.5%)
Macrolide resistance	845 (9.6%)	261 (0.8%)
Quinolone resistance	2,520 (28.7%)	10,276 (31.9%)
Tetracycline resistance	4,837 (55.1%)	14,283 (44.3%)

* the majority of isolates were aminoglycoside resistant (*C. coli*: 17%), quinolone resistant (*C. coli*: 26%, *C. jejuni*: 31%) or tetracycline resistant (*C. coli*: 54%, *C. jejuni*: 67%).

† the majority of isolates were aminoglycoside+tetracycline resistant (*C. coli*: 55%, *C. jejuni*: 11%) or quinolone+tetracycline resistant (*C. coli*: 34%, *C. jejuni*: 88%).

‡ the majority of isolates were aminoglycoside+macrolide+tetracycline resistant (*C. coli* only: 28%) or aminoglycoside+quinolone+tetracycline resistant (*C. coli*: 64%, *C. jejuni*: 86%). MDR = multi-drug resistant.

LEGENDS TO FIGURES

Figure 1. Breakdown of the UK and US *C. coli* and *C. jejuni* samples obtained in surveillance programs from 2015-2018. A) Number of genome sequences per individual year from 2015-2018. The number of US genome sequences shows steady growth each year, whereas the number of UK genome sequences varies per year without a clear trend. B) Comparison of the isolation source category of the UK and US samples from 2015-2018, with the 2001-2014 historical isolates included. Within the UK samples, the human source category is dominant, consistent with the major surveillance category being the Oxfordshire sentinel surveillance (25, 26), while US isolates have a much higher contribution of farm animals (pigs, ruminants) and poultry (27, 28).

Figure 2. Comparison of the proportion of UK and US *C. coli* and *C. jejuni* samples resistant to aminoglycosides, macrolides, quinolones and tetracycline for individual years from 2015-2018, with the average of the 2001-2014 period provided for comparative purposes. Error bars show the 2.5% and 97.5% quantiles, based on 500 bootstraps.

Figure 3. Tornado plot displaying the relative contributions of the source categories (A) and *C. jejuni* MLST clonal complexes (B) to the individual antibiotic classes (aminoglycosides, macrolides, quinolones and tetracycline). Only the 2015-2018 samples have been included.

Figure 1

A

B

Figure 2

568 **Figure 3**
569

570
571