Gut Pathogens

REVIEW

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Genomic insights into zoonotic transmission and antimicrobial resistance in *Campylobacter jejuni* from farm to fork: a one health perspective

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Abstract

Background: Campylobacteriosis represents a global public health threat with various socio-economic impacts. Among different *Campylobacter* species, *Campylobacter jejuni* (*C. jejuni*) is considered to be the foremost *Campylobacter* species responsible for most of gastrointestinal-related infections. Although these species are reported to primarily inhabit birds, its high genetic and phenotypic diversity allowed their adaptation to other animal reservoirs and to the environment that may impact on human infection.

Main body: A stringent and consistent surveillance program based on high resolution subtyping is crucial. Recently, different epidemiological investigations have implemented high-throughput sequencing technologies and analytical pipelines for higher resolution subtyping, accurate source attribution, and detection of antimicrobial resistance determinants among these species. In this review, we aim to present a comprehensive overview on the epidemiology, clinical presentation, antibiotic resistance, and transmission dynamics of *Campylobacter*, with specific focus on *C. jejuni*. This review also summarizes recent attempts of applying whole-genome sequencing (WGS) coupled with bioinformatic algorithms to identify and provide deeper insights into evolutionary and epidemiological dynamics of *C. jejuni* precisely along the farm-to-fork continuum.

Conclusion: WGS is a valuable addition to traditional surveillance methods for *Campylobacter*. It enables accurate typing of this pathogen and allows tracking of its transmission sources. It is also advantageous for in silico characterization of antibiotic resistance and virulence determinants, and hence implementation of control measures for containment of infection.

Keywords: Campylobacteriosis, *Campylobacter jejuni*, Whole genome sequencing, Molecular subtyping, Surveillance

Introduction

The *Campylobacter* genus has approximately 57 species and some of these species are of clinical and veterinary relevance [1]. Among these species, thermophilic *Campylobacter* including *Campylobacter jejuni*, are the most common causative agents of campylobacteriosis. Other emerging *Campylobacter* species, such as *C. sputorum*, *C. upsaliensis*, *C. ureolyticus*, *C. lari*, and *C.*

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hyointestinalis also contribute to a wide range of gastrointestinal diseases [2]. *Campylobacter* is a Gram-negative bacillus with a characteristic spiral shape and polar flagella that propel the cells in a corkscrew-like fashion [3]. They have an optimal growth between 37 and 42° C [4]. *C. jejuni* colonizes the gastrointestinal tract of most warm-blooded animals as commensals [5]. Chickens are reported to be one of the main sources of infection to humans. However, recent reports have also highlighted the role of wildlife and the environment (e.g. soil and water) in disease transmission [6]. The prevalence of *Campylobacter* infections is a critical global health concern. The World Health Organization (WHO) declared that *Campylobacter* species are responsible for 96 million cases of enteric infection worldwide [7]. The European Union (EU) tagged campylobacteriosis as the most reported zoonotic infection in 2020, responsible for over 60% of all documented cases [8]. The clinical presentations of *C. jejuni*-mediated infection vary from self-limiting diarrhea and abdominal pain to more serious extraintestinal infections [9].

Several molecular subtyping approaches (i.e., amplicon-based typing, sequence-based typing, and restriction-based typing) have been implemented to investigate the epidemiology of *C. jejuni* [10]. However, systematic surveillance and epidemiological studies of *C. jejuni* are burdensome because of the sporadic nature of *Campylobacter* infections and the low discriminatory resolution of the traditional subtyping methods [10]. Thus, there is a demanding need for developing rapid subtyping methods with higher discrimination to track outbreak-causing lineages, predict antimicrobial resistance, determine accurate source attribution, and identify transmission dynamics. Whole genome sequencing (WGS) is a cutting-edge analytical method that enables reliable identification and characterization of foodborne pathogens. It can be used to tackle the challenges of the traditional molecular subtyping approaches. For example, WGS enabled the representation of global *Campylobacter* isolates and provided new means to detect disease-causing variants and host-related risks [11]. WGS opened new frontiers to explore the epidemiology of *C. jejuni* in populations, its capabilities for host adaptation, and its transmission from animal reservoirs to humans. Moreover, in the light of WGS, antimicrobial resistance (AMR) determinants can be predicted taking into consideration their composite transmission dynamics [12].

In this review, we highlight the impact of campylobacteriosis on human health, transmission of *C. jejuni* from animals to humans, genomic plasticity, and relevant information on epidemiological surveillance and antibiotic resistance. We will compare the performance of traditional techniques with that of whole genome sequencing in addressing different epidemiological questions related to *C. jejuni*.

Impact of *campylobacter* on human health

Compared to other gastrointestinal pathogens, *Campylobacter* is highly infectious with an infective dose of 500 to 800 organisms for *C. jejuni* [13]. Although most of the infections caused by *C. jejuni* to humans are observed as isolated cases, outbreaks can take place [14]. *Campylobacter* infection varies from a self-limiting disease to serious extraintestinal infection. The most frequent clinical symptoms of campylobacteriosis are unspecific including fever, abdominal cramps, general malaise, muscle pain, diarrhea, and acute uncomplicated enterocolitis. Chronic gastrointestinal complications of *Campylobacter* infection include irritable bowel syndrome (IBS), inflammatory bowel disease, functional dyspepsia, and colitis [15]. While the disease is typically mild in healthy adults, severe and extended course of the disease, including bacteriemia, is a potential threat to young children, elderly adults, and immunocompromised patients [16].

Campylobacter infection and post-infection complications are quite rare. Nevertheless, many of these complications have a worse prognosis than the acute disease itself. The intensity of symptoms is thought to be affected by co-infection with another foodborne bacteria [17]. The most important extraintestinal complications are bacteremia, meningitis, hepatitis, endocarditis, and pulmonary infection [9]. The evolution of *Campylobacter* infections to a critical systemic illness, resulting in sepsis and death, is remarkably uncommon with a case-fatality rate of 0.05 in every 1000 infections [18]. Guillain–Barre syndrome (GBS) is a rare autoimmune neurological disorder in which peripheral nerves are demyelinated [19]. *C. jejuni* infection is the most common preceding infection and was reported in about 30% of GBS cases [19]. Specific *C. jejuni* serotypes have been associated with an increased risk of GBS (capsular types HS19, HS2, HS41, HS1/44c, HS4c, HS23/36c) [19]. Not all patients with cross-reactive antibodies develop neurologic manifestations. This can be explained by host determinants of post-*Campylobacter* GBS, particularly human lymphocyte antigen type [20]. The clinical isolate 81–176 of *C. jejuni* may be involved in development of colorectal cancer due to the cytolethal distending toxin [21].

Zoonotic transmission of *Campylobacter jejuni*: one species, different hosts

Campylobacter is primarily a zoonotic disease-causing bacterium [13]. Poultry are the main natural reservoirs, especially for *C. jejuni*, with a cecal content of up to 1×10^8 CFU/g [22]. By contaminating the carcass and surviving processing in slaughterhouses, *C. jejuni* can be

transferred to humans via undercooked chicken meat or through cross-contamination of other foodstuffs at the kitchen [23]. Contaminated water, milk, and dairy products are other sources of infection [24]. In addition, *C. jejuni* and other *Campylobacter* species are frequently found in other animal reservoirs, in up to 90% of cattle, 85% of pigs, and 17.5% of sheep and goats [9]. Cattle isolates are usually clustered in *C. jejuni* clonal complexes (CC) CC-21, CC-45, CC-48, CC-42, CC-61 and CC-206 [25].

Although cross contamination of food represents the most common source to contact *Campylobacter*, different reservoirs of human campylobacteriosis may potentially play a role in the epidemiology and transmission of *Campylobacter*. Other unconventional routes of transmission of *Campylobacter* spp. are surface water, pets, and wild birds [26]. Surface water accounts for a significant number of human cases. Contamination of surface water with wild animal feces and agricultural waste makes water a collection vessel of different *Campylobacter* strains from various hosts [26]. Pets were found to cause considerable number of human cases where the transmission of *Campylobacter* can be bi-directional from owners to pets and vice versa. Pets may acquire infection in parallel with their owners from a common source [26]. While a genomic characterization and profiling of *C. jejuni* showed a partial overlap between isolates from livestock, pets, and clinical cases, isolates from pets showed specific genomic profiles. Thus, pets can be a potential reservoir for *C. jejuni* [27]. Wild birds acquire *C. jejuni* from contaminated water, refuse dumps, and waste from animal farms, pets, and humans [28]. Their body temperatures, in addition to foraging and breeding habits, enable wild birds to be potential reservoirs and spreading routes of *Campylobacter* [28]. Some studies detected antibiotic resistant *C. jejuni* in wild birds in many geographical locations, which poses a concern in urban areas and agricultural farms with increased wild birds population [29]. Other studies showed that food and human *C. jejuni* isolates differed from those of wild birds [30]. Other environmental habitats such as soil are directly or indirectly implicated in human campylobacteriosis [31]. Intriguingly, *Campylobacter* can be also transmitted through flies to chicken flocks and possibly to humans [32].

Although it has strenuous growing conditions in the lab, *C. jejuni* acquired resistance to a plethora of stressors, such as low pH, temperature variability, oxidative stress, osmotic pressure, and antimicrobials [33]. These mechanisms enable *C. jejuni* to survive and transmit between diverse hosts [33]. Tolerance to environmental stressors is frequent among disease-causing lineages and can result in more adapted isolates, with an impact in the general epidemiology of *C. jejuni* [33]. Among the resistance mechanisms to environmental stressors are the transformation into a viable non culturable state (VBNC), biofilm formation, and mutations specific to certain lineages [33, 34]. Some of the host-generalist and host-specific strains of *C. jejuni* were proved to survive in aerobic conditions and under oxidative stress [35].

Mechanisms underlying genomic plasticity and host adaptation

Pan-genome is a term used to describe the entire gene collection identified in a species. The term encompasses two classes: core genome and accessory genome [36]. The core genome represents the set of genes present in every isolate of the species and carries out the necessary cellular functions [36]. The accessory genome constitutes the variable dispensable genome acquired for adaptation and is present only in a few strains or even unique to one strain. Pan-genomic studies highlight the marked variations of bacterial genomes between different genera and species and even between different strains of the same species. These variations can be referred to as genomic plasticity [37].

Almost all bacterial genomes have mosaic structures that are assembled from different DNA segments during evolution and adaptation [38]. The exchange of DNA between bacterial cells occurs via horizontal gene transfer (HGT) either by conjugation, transformation, or transduction [38]. While plasmids and conjugative transposons mediate conjugation, phages that infect bacterial cells mediate transduction [38]. Transformation, on the other hand, occurs when naturally competent bacteria take up extracellular DNA from the environment [38]. The genome of *C. jejuni* is relatively small, however, it is characterized by high variation even at the strain level [31]. *C. jejuni* is naturally competent as it uses a DNA uptake system called type II secretion system to transport foreign extracellular DNA to the cytoplasm [5]. To integrate the homologous DNA into the chromosome, *C. jejuni* uses RecA recombinase, a protein that promotes homologous recombination [5].

A recent study elucidated the role of the chicken gut environment, particularly that of the ceca, in providing suitable conditions for recombination to occur [39]. The results suggested that increased HGT in chicken gut promotes the genetic diversity and hence the adaptability of *C. jejuni* to the constantly challenging gut environment [39]. The exchange of DNA between bacterial cells contributes to bacterial adaptation to a wide range of environmental conditions and to the colonization of multiple niches. Moreover, it plays an essential role in the evolution of antibiotic resistance and bacterial virulence [38]. When comparing the pattern of

genetic variations observed in human pathogenic isolates to that of poultry isolates belonging to the same clonal complex, evidence is further supporting that host-specific mutations develop within certain hosts [5]. Although host specialists are mainly found in only one host species while host generalists are commonly associated with multiple hosts, host specialists have been recently shown to infect more than one definite host [40, 41]. This host adaptation was recently supported by Nennig et al. by implementing different typing schemes based on WGS gene-by-gene approach. They concluded that some *C. jejuni* lineages have clonally expanded and can colonize or infect multiple hosts as they show adaptation to different niches [42]. Remarkably, a recent study has found that host generalist lineages are better equipped to withstand hostile environmental conditions compared to host specialists, but this needs to be further characterized at the molecular level [33]. To provide better insights into the emergence of generalists, Woodcock et al. investigated the role of genomic plasticity in the coexistence of generalist and specialist *Campylobacter* lineages. They concluded that the ecological generalism observed in some *C. jejuni* isolates reflected their genotypic and phenotypic plasticity and resulted in their rapid host adaptation in different host environments [37].

WGS and *Campylobacter jejuni* genomic diversity

As mentioned in the previous section, certain lineages of *C. jejuni* are specific to a particular host species that is related to host adaptation [40–43]. Another interesting example is the recent study conducted by Parker et al. where two strains of *C. jejuni* colonizing guinea pigs were compared with well characterized *Campylobacter* strains [44]. They found that isolates from guinea pigs were of novel sequence type, distinct from other known *Campylobacter* strains, and had genes gain and loss in their genomes. This can further support that genomic divergence occurs as a result of host adaptation mechanisms [44]. This extensive genome variability may play an important role in *C. jejuni* survival and host adaptation [31].

One application for WGS is studying bacterial genomic diversity accrued by animal colonization and human infection [45–48]. Golz et al. identified hybrid strains of *C. jejuni* where extensive gene transfer between the two species interfered with the analysis of species differentiation and multilocus sequence typing (MLST) [49]. These adaptation mechanisms lead to the emergence of host-associated genes or clusters of genes that can be resolved by WGS, which can be of a remarkable use to detect and infer host adaptation mechanisms in *C. jejuni* [50].

Epidemiological surveillance

Systematic surveillance of *C. jejuni* infection is a complex process due to high genomic diversity of the bacteria and interactions between different routes of transmission [31, 43]. In the context of epidemiological surveillance of *C. jejuni,* bacterial subtyping is crucial to differentiate bacteria sharing certain genomic similarities and link them to the same source [51]. The distinction between epidemiologically related incidents and sporadic cases requires high-resolution detection and typing techniques [10]. This is especially important to track both point source and diffuse outbreaks.

Molecular typing schemes

Consistent detection and identification of *C. jejuni* genotypes is challenging due to their high variability. Additionally, traditional culture-based methods fail to detect bacterial variations [52]. They are time-consuming, low throughput, laborious, of low sensitivity, and may yield false negative results if the bacteria are in VBNC state [52]. Without accurate diagnostic tests to detect the presence of *Campylobacter*, precise differentiation and diagnosis of enteric illnesses caused by other bacteria including *Salmonella*, *Shigella*, and *Yersinia* can be challenging [53]. *Campylobacter* species, specifically *C. jejuni*, possess highly changeable physiology, metabolism, and phenotypic diversity. Consequently, traditional detection methods are inadequate, inaccurate, and not sensitive enough [52]. Thus, research is now driven towards devising more accurate, cost- and time-effective detection methods, especially in the food industry where screening is crucial to prevent transmission [54].

Molecular typing schemes have been previously used for *C. jejuni*, including in outbreak investigations, host-association, and population structure studies. Restriction fragment length polymorphism, ribotyping, PCR-based methods, pulsed-field gel electrophoresis, and antigen gene sequence typing (as for *flaA* and *porA* genes) are considered as robust and reproducible genotyping methods in understanding the biology of *C. jejuni*. These typing methods can be implemented for different epidemiological purposes, including for *Campylobacter* subtyping, phylogenetics, identification of outbreak-inducing lineages, and epidemiologic tracking [55]. Nonetheless, the aforementioned subtyping methods have limited discrimination capacity in epidemiological investigations and have several drawbacks such as poor discriminatory power and incompatibility with high throughput applications [55]. MLST has been previously employed over the past decades as the gold standard subtyping method in studying relationships between *Campylobacter spp*. strains, investigating the evolution, population

structure, and the molecular epidemiology of the disease and exploring the potential host reservoirs and host associations [56]. MLST classifies isolates based on polymorphisms present in certain regions of housekeeping genes. Closely related sequence types are grouped under clonal complexes [57]. According to MLST genotyping, strong associations were found between *C. jejuni* host generalists and some clonal complexes (CC) including: ST-21, ST-48, ST-206, and ST-45 [41]. These lineages are causative sources of human diseases [58]. Despite broad geographical distinction between *Campylobacter* species, specific STs are found to be associated with infections in specific countries. For instance, ST-22 and ST-4526 were found in Finland and Japan, respectively, while ST-190 and ST-474 emerged in New Zealand [36]. The comparison of geographically distinctive *Campylobacter* isolates is made possible by molecular typing and WGS. One example is the analysis of *Campylobacter* genomes in UK and North America [59]. The analysis concluded the clustering of these isolates based on variations of highly recombining genes while the isolates were geographically distant [59]. Another study compared *C. jejuni* isolates in Egypt and UK. CC21 isolates from the same country shared more accessory genome genes that were lineage-specific; thus, isolates were geographically clustered [60]. Therefore, biogeographical identification of signatures from *Campylobacter* genomes can help improve campylobacteriosis source attribution and implement reliable intervention strategies.

While MLST is superior to other classic typing methods in studying the population structure for source attribution and the identification of transmission routes in outbreaks, it has several drawbacks [57]. MLST alone may not be sufficient to resolve closely related bacterial strains and, in this case, a specific MLST scheme should be devised [57]. Therefore, new tools and screening techniques were needed for epidemiological surveillance of *C. jejuni* to address the limitations of the classic typing methods.

WGS in the surveillance of *Campylobacter*

WGS technologies are continuously evolving to sequence nucleotides at reasonable speed and low cost. As a result, more and more bacterial genomes are becoming available for analysis and routine surveillance and outbreak tracking are becoming more feasible [61]. Instead of conventional genotyping, which is restricted to only some parts of the genome, WGS provides information on the entire genomic content of isolates. WGS can thus enhance microbial safety surveillance to help control foodborne outbreaks [61]. WGS is characterized by enhanced discriminatory power at the strain level, also enabling the association of specific genotypes with phenotypes that are clinically and epidemiologically relevant [62]. WGS based subtyping demonstrates several advantages over traditional genotyping methods, including in silico prediction of antimicrobial resistance determinants, attribution of transmission sources and routes, and enhanced surveillance of food-borne pathogens [63–65]. Therefore, WGS serves as an effective measure for controlling and preventing foodborne infections [62]. This is especially demonstrated during infectious disease outbreaks where WGS was used for typing [62]. WGS paves the way to characterize the genomic diversity among *Campylobacter* isolates; thus, improving decision making and intervention to control outbreaks [10].

WGS and *C. jejuni* epidemiology

WGS-based subtyping

WGS analysis can be applied in real time to investigate epidemiologically-linked campylobacteriosis cases showing high similarities at the genomic level [66]. Coupling de novo assembly of genomes with a gene-by-gene analysis can expand from the classic MLST scheme to the core genome MLST scheme [11] (cgMLST), based on the analysis of a large number of genes shared by most of the members of a given bacterial group. cgMLST typing is routinely used to align *C. jejuni* genes for the identification of clonal complexes and has greater discriminatory power than conventional MLST, thus aiding in providing better insights into the origin of human campylobacteriosis cases [11].

A study by Cody et al. performed the first real-time genomic epidemiological investigation using a hierarchical whole genome MLST approach [67]. Over 1000 loci were extracted using a BIGSdb Genome Comparator in PubMLST. These loci were compared against 1643 publicly listed loci and complemented with a whole genome MLST analysis. The analysis aimed to identify diversity within the detected clusters to allow for the identification of temporal links between clusters in seemingly epidemiologically unrelated cases [67]. Further support to these findings was a study by Fernandes et al. where comparison of *C. jejuni* isolates against a reference non-related population showed that most of the apparently sporadic cases belonged to a cluster with fewer than 8 allele dissimilarities out of 1577 shared loci [68]. Another study used reference-based core-genome MLST analysis to examine a chicken-associated outbreak in Australia over 1271 loci, and found no more than one allele difference between the clinical isolates [69]. A study on the Walkerton outbreak in Canada indicated that four isolates were related on the clonal level and of limited variation on the genomic level. The isolates were different from one another by 15 single nucleotide variations

and approximately 4 allele differences in a core genome scheme over 732 core loci [70].

WGS based source attribution

Source attribution of zoonotic diseases is defined as the assignment of the human clinical cases of infection to their reservoirs and transmission routes [71]. Different source attribution methods have been developed for foodborne pathogens. These methods can be defined as either top-down or bottom-up approaches. Top–down approaches assign human cases to the sources of infection and aid in predicting the risk of food production animals and other sources for causing infections in humans, advancing intervention strategies and public health in general [71–74]. The data for these methods can be provided by epidemiological methods, microbiological subtyping based methods, or both together [71]. On the other hand, bottom–up approaches predict the number of human cases caused by each source by first analyzing the contamination level and then moving upwards through the transmission chain [71]. The genetic analysis of foodborne pathogens plays a pivotal role in source attribution. In terms of foodborne pathogens, population structure defines the systematic differences in allele and phenotype frequencies in populations and subpopulations of a pathogen [75]. Consequently, probable risk factors and relative contribution of different sources can be determined. While source attribution depends on the accurate estimation of the frequency of different subtypes in each host reservoir, it may be challenging for some organisms such as *Campylobacter* to find specific host associated markers as the population is not properly structured into differentiated clusters [76, 77].

One approach to study population genetics is microbial genotyping of isolates from both human cases and possible sources in the food chain [77]. This approach depends on the bacteria being adapted to different hosts or ecological niches which leads to uneven distribution of sequence subtypes among host reservoirs [77]. These genomic signatures would help to understand *C. jejuni* evolution and track sources of human infection [76]. For proper source attribution, a typing method should be standard and valid to help reliable knowledge transfer among laboratories working on the analysis [71]. Moreover, the method should also be automated with a reference data set allowing for the establishment of nomenclature within the microbial species [71].

To apply WGS in source attribution, there have been several successful attempts to develop algorithms that provide optimal discriminatory power and proper modeling [73]. Recently, allelic variation has been analyzed using 15 host-segregating marker loci (including seven core genes, seven soft-core genes, and one accessory gene) derived from the pan-genome of *C. jejuni* reference strains [73]. These loci have been used in source attribution analysis as they retain high accuracy of attribution even between host specialist and generalist genotypes [73, 78, 79]. Six of the host-segregating loci encode hypothetical proteins and the remaining loci are involved in metabolic activities, signal transduction, protein modification, and stress response [73]. This typing method was reported to be of higher accuracy and segregation power than MLST [78]. It is advisable to use more than one molecular typing method for the investigation of *Campylobacter* populations [80].

Source attribution based on microbial subtyping can be classified according to the computational modeling used. The model-based molecular attribution can be applied to assess interventions used to halt disease transmission from farms to retail outlets to final human consumption (farm-to-fork) [74]. The differences in genotype frequency between various populations enables probabilistic assignment of isolates to populations [77]. Models can be frequency-matching models or population genetics models [71]. In frequency-matching models, subtype frequencies are compared and weighted assuming that subtypes are stable from their sources [71]. The population genetics models are probabilistic, and the parameters are assumed to be unknown [71]. The comparison of the genomic data available for strains may infer the link between strains from human and different sources. Examples of current population genetics models available are the STRUCTURE model and the Asymmetric Island Model [71]. STRUCTURE is a model-based clustering method designed to infer population structure and assign individuals to populations using genotype data [81]. STRUCTURE estimates genotype frequencies in each host species based on all the isolates. It estimates the population of origin for isolates of unknown origin [81]. The principle of this model is to estimate the allelic frequencies in different populations and their admixtures using Bayesian approach [81]. Tracing the sources of human cases is a use case of this model without admixture of the source strains. The strains should belong only to one of each population and each population should be of a specific source [71].

Antimicrobial resistance in Campylobacter

Although campylobacteriosis is typically self-limiting, with a short-duration, and rarely requires antimicrobial therapy, high-risk patients may receive an early antibiotic intervention to avoid serious complications [82]. Macrolides are the antibiotics of choice when treating *C. jejuni* infections and fluoroquinolones are used as an alternative therapy [83]. Tetracyclines are another alternative treatment for campylobacteriosis but not

commonly used in clinical practice. Severe systemic campylobacteriosis may be treated with intravenous aminoglycosides [84]. *C. jejuni* has intrinsic resistance to a wide range of antibiotics including penicillin, most of the cephalosporins, vancomycin, cotrimoxazole, and rifampicin [83]. Moreover, a growing number of *Campylobacter* strains are developing resistance against quinolones and macrolides which are critically valuable antimicrobials in managing human infections [83]. In the past decades, the rise of antimicrobial resistance has become a significant global concern in both developed and developing countries. Resistance to antimicrobials is acquired and are mainly disseminated among *Campylobacter* strains via HGT and mutation-based mechanisms. Antibiotic-resistant strains are capable of modifying the antibiotic target sites, reducing cellular permeability to antibiotics, or hydrolyzing or effluxing antibiotic compounds [83].

Role of animals and food in transmission of antimicrobial resistance

The role of animals in the spread and transmission of AMR in humans is evident by studies that correlated the emergence and clonal expansion of resistant *C. jejuni* strains with the dissemination of resistance genes among various lineages as revealed by the association between different clones and antimicrobial resistance [85–88]. One of the main sources of AMR transmission from animal to human is the use of antibiotics in agriculture and veterinary fields. How the antibiotics are selected for use in these fields depends on the animal species itself, whether farming is commercial or domestic, and the availability of the antimicrobials under strict legalization work frame [89]. Multiple studies have detected AMR in *C. jejuni* not only in broiler products but also in livestock animals in different geographical locations suggesting their role as a probable source of clinically relevant antimicrobial-resistant *Campylobacter* spp. [90–92]. In an attempt to investigate the AMR genes transfer between bacterial isolates, a study explored the genomic determinants of AMR in *C. jejuni* isolated from humans, livestock, and sewage [93]. The results indicated the spread of some AMR determinants between *Campylobacter* species and the niches from which they are isolated. These study findings were in agreement with the results of resistome analysis obtained by Cobo-Díaz et al. [94]. A total of 39,798 publicly available *Campylobacter jejuni* genomes were studied, focusing on their sequence types and resistome profiles. These studies highlighted the association between the use of antimicrobial agents in veterinary settings, particularly poultry production, and the subsequent spread of AMR genes between *Campylobacter* isolates residing in humans, animals, and environment.

AMR and WGS

WGS analyses have served as a powerful tool for the accurate characterization and prediction of AMR within members of the *Campylobacter* genus [95–97]. In *Campylobacter*, antimicrobial resistance develops from either spontaneous mutations, acquisition of AMR genes, or both [83]. WGS is successfully applied to detect putative gene mutations that result in resistant phenotypes. It can also detect acquisition of DNA sequences associated with antibiotic resistance. The prediction can be further improved by verifying resistance markers and constructing a reliable pipeline. Several databases are available to detect AMR genes based on WGS technology such as ResFinder [98], Resfams [99], ARG-ANNOT [100], CARD [101], or NCBI AMRFinder [102]. Jointly with comparative genomic studies, the data obtained can unravel much about the unknown mechanisms of resistance and the role animals play in disseminating resistant strains in humans [95, 103].

Genome wide association studies and Campylobacter

Genome-wide association studies (GWAS) are increasingly being implemented in microbial genomics to statistically associate genetic elements with particular phenotypes [104]. With the cost effective availability of WGS, GWAS can be performed to identify the genetic components of any measurable heritable phenotype in a hypothesis-free manner [75]. Microbial GWAS analysis could reveal genes and mutations that are linked to antibiotic resistance, virulence, and host tropism [75]. GWAS is an example of a top-down approach because the genomic content of test and control groups is compared and analyzed to identify genetic variation that is associated with a specific trait. Bacteria are characterized by unique population genetics that impose challenges in applying microbial GWAS analyses [75]. Among these challenges are the genetic content and its high diversity. Early GWAS depended on expensive genotyping chips with known DNA probes which became obsolete by time due to the plasticity of bacterial genomes. WGS is a cheaper and more comprehensive for production of full sequences fast and in high throughput [105].

Microbial GWAS analyses are divided into either phylogeny-based, non phylogeny-based, or can be a combination of both. Machine learning predictive models can also be applied [105]. GWAS that were applied to identify SNPs and k- mers in microbial genomes have identified mutations and genes associated with antibiotic resistance, cancer, virulence and host preference [75]. Among the tools used for microbial GWAS are Scoary, TreeWAS,

bugwas, and PySEER [106–109]. *C. jejuni* population lineages are clustered into clonal complexes that share genetic elements. Not all of these genetic elements are correlated to particular phenotypes as some elements are passed through clonal descent and not associated with the phenotype of interest [104]. GWAS analysis of *C. jejuni* revealed the association of the *cj1377c* gene with survival where protein expressed by cj1377c gene is involved in *C. jejuni* respiration and formate metabolism [104]. Another study showed that the gain and loss of the *pan*BCD genes, encoding the vitamin B5 biosynthesis pathway, is associated with rapid host adaptation. On one hand, vitamin B5 is present in cereals and grains, which are part of the chicken diet. On the other hand, it is found in a very low concentration in grasses on which cattle feed. The *pan*BCD genes were found almost globally in cattle isolates as *Campylobacter* needs to produce the vitamin to persist in cattle. Thus, host generalism in *Campylobacter* lineages linked to agricultural niches is probable as *pan*BCD genes persist in some isolates in chickens [110]. GWAS on *C.jejuni* isolates distinguished 28 genes that are significantly associated with highly prevalent and clinically related *C. jejuni* subtypes. Those genes are associated with iron acquisition, vitamin B5 biosynthesis, catalysis, and transport [111]. WGS together with GWAS could reveal novel source attribution markers that differentiated *C. jejuni* isolates from UK and France [74]. GWAS helped determining marker genes, where the absence/presence or mutations were associated with the adaptation of certain lineages of *C. jejuni* to specific host niches [112].

Conclusion

The review summaries the WGS applications in the post genomic era to understand *C. jejuni* adaptation, antimicrobial resistance determinants, and transmission dynamics along the farm-to-fork continuum. The increasing use of WGS for epidemiological purposes can contribute to improve current surveillance programs. WGS provides high discriminatory resolution in comparison with traditional subtyping methods and will gradually replace these methods in surveillance studies. It should allow a more accurate identification of possible case clusters and resistome patterns to control and prevent more cases of campylobacteriosis. WGS can drive "One Health" epidemiological investigations by providing an unprecedented level of data that can be used to describe emerging trends. It can guide the establishment of links between animal and human health and the environment and clarify the direct or indirect role of *Campylobacter* ecology in its transmission to humans.

Abbreviations

AMR: Antimicrobial resistance; BIGSdb: Bacterial Isolate Genome Sequence Database (BIGSdb); CC: Clonal complex; C. jejuni: Campylobacter jejuni; cgMLST: Core genome multi-locus sequence typing; EU: European Union; GBS: Guillain–Barre syndrome; GWAS: Genome-wide association studies; HGT: Horizontal gene transfer; IBS: Irritable bowel syndrome; MFS: Miller Fisher Syndrome; MLST: Multi-locus sequence typing; PCR: Polymerase chain reaction; QRDR: Quinolone resistance determining region; RPP: Ribosomal protection proteins; ST: Sequence type; VBNC: Viable non culturable state; WGS: Whole genome sequencing; WHO: World Health Organization.

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