

Use of whole-genome sequencing in the epidemiology of *Campylobacter jejuni* infections: state-of-knowledge

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ABSTRACT

High-throughput whole-genome sequencing (WGS) is a revolutionary tool in public health microbiology and is gradually substituting classical typing methods in surveillance of infectious diseases. In combination with epidemiological methods, WGS is able to identify both sources and transmission-pathways during disease outbreak investigations. This review provides the current state of knowledge on the application of WGS in the epidemiology of *Campylobacter jejuni*, the leading cause of bacterial gastroenteritis in the European Union. We describe how WGS has improved surveillance and outbreak detection of *C. jejuni* infections and how WGS has increased our understanding of the evolutionary and epidemiological dynamics of this pathogen. However, the full implementation of this methodology in real-time is still hampered by a few hurdles. The limited insight into the genetic diversity of different lineages of *C. jejuni* impedes the validity of assumed genetic relationships. Furthermore, efforts are needed to reach a consensus on which analytic pipeline to use and how to define the strains cut-off value for epidemiological association while taking the needs and realities of public health microbiology in consideration. Even so, we claim that ample evidence is available to support the benefit of integrating WGS in the monitoring of *C. jejuni* infections and outbreak investigations.

Abbreviations

CC: clonal complex

cgMLST: core-genome MLST

DALY: Disability-adjusted life year

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

Epi-linked: epidemiologically linked

GWAS: genome-wide association study

MALDI-TOF: Matrix Associated Laser Desorption Ionization–Time of Flight

MLST: multilocus sequence typing

NGS: Next generation sequencing

SNPs: single nucleotide polymorphisms

SNV: single nucleotide variant

ST: Sequence type

PFGE: Pulsed-field gel electrophoresis

wgMLST: whole-genome MLST

WGS: Whole-genome sequencing

INTRODUCTION

The ability to conduct epidemiological investigations and intervene to control and prevent food- and environmentally transmitted diseases is a major task of public health authorities. In this regard, identifying epidemiologically (epi)-linked cases and differentiating them from concurrent, sporadic incidences are essential in risk assessment, outbreak investigations, and source attribution. To do so, traditional epidemiological investigations is concurrently used with molecular subtyping of the etiological agent, and no other method offers a higher degree of resolution than whole-genome sequencing (WGS). The recent development of high throughput sequencing technologies for WGS (next-generation sequencing, NGS) has prompted large-scale detailed studies of entire pathogen genomes, replacing the need for traditional typing methods [e.g. pulsed-field gel electrophoresis (PFGE), serotyping, biotyping] and sequence-based investigations (e.g. resistance or virulence gene detection). Moreover, the declining costs of NGS and availability of bench-top analyzers facilitate the application of WGS in routine surveillance and outbreak investigations of bacterial and viral infectious disease by public health authorities (1). As a result, WGS analysis is currently used in real-time surveillance of *Listeria monocytogenes* and *Salmonella* Enteritidis by the American Centers for Disease Control and Prevention and the U.S Food and Drug Administration (<http://www.fda.gov/Food>) and such approaches for other foodborne pathogens are expected to come in use shortly. Furthermore, both the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have stated that WGS should be the analysis method of choice in public health activities (2,3).

Campylobacter jejuni is the one of the most frequent causes of bacterial gastroenteritis globally, with an estimated burden of illness of 7.5 Disability-adjusted life year (DALY) (4,5). The epidemiology of *C. jejuni* is only partially understood and shedding new light on this area is difficult as most cases appear to be sporadic and remain unreported (6). Additionally, the occurrence of immune individuals in the population coupled with the many possible reservoirs, transmission pathways, and regional epidemiological differences makes source tracing and risk assessment difficult (7). Therefore, more knowledge on these matters is needed to curb the current campylobacteriosis epidemic. Until now, the two most common molecular typing methods [PFGE and the seven-loci multilocus sequence typing (MLST) scheme], have been irreplaceable working tools in studying the epidemiology of *C. jejuni* infections, and have greatly contributed to the current knowledge on the nature of *C. jejuni* infections in human patients and their potential reservoirs (8–19). However, limitations of these methods has been uncovered which deem PFGE and MLST unsuitable as sole subtyping methods for assessing epidemiological links between *C. jejuni* isolates (20–22). Therefore, developing an efficient “second generation” molecular-guided epidemiological surveillance is clearly necessary for *C. jejuni* and of high priority to overcome the limitations of available typing methods (23). As international standardization is needed due to the global and regional nature of campylobacteriosis, full comparability of molecular data of strains collected from different areas must be possible. Great hopes have been placed on WGS approaches in these regards, as WGS offers a high discriminatory power that allows detection of all possible epidemiologically significant variation between strains (23–25) and the global and regional standardization and comparison achievable through WGS, which today is facilitated by public databases (26,27).

In this review we show how WGS has improved, or has the capacity to improve, surveillance and outbreak detection of *C. jejuni* infections and discuss how WGS has increased our understanding of both the evolutionary and epidemiological dynamics of campylobacteriosis caused by *C. jejuni*.

METHODS

A scientific literature review was carried out by searching the Google Scholar database (scholar.google.com), using the qualifiers ‘Whole-genome sequencing AND *Campylobacter jejuni*’, and ‘epidemiology OR ecology’. Search was done in August-September 2016 resulting in a list of 2,640 publications. Inclusion criteria were based on title and abstract, leading, if related to the use of WGS in epidemiological investigation of campylobacteriosis, to retrieval and analysis of the full paper. Non-peer-reviewed studies and studies focused solely on functional genomics were not included. In addition, the authors integrated their own knowledge and opinions with the scientific literature review.

SUBTYPING OF *C. JEJUNI* IN OUTBREAKS AND SURVEILLANCE; ADAPTING WGS TO THE MOLECULAR EPIDEMIOLOGY OF CAMPYLOBACTERIOSIS

An outbreak is the sudden increase in the number of expected cases of a disease in a population in a limited geographical area over a short period of time. Outbreak detection depends on knowledge of the baseline level of that disease, which is usually acquired through surveillance (28). Additionally, through data analysis and isolate collection from monitoring programs it is possible to detect so-called diffuse outbreaks, i.e. clusters of cases with a presumptive common source not necessarily clustered geographically and/or represented by an increase in cases above the expected baseline level (29). To be effective, surveillance depends on correct reporting of communicable diseases, and the EU has therefore provided a case definition and clinical and laboratory criteria for diagnosis of *C. jejuni* infections to secure and unify reporting of campylobacteriosis (30).

Upon outbreak detection, epidemiologists and microbiologists work together to trace the source and include or exclude patients as part of a given outbreak by combining traditional epidemiological tools and the analysis of samples collected from sources and patients. From a microbiologists’ point of view, isolation of the causative pathogen with subsequent subtyping of that agent is critical. As mentioned above, one of the subtyping method most commonly used in campylobacteriosis outbreaks investigations has been PFGE (16,17,19,31). PFGE is highly discriminatory when used with certain restriction enzymes (31), and the method of Tenover *et al.* (1995) is commonly applied to decide whether two isolates are related or not (32). However, PFGE can both over- and underestimate the clonal relationship between *C. jejuni* strains (20).

Additionally, MLST has been the essential subtyping method for longitudinal analysis of *C. jejuni* isolates acquired through monitoring programs (8,9,11–15), but lacks the necessary resolution for cluster analysis as extensive genomic mosaicism between highly related strains has been demonstrated (33).

WGS is currently starting to take over as the typing method of choice for *C. jejuni* outbreaks investigation, and has the potential to substitute MLST in routine surveillance of

campylobacteriosis. However, several issues regarding the epidemiology and genomic diversity need to be addressed before WGS can be a useful and reliable working tool in the public health sector. In particular, understanding baseline ecological diversity and population structure of *C. jejuni* and the genomic diversity within a single human infection is a necessary prerequisite for determining if isolates are clonal, as would be expected in a cluster of cases or outbreak with a common source. Moreover, the resolution depth of WGS should be adjusted to balance the power to resolve isolates from the main *C. jejuni* lineages while maintaining clonal stability between epidemiologically linked strains from outbreaks and source and patient. The latter might include creating and agreeing on a suitable nomenclature system for *C. jejuni*.

Here we review how laboratory diagnosis and surveillance of *C. jejuni* infections (species determination, subtyping and the definition of clonal isolates) are currently performed and how the implementation of WGS could change, or have already changed, this field of public health.

Species determination

Determining the causative agent present in a sample of stool or food is crucial in making effective clinical decisions and public health interventions. Today, species determination is accomplished by culturing the pathogen on selective or non-selective media, identifying suspicious colonies, and producing a mass of pure organism for further use. For *C. jejuni*, growth on selective media containing antibiotics and blood or charcoal antioxidants in a microaerophilic atmosphere is the most common choice, and was instrumental in establishing *C. jejuni* as a major cause of human gastroenteritis in the late 1970s and early 1980s (34,35). Thereafter, colony morphology, Gram stain, motility-, oxidase- and hippurate hydrolysis tests aid to identify *C. jejuni* (36,37). Molecular methods, such as genus-specific PCRs detecting the *hipO*-gene (38) or sequencing the 16sRNA, *groEL* (39) or the *rpoB* (40) genes have been used to differentiate between *Campylobacter* species. The entire process can take approximately one to two weeks, which is a considerable amount of time when immediate intervention or treatment is needed.

The recent introduction of Matrix Associated Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry for rapid and cost effective species determination of bacterial pathogens (41,42) has considerably shortened the time needed for identification of *Campylobacter* species, especially in clinical settings. The method allows precise identification [99.4% accuracy for *Campylobacter jejuni* (42)] of bacterial colonies collected directly from the primary isolation plate. As MALDI-TOF mass spectrometry is faster and more accurate than standard methods, it will probably remain the reference methodology in the future, regardless of the WGS revolution.

Although the use of WGS would increase the confidence of species delineation in most cases, current detection methodologies for *Campylobacter*, coupled with MALDI-TOF, are generally cheap and fast enough to satisfy the need for both clinical diagnostic and surveillance. In this scenario, implementation of WGS for species determination would advance the ability of the clinical laboratory in performing diagnosis when culture-independent methods are needed (e.g. in case of bacteremia, septicemia and meningitis), which are rare in the case of campylobacteriosis. The real advantage of implementing WGS in clinical and public health setting, for what concern *Campylobacter*, is the provision of high-resolution genotyping (including predicting phenotyping)

in a faster and, most importantly, automatized way (25). In this contest, species confirmation could function as the first quality assurance step in the analytic pipeline, by using for instance methods especially designed for metagenomics datasets (43) or Average Nucleotide Identity (44).

Genomic diversity during *C. jejuni* infection and colonization

The genomic variants that accumulate during human passage should be considered when establishing the level of genetic similarity between epidemiologically linked isolates. The generation of diversity in this population is dependent on the mutation rate and mutational patterns of *C. jejuni*. The fixation of such mutations in the population is again determined by evolutionary processes like genetic drift, bottleneck effect, and selection due to for instance host adaptation (45). WGS has been applied to study the genomic diversity and changes accumulating during human infection (46,47) and animal colonization (48,49), of which the latter has been used both as a model organism for human infection [mice; (48,49)] and a reservoir with source attribution [chicken; (49)] in mind. Revez *et al.* (2013) published the first study in which the inoculum isolate (*C. jejuni* NCTC 11168) was subjected to WGS comparison with the excreted isolate-population after human passage (46). The authors demonstrated that the genomic changes occurring during human passage were small and, except for one single nucleotide variant (SNV) in Cj0184c, limited to indels in homopolymeric tracts in contingency loci. These frameshift mutations were generally found in genes regulating surface structures. Bayliss *et al.* (2012) showed that such mutations accumulated rapidly in the population even in the absence of selective pressure, demonstrating mutation rates between ten to hundred times faster than mutation rates in other parts of the genome (45). The same regulatory mechanisms in contingency loci were confirmed in another human-passage study by Thomas *et al.* (2014) (47). Furthermore, the genetic changes observed in *C. jejuni* strains subjected to passage through C57BL/6 IL-10^{-/-} mice and IL-10 KO mice were of similar nature: indels in homopolymeric tracts in contingency loci (48,49). However, different loci were affected in mice and chicken relative to humans (48,49), indicating possible differences in adaptation mechanisms in animal and human hosts. Thus, care should be taken when extrapolating results from animal colonization experiments to humans. Summarizing, a growing body of evidence points out that phase variation is the major genetic regulatory mechanism during human and animal single passage, enabling *C. jejuni* rapid access to a significant amount of genetic diversity influencing host adaptation, virulence and immune evasion by rapid changes in surface structures (45,50).

From an epidemiological standpoint, we propose to exclude such homopolymeric tracts from the WGS comparison in public health operations to minimize the bias introduced in the genetic diversity of the *C. jejuni* strain by the patient itself (20,51–53). In this way, tracing and source attribution of isolates would be independent from genomic changes introduced in the host, and the genomes would more closely resemble that the inoculum isolates.

Application of WGS of *C. jejuni* in point-source outbreak investigations

Several studies aim to investigate the applicability of WGS analysis in identifying clonal *C. jejuni* isolates in point-source outbreaks and the retrospective examination of food-, water- and milk-borne outbreaks of campylobacteriosis has gained a lot of research attention. Revez *et al.* (2014) analyzed a resolved milk-borne outbreak of the ST-50 lineage [ST-21 clonal complex (CC)], in which three

SNVs and phase variations in 12 loci were identified between the milk- and the patients-isolates by PubMLST-hosted wgMLST-schema featuring 1738 loci (51). The non-related control strains included in the analyses differed by 420-454 alleles from the epidemiologically related strains. In a second study, Revez *et al* (2014) redefined a waterborne outbreak by proving that one of the two investigated cases was a separate, non-related sporadic case; the *C. jejuni* isolated from this patient was deemed too genetically different (69 SNVs) from the assumed source-isolate, while the isolate of the other case differed by only three SNVs compared with the source-isolate (20). In this study, the analysis of homopolymeric tracts was excluded and what was perceived to be homologous recombination was counted as one allele difference (20). Zhang *et al.* (2015) confirmed the findings of Revez *et al.* (2014a,b) by identifying up to seven allele differences, mainly in homopolymeric runs, between clonal isolates collected from three waterborne outbreaks by the use of an *ad hoc* reference based gene-by-gene approach (Genome profiler; <https://sourceforge.net/projects/genomeprofiler/>)(54).

Lahti *et al.* (2016) investigated a foodborne outbreak using WGS in parallel with PFGE and applied a reference-based core genome (cg)-MLST approach using SeqSphere+™ (Ridom© GmbH) to compare the outbreak strains (total of 1271 shared-loci) (55). The authors described a maximum of one allele difference between the clinical isolates. Contrastingly, a *C. jejuni* isolate originating from the same farm as the presumed source (chicken liver paté), but collected 5 months prior to the outbreak, differed by 15 alleles from the patient isolates (55). Furthermore, an Australian study investigated a foodborne outbreak in real-time using WGS and SNVs detection using Snippy v. 3.0 (<https://github.com/tseemann/snippy>) (56). The study proved the circulation of at least two different STs among patients, forming two distinct clusters. The ST-528 cluster showed SNVs in the range from three to eight, while the ST-535 cluster exhibited 30 SNVs. Moreover, the authors concluded that the source isolate was not “caught” as the *C. jejuni* collected from the suspected source was of a different ST and differed from the case isolates by at least 22,940 SNVs (56). Nevertheless, these results should be interpreted with care as the authors operated with very low coverage (minimum coverage of 10x), they didn’t indicate the overall number of compared nucleotides, and avoid to specify how recombination and homopolymeric tracts were handled (56).

Application of WGS in surveillance of C. jejuni infections and detection of diffuse outbreaks

The occurrence of diffuse outbreaks, meaning individual cases occurring over a larger area linked to a single vehicle of infection (29), is difficult to detect through routine surveillance, as these cases are masked among the background sporadic cases. However, earlier studies have verified the existence of diffuse outbreaks of campylobacteriosis by demonstrating the presence of temporal, spatial, and genotypic clusters among apparently sporadic cases. Such clusters were two to five times more common than point-source outbreaks in Scotland (29), and accounted for approximately 13% of the reported cases. Similarly, up to 19.6% of the annual campylobacteriosis cases in Norway occurred in spatial-temporal clusters (57). Furthermore, an Alaskan study estimated 32% of the campylobacteriosis cases collected during a decade to be temporal clusters of related PFGE profiles (58). It follows that more emphasis should be put on the detection of such outbreaks, as interventions against the mechanisms creating campylobacteriosis-clusters would be more effective at reducing the burden of illness than actions taken against sporadic transmissions. High-resolution genotyping can improve the detection of clusters of epidemiologically related cases occurring in a

less geographically separated group (48) and since WGS offers the highest possible genotyping discrimination, the integration of genome sequencing in surveillance would probably facilitate the implementation of more effective intervention strategies targeted at campylobacteriosis.

Some studies have already integrated genomics in *Campylobacter* surveillance, and all have applied a wgMLST methodology. Cody *et al.* (2013) performed the first real-time genomic epidemiological investigation of *C. jejuni* collected through a surveillance program using a hierarchical wgMLST approach (26,59). In the first all-against-all analysis, BISGdb Genome Comparator, implemented in PubMLST (<http://pubmlst.org/>), was used to extract the 1026 loci shared among the 379 investigated isolates obtained from the Oxfordshire monitoring program from a total of 1,643 loci listed in the “Gundogdu” schema (<http://pubmlst.org/campylobacter>), which was established on the re-annotation of *C. jejuni* NCTC 11168 (60). Based on the level of genomic diversity found between isolates collected from a single patient, a cut-off value for investigating the presence of possible clusters was set to 20 allele differences among the 1026 shared-loci (59). Thereafter, an *ad hoc* wgMLST analysis, again using the “Gundogdu schema”, was used to define diversity within the discovered clusters. With this approach, the authors were able to identify temporally associated clusters of strains from cases with apparently no epidemiological links but showing similar genetic diversity as observed between same-patient isolates (3 to 14 loci differences among 1,478 to 1,586 loci shared loci) (59). As observed in point-source outbreaks (20,51,54), the loci that recurrently resulted in differentiation between same-patient and epi-linked isolates were homopolymeric tracts in contingency genes. Similar results were reported by Kovanen *et al.* (2014), who also used a hierarchical wgMLST approach to investigate the genomic relationship of apparently sporadic cases collected during a seasonal peak in Finland (52). First, the authors clustered the isolates based on the 7-loci MLST scheme and performed an *ad hoc* wgMLST analysis using BIGSdb to extract allele information of all 1738 loci for each ST-group. This approach allowed the authors to identify sub-clusters of isolates, which varied only in a few loci, obtained from cases spread over a large geographical area lacking an apparent epidemiological link (52). In their following study, Kovanen *et al.* (2016) attempted to identify the possible source of these predicted diffuse outbreaks, again applying a hierarchical wgMLST approach for each ST group, using the references based gene-by-gene method implemented Genome Profiler (53). Thereafter, the authors manually screened for allele differences, using five SNVs as a cut-off value, while excluding indels in homopolymeric tracts, to define clusters of genetically indistinguishable isolates. The rationale for the low cut-off value was the observed genomic diversity between chicken isolates collected from the same farm during one rearing cycle (61), within point-source outbreaks (20,51,54) and during a human infection (46). When accounting for temporal clustering, the authors were able to link up to 24% of the human cases to specific chicken slaughter batches (53). To identify the possible existence of a diffuse outbreak, Fernandes *et al.* (2015) applied the method introduced by Cody *et al.* (2013) to compared *C. jejuni* isolates collected through a surveillance program with non-related isolates (reference population) of the same ST-21 (62). They showed that 20 of the 23 apparently sporadic cases were indeed part of a cluster, with less than eight (with a mean of four) allele differences across 1577 shared-loci. Simultaneously, the authors demonstrated that the genetic distance between the cluster isolates and the reference population were at least 20 alleles, and proposed faulty pasteurized milk distributed over the investigated catchment area as the source of the clustered cases (62).

The results from these studies support the idea that putative diffuse outbreaks have a clear impact on the epidemiology of campylobacteriosis, and by integrating WGS in the surveillance of *C. jejuni* infections we can potentially distinguish between clustered and sporadic cases. The common belief is that the gene-by-gene method is more suitable for this task than an SNV-calling approach since the gene-by-gene approach is efficient, easy to automate and computationally less intensive. Although a consensus on the analytic pipeline (experimental and bioinformatical) to use in the gene-by-gene approach and the strain definition cut-off to apply are still under evaluation, it seems clear that the hierarchical wgMLST-pipeline, where a first level genomic clustering based on a core set of loci is performed, followed by an *ad hoc* analysis with an increased number of loci, will be the future reference approach for these types of investigations (59).

DEFINING THE BASELINE GENOMIC DIVERSITY FOR *C. JEJUNI*

Deciding the genomic diversity present in a population of *C. jejuni* is necessary for deducing whether genomic differences between any two *C. jejuni* isolates are sufficiently small to assume epidemiological relatedness, i.e. the isolates share a common source (3). As seen above, earlier studies have utilized a fairly arbitrary impromptu value for defining epidemiological relationships and genetic associations between strains to detect diffuse outbreaks and trace isolates to possible vehicles and reservoirs. However, the diversity level present within the lineage of which the investigated isolates reside is rarely discussed. Yet, defining such diversity is a necessary prerequisite: if the lineage diversity is generally low, the genetic distinction between two non-linked isolates might fall below the preset cut-off value even when no epidemiological link exists. Evidence proving variation in genetic diversity between *C. jejuni* lineages has recently arisen. For instance, Kovanen *et al.* (2014) found limited genetic diversity within three common STs, namely ST-230, ST-267 and ST-677, while ST-45 were considerably more diverse and separated into three main lineages (52). In a follow-up paper, Llarena *et al.* (2016) investigated the nature of the population structure of ST-45 CC, focusing on identifying possible spatial-temporal evolution signals (21). The authors found that the occurrence and strength of the geographical signal varied between sub-lineages of ST-45 CC strains. Yet, no evidence of a temporal signal was found. In addition, the authors unexpectedly identified certain sub-lineages of ST-45 with extremely similar genomes regardless of time and location of sampling. These successful monomorphic clones were persistently isolated from animal hosts and human patients over a decade from several countries around the globe (21). There is no reason to believe that this clonal nature is limited to ST-45. Indeed, in a recent publication Wu *et al.* (2016) demonstrated the clonal expansion of a highly virulent, ST-8 lineage during the 1970s, which is now causing vast numbers of ovine abortions in the USA (63).

The presence of clonal populations makes genomic distinction between epidemiologically associated and non-related isolates difficult, and seriously hampers the implementation of WGS methodology in surveillance and outbreak investigations for public health purposes. For example, a cluster of ST-45 isolates identified as a diffuse *C. jejuni* outbreak by Kovanen *et al.* (2014) (52) consisted of isolates belonging to a ST-45 population of monomorphic clones (21). Thus, it is uncertain whether these isolates were indeed representatives of a diffuse outbreak, based only on

WGS analysis. Clearly, more studies on the genomic diversity and possible occurrence of clonal populations in other *C. jejuni* lineages are needed to resolve this predicament.

WGS IN *C. JEJUNI* SOURCE ATTRIBUTION AND HOST ADAPTION

Identification of the most frequent transmission routes and foodborne sources of campylobacteriosis is of utmost importance for prioritizing food safety interventions and setting public health goals (64). *C. jejuni* has a complex epidemiology and transmission can occur in numerous ways, including contaminated food, water, and raw milk, and direct animal- and environmental contact. To overcome this problem, methods to quantify the relationship between human patient data and possible infection reservoirs, transmission routes, and risk factors have been developed (65,66). Approaches based on genotypic methods compare the proportion of *C. jejuni* subtypes in different sources and reservoirs with isolates subtypes collected from human patients. These methods rely on different subtypes having variable ability to colonize different hosts or that existing ecological restrictions prevent an equal subtype distribution between reservoirs; often referred to as host adaption (67). Adaptation to a specific host might result in the selection of reservoir-associated traits, such as the presence of specific genes or clusters of genes (68). Therefore, detection of these traits in *C. jejuni* isolates from human patients could theoretically make source attribution simple and straightforward. Although source attribution models using WGS data as input are yet to be applied to campylobacteriosis cases, phylogenic and population structure analyses of *C. jejuni* isolates collected from different studies have been used to trace the source of sporadic cases, as described above (53). On the other hand, major efforts have been devoted to detect and elucidate the mechanisms of host-adaptation in *C. jejuni* with the use of WGS, especially for identifying source-specific traits (67,69–71). Using 7-loci MLST, Sheppard *et al.* (2011) argued that certain lineages of *C. jejuni* were adapted to wild birds and agricultural animals while other lineages were generalists lacking host adaption (72), and confirmed these findings and attempted to detect the underlying mechanisms of host adaption and generalist lifestyles by WGS and genome-wide association study (GWAS) of *C. jejuni* isolates collected from cattle and chickens (67). The search for host-specific traits within generalist lineages identified a predicted bovine-specific seven-gene region encoding for vitamin B₅ biosynthesis, and suggested that the overrepresentation of these seven genes in bovines denoted a bacterial adaption to the low vitamin B₅-containing grass-based bovine-diet. However, the authors pointed out that even though the gene cluster seemed to be necessary in bovines, the fitness costs of such a cluster must have been low as chicken isolates of generalist lineages frequently harboured the same gene region, possibly as a result of a recent host switch from bovines (67). Morley *et al.* (2015) went further in this direction by investigating the molecular mechanisms of host-adaption by analysing one porcine-restricted lineage (ST-403 CC). The authors found three restriction-modification loci unique to this lineage and showed that a number of coding sequences present in other lineages were absent. Moreover, the authors showed that ST-403 CC underwent lineage specific decay and pseudogenization, and they argued that these events were the result of the selective pressure enforced by moving away from avian to porcine host, reflecting the ability of ST-403 CC to differentiate according to niche (71).

The discovery of host-specific loci or gene loss makes these genetic elements possible targets for source tracking. Hence, the genetic make-up of a human isolate could theoretically help determine the source of that isolate. However, we are still far from that possibility, as only a few host-

associated loci have been found in *C. jejuni* and the interpretation of their presence is complicated. Additionally, the commonness of generalist-lineages complicates source attribution because of lacking host adaption and signals, probably due to frequent host-jumps (70). Dearlove *et al.* (2015) found that generalists ST-21 CC and ST-45 CC undergo so rapid host jumps that the host signal is totally eroded, and WGS is therefore no more informative than 7-genes MLST in defining the source of infections (70).

WGS FOR THE DETECTION OF *C. JEJUNI* VIRULOME

Differences in strains virulence might explain why certain *C. jejuni* cause more human diseases than others. Consequently, the application of WGS in clinical microbiology might have an advantage in providing a fast and accurate *in silicio* typing (23) of the virulome as defined by several studies. Furthermore, such virulence traits can be used for pinpointing intervention targets for vaccine development. For example, a pan-genome approach led to the discovery of a set of unique loci, or loci with unique alleles, overrepresented in hyper-invasive *C. jejuni* strains which were not related to neutral variation caused by demographic processes (73). Also, bacteremia associated strains showed a linkage between the hyper-invasive phenotype and specific genes within the capsule region (74,75). Moreover, lineages expressing certain lipooligosaccharide structures defined by a specific set of loci are overrepresented in cases of Guillain-Barre syndrome (76,77).

CONCLUSION

Currently, a few issues are hurdling the full implementation of WGS in real-time surveillance of *C. jejuni* infections: the lack of a common nomenclature and a Tenover-like cut-off value for defining the relationship between *C. jejuni* isolates, and common data analysis tools for enabling comparison of isolates at national and international level. Within these hurdles resides the issue of a limited knowledge on the genetic diversity within different lineages of *C. jejuni*, which impedes the validity of the assumed genetic relationship when WGS is used alone. Additionally, different studies use diverse approaches to compare strains with no clear consensus of which method would be more suitable for meeting the needs and the realities of public health microbiology. Nevertheless, existing evidence supports the benefit of integrating WGS in the surveillance of *C. jejuni* infections and in point-source and diffuse outbreak investigations. Advances in WGS and statistical genetics provide new opportunities to improve our understanding of *C. jejuni* ecology, evolution, and pathogenesis, and have revealed a more complex epidemiological scenario and ecological dynamic than previously believed.

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DISCLAIMER

The conclusions, findings, and opinions expressed in this review paper reflect only the view of the authors and not the official position of the European Food Safety Authority (EFSA).

CONFLICT OF INTEREST

None declared

AUTHORS' CONTRIBUTIONS

AKL performed the literature search. Both authors contribute in drafting and revising the manuscript. All approved the final draft for publication.

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