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REVIEW

Pathogenomics of Emerging *Campylobacter* Species

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SUMMARY *Campylobacter* is among the four main causes of gastroenteritis worldwide and has increased in both developed and developing countries over the last 10 years. The vast majority of reported *Campylobacter* infections are caused by *Campylobacter jejuni* and, to a lesser extent, *C. coli*; however, the increasing recognition of other emerging *Campylobacter* pathogens is urgently demanding a better understanding of how these underestimated species cause disease, transmit, and evolve. In parallel to the enhanced clinical awareness of campylobacteriosis due to improved diagnostic protocols, the application of high-throughput sequencing has increased the number of whole-genome sequences available to dozens of strains of many emerging campylobacters. This has allowed for comprehensive comparative pathogenomic analyses for several species, such as *C. fetus* and *C. concisus*. These studies have started to reveal the evolutionary forces shaping their genomes and have brought to light many genomic features related to pathogenicity in these neglected species, promoting the development of new tools and approaches relevant for clinical microbiology. Despite the need for additional characterization of genomic diversity in emerging campylobacters, the increasing body of literature describing pathogenomic studies on these species deserves to be discussed from an integrative perspective. This review compiles the current knowledge and highlights future work toward deepening our understanding about genome dynamics and the mechanisms

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governing the evolution of pathogenicity in emerging *Campylobacter* species, which is urgently needed to develop strategies to prevent or control the spread of these pathogens.

KEYWORDS *Campylobacter*, emerging pathogens, genome evolution, pathogenomics, whole-genome sequencing

INTRODUCTION

The first recognized *Campylobacter* infection was reported in 1913 by McFaydean and Stockman, as they found a curved-shaped microorganism causing abortion in sheep and cattle (1). This bacterium remained unnamed until 1919, when Smith and Taylor isolated the same microorganism from bovine fetal fluids and named it *Vibrio fetus* (2). Then, in 1973 Véron and Chatelain proposed the genus *Campylobacter* by reclassifying *V. fetus* to *Campylobacter fetus* (3). In addition to this long and recognized importance as a veterinary pathogen since the beginning of the 20th century, *C. fetus* was subsequently identified as the causative agent of bloodstream infections in humans (4, 5). However, the major relevance of campylobacters as a main cause of human disease was just uncovered in the early 1980s, after the development and widespread implementation of selective media for the isolation of *Campylobacter* from stool samples. Today, the most relevant species within the genus is *C. jejuni*, a leading cause of bacterial gastroenteritis in humans whose worldwide incidence is even higher than that of very well-known pathogens that cause acute gastrointestinal infections, such as *Escherichia coli*, *Shigella*, or *Salmonella*. A close relative to *C. jejuni* is *C. coli*, which causes 1 to 25% of all *Campylobacter*-related diarrheal diseases (6). The remaining species of the genus have been much less studied, but the enhanced ability to detect campylobacters caused by the routine implementation of molecular techniques and the improvement of culture media and growth conditions allowed for the description and identification of a growing diversity of *Campylobacter* species distinct from *C. jejuni* and *C. coli* as relevant pathogens for humans and other animals.

The clinical awareness of many of these emerging *Campylobacter* species coincided with the advent of high-throughput sequencing as a popular tool for studying the microbial world, which fed the interest in applying whole-genome sequencing and comparative genomics to elucidate how emerging campylobacters cause disease, transmit, and evolve. The first complete genome sequence of *C. jejuni* was published almost 20 years ago (7), and today, several thousands of *C. jejuni* and *C. coli* genomes can be accessed through public databases. Accordingly, the increasing number of whole-genome sequences has allowed a transition in comparative genomics studies that initially included only a few genomes and now comprise hundreds to thousands of them. However, the availability of genomic data for emerging *Campylobacter* species is still lagging and more fragmented, hindering the improvement of our understanding of the biology of nonclassical *Campylobacter* pathogens. In this review, we summarize the state-of-the-art literature about emerging campylobacters in light of comparative genomics, discuss how these data are helping to uncover basic aspects of *Campylobacter* pathobiology and its applications in clinical microbiology, and highlight upcoming challenges in the field, including future work needed to mitigate sequencing bias in favor of well-known species. This review constitutes a comprehensive resource for researchers working on *Campylobacter* genomics and emerging pathogens, aiming to integrate the knowledge and future challenges in the field of emerging *Campylobacter* pathogens.

CURRENT TAXONOMY AND AVAILABLE GENOMIC DATA

To date, the genus *Campylobacter* consists of 32 officially described species and 9 subspecies, namely, *C. avium* (8), *C. blaseri* (9), *C. canadensis* (10), *C. coli*, *C. concisus* (11), *C. corcagiensis* (12), *C. cuniculorum* (13), *C. curvus* (14) *C. fetus* subsp. *fetus* (3), *C. fetus* subsp. *venerealis* (3), *C. fetus* subsp. *testudinum* (15), *C. geochelonis* (16), *C. gracilis* (17), *C. helveticus* (18), *C. hepaticus* (19), *C. hominis* (20), *C. hyointestinalis* subsp. *hyointestinalis*

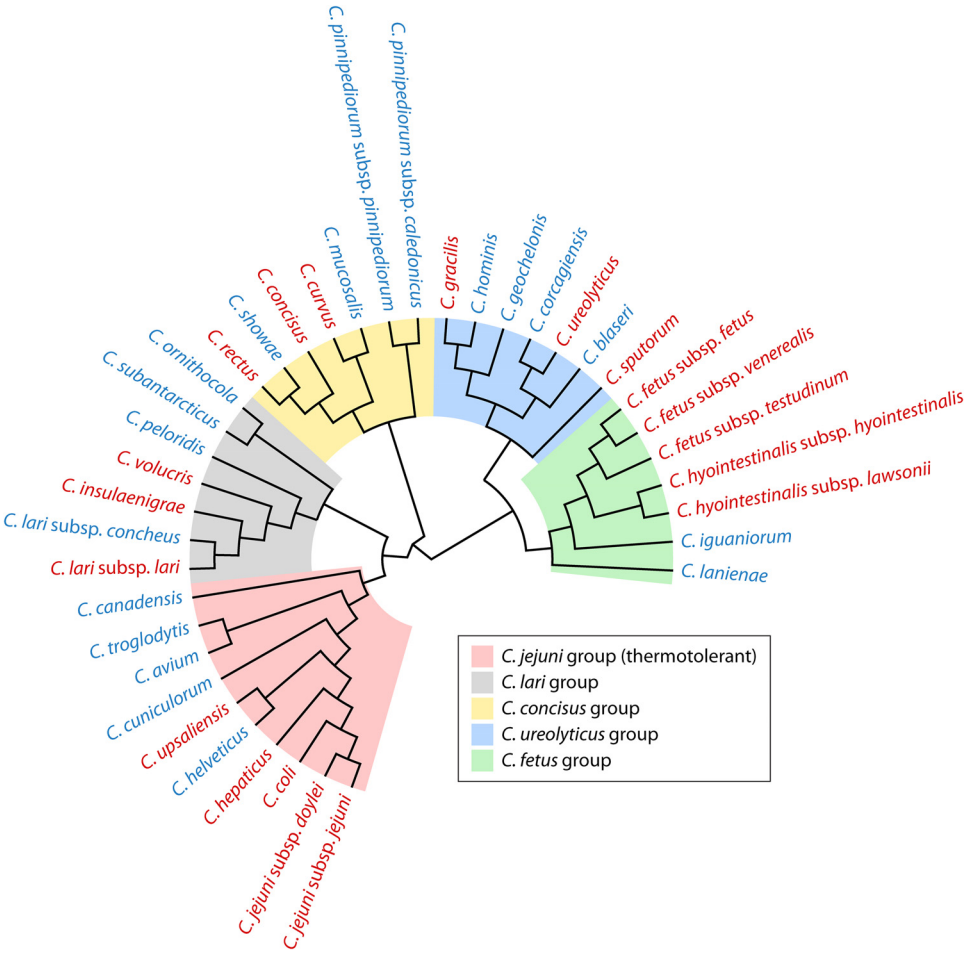

FIG 1 Phylogenetic relationships between described *Campylobacter* species. A phylogenetic tree of *Campylobacter* species dividing the genus into five distinct groups, namely, the *C. fetus* group, *C. jejuni* group, *C. lari* group, *C. concisus* group, and *C. ureolyticus* group, is shown. Names were assigned by considering the most clinically relevant species within each group. Tip labels are colored in red for species documented to cause infections in human and/or other animals or blue for species not documented to cause infections.

(21), *C. hyointestinalis* subsp. *lawsonii* (22), *C. iguaniorum* (23), *C. insulaenigrae* (24), *C. jejuni* subsp. *jejuni* (3), *C. jejuni* subsp. *doylei* (25), *C. lanienae* (26), *C. lari* subsp. *lari* (27), *C. lari* subsp. *concheus* (27), *C. mucosalis* (28), *C. ornithocola* (29), *C. peloridis* (27), *C. pinnipediorum* subsp. *pinnipediorum* (30), *C. pinnipediorum* subsp. *caledonicus* (30), *C. rectus* (31), *C. showae* (32), *C. sputorum* (33), *C. subantarcticus* (34), *C. troglodytis* (35), *C. upsaliensis* (36), *C. ureolyticus* (37), and *C. volucris* (38). These species cluster in five discrete phylogenetic groups, which all contain pathogenic microorganisms (Fig. 1), highlighting the clinical relevance of the whole genus. Despite this scenario clearly reflecting the taxonomic diversity and the widespread presence of pathogenic lineages in the genus *Campylobacter*, not a single genome is available for some species, like *C. canadensis*, *C. troglodytis*, and *C. mucosalis*. Also, for many others, including *C. volucris*, *C. peloridis*, *C. rectus*, *C. insulaenigrae*, *C. hominis*, *C. helveticus*, *C. cuniculorum*, *C. corcagiensis*, *C. ornithocola*, and *C. avium* (31% of the genus), only a single representative genome per species is available (Table 1). Importantly, when we exclude *C. jejuni* and *C. coli*, 13 out of the remaining 30 species (43%) have been at least sporadically reported to be the causative agent of infections in humans and/or other animals, and many of them are frequently associated with diverse clinical presentations, such as invasive blood infections, periodontal infections, abscesses, meningitis, diarrhea, or gastroenteritis (Table 2). The lack of sufficient genomic information on the causative agents of these infections prevents the exploration of intraspecific genetic variability

TABLE 1 Reported hosts and available genomic information for members of the genus *Campylobacter*

Species or subspecies	Reported host(s) (reference)	No. of genomes at:	
		PATRIC	NCBI
C. avium	Chicken, turkey (8)	3	3
C. blaseri	Seal (9)	2	1
C. canadensis	Whooping crane (10)	0	0
C. coli	Cattle (120), chicken (121), dog (122), duck (123), goat (124), monkey (125), pig (126), seagull (127), sheep (128), human (129)	1,571	981
C. concisus	Cat (126), dog (122), human (130, 131)	168	163
C. corcagiensis	Lion-tailed macaque (12)	1	2
C. cuniculorum	Rabbit (13)	2	3
C. curvus	Dog (122), human (14, 132)	8	3
C. fetus subsp. *fetus*	Cattle (3), sheep (133), human (65)	28	24
C. fetus subsp. *venerealis*	Cattle (3), human (134)	44	26
C. fetus subsp. *testudinum*	Reptiles (15), human (68), monkey (64)	33	23
C. geochelonis	Hermann's tortoise (16)	3	3
C. gracilis	Dog (122), human (40, 130)	3	3
C. helveticus	Dog (18), cat (18), human (135)	3	3
C. hepaticus	Chicken (19)	13	16
C. hominis	Human (20)	2	2
C. hyointestinalis subsp. *hyointestinalis*	Cattle (136, 137), human (138, 139), deer and reindeer (140), swine (137), sheep (137), hamster (136), dog (122)	21	18
C. hyointestinalis subsp. *lawsonii*	Swine (22), cattle (81)	10	10
C. iguaniorum	Reptiles (23), alpaca (141)	3	3
C. insulaenigrae	Pinnipeds (24, 142, 143), cetaceans (24), human (86)	1	2
C. jejuni subsp. *jejuni*	Cattle (120), chicken (121)	2,595	1,602
C. jejuni subsp. *doylei*	Human (25, 144, 145)	6	6
C. lanienae	Cattle (83), swine (83), sheep (137), human (26)	27	27
C. lari subsp. *lari*		13	13
C. lari subsp. *concheus*	Shellfish (27)	1	1
C. mucosalis	Dog (122), pig (28), human (146)	1	1
C. ornithocola	Wild birds (29)	1	1
C. peloridis	Shellfish (27)	1	1
C. pinnipediorum subsp. *pinnipediorum*	Sea lion (30)	5	8
C. pinnipediorum subsp. *caledonicus*	Seal (30)	1	3
C. rectus	Dog (122), human (147)	1	2
C. showae	Dog (122), human (32)	10	10
C. sputorum	Cattle (137, 148), sheep (33), swine (149), dog (122, 135), human (33)	7	7
C. subantarcticus	Wild birds (34)	2	2
C. troglodytis	Chimpanzee (35), human (150)	0	0
C. upsaliensis	Cat (151, 152), dog (97, 151), human (96)	6	6
C. ureolyticus	Cattle (153), horse (154), human (37)	7	7
C. volucris	Black-headed gull (38), human (87)	1	1

and patterns of genomic evolution. Consequently, relevant information about how a vast number of emerging *Campylobacter* species cause disease and transmit between hosts is currently unavailable. However, several groups have made a considerable effort to generate whole-genome sequences for some emerging campylobacters whose relevance for public health is frequently underestimated, uncovering genomic features that represent valuable contributions to understanding the disease biology and epidemiology of these microorganisms. Thus, these cases are discussed for each individual species that have deserved attention from the field of comparative genomics.

Campylobacter concisus

Campylobacter concisus was originally reported in 1981 from periodontal lesions (11); however, its role as an oral pathogen has remained uncertain since healthy individuals have been found to carry this species in the saliva (39). Additionally, *C. concisus* has been detected in fecal samples from diarrheic patients but also in healthy

TABLE 2 Emerging *Campylobacter* species with reported infections in humans or other animals and current record of pathogenomic studies

Species or subspecies	Human disease(s) [reference(s)]	Animal disease	No. of genomic studies [reference(s)]
C. concisus	Gastroenteritis (131, 155), brain abscess (156), arthritis, Crohn's disease and ulcerative colitis (48, 157), Barrett's esophagitis (158)	Not reported	5 (44, 47, 52, 53, 159)
C. curvus	Preterm birth (160), empyema (161), alveolar abscess (126), liver abscess (162), gastroenteritis (14)	Not reported	Not available
C. fetus subsp. *fetus*	Gastroenteritis, bacteremia, cellulitis, neurological infections (meningitis, meningoencephalitis, subdural empyema, brain abscess), perinatal infections (uterus infection, abortion, placentitis), vascular infections (endocarditis, vasculitis, thrombophlebitis, pericarditis) (6, 65)	Sporadic abortion (sheep and cow) (163)	6 (56, 58, 61–64)
C. fetus subsp. *venerealis*	Vaginosis (134)	Infertility and abortion (cow) (164)	7 (55–58, 60, 61, 68)
C. fetus subsp. *testudinum*	Bacteremia, subdural hematoma (68)	Not reported	4 (69–72)
C. gracilis	Bacteremia (165), empyema (166), brain abscess (156), head infection (167), Crohn's disease (40), ulcerative colitis (168), periodontitis (130)	Dog (diarrhea) (122)	1 (169)
C. helveticus	Diarrhea (135)	Cat (diarrhea) (18), dog (diarrhea) (122)	Not available
C. hepaticus	Not reported	Chicken (spotty liver disease) (19)	2 (19, 74)
C. hyointestinalis subsp. *hyointestinalis*	Gastroenteritis (138, 139)	Proliferative ileitis (pig) (21)	(77, 78, 82)
C. insulaenigrae	Gastroenteritis (135), septicemia (86)	Not reported	Not available
C. lari subsp. *lari*	Gastroenteritis (153, 170), bacteremia (88, 89, 171)	Not reported	2 (85, 86)
C. mucosalis	Gastroenteritis (50, 135)	Diarrhea (dog) (122)	Not available
C. pinnipediorum subsp. *pinnipediorum*	Not reported	Pinniped (abscess) (30)	1 (30)
C. pinnipediorum subsp. *caledonicus*	Not reported	Pinniped (abscess) (30)	1 (30)
C. rectus	Gastroenteritis (172), Crohn's disease (40, 42), ulcerative colitis (168), periodontal disease (147, 173), bacteremia (174), oral abscess (175), bone abscess (156), empyema thoracis (176)	Not reported	Not available
C. showae	Crohn's disease (40, 42), ulcerative colitis (168), abscess (156)	Diarrhea (dog) (122)	Not available
C. sputorum	Gastroenteritis (177, 178), abscess (179), bacteremia (180)	Diarrhea (dog) (122), sheep (abortion) (33)	2 (116, 181)
C. upsaliensis	Gastroenteritis (96, 182–184), abortion (185), bacteremia (186), breast abscess (187)	Not reported	Not available
C. ureolyticus	Gastroenteritis (92, 153), Crohn's disease (40, 168), ulcerative colitis (168), oral and perianal abscesses (167)	Not reported	3 (94)
C. volucris	Bacteremia (87)	Not reported	Not available

individuals, questioning its role in diarrheic disease. More recently, the prevalence of *C. concisus* has been found to be increased in both children and adult patients with inflammatory bowel disease (IBD) (40–42), suggesting that *C. concisus* may be implicated in its development and progression. Together, these studies show that the role of this species as a human pathogen is still unclear. This has motivated the development of several whole-genome sequencing projects aiming to uncover the genetic variability of this species with greater resolution and its relationship with disease.

First attempts to characterize the genetic diversity of *C. concisus* and its relationship with pathogenicity started with pulsed-field gel electrophoresis (PFGE), DNA-DNA hybridization, and ribosomal gene analyses (43). These results revealed a complex intraspecific taxonomy with high genetic heterogeneity that led to the description of genomospecies, defined as groups of genetically divergent strains without an apparent

phenotypic distinction. In 2011, two papers came out describing the whole-genome sequencing and comparison of two *C. concisus* strains: BAA-1457 (also referred as 13826), isolated from a patient with acute gastroenteritis, and UNSWCD, isolated from a biopsy specimen from a child with Crohn's disease (CD) (44, 45). These initial genomic comparisons confirmed previous findings based on nongenomic approaches and concluded that the two strains presented enough genetic diversity to be classified as distinct species. Also, these studies identified novel genetic features, like the presence of potentially secreted proteins exclusive to the species *C. concisus*, that could be used as disease markers or diagnostic targets. The assessment of strain-specific genomic regions that were potentially associated with virulence uncovered the presence of type VI secretion system (T6SS) genes in strain BAA-1475. This macromolecular system is implied in host-pathogen interactions and virulence and has been identified in many well-known bacterial pathogens, like *Salmonella*, *Pseudomonas*, *Yersinia*, or *Vibrio* (46). Another important difference between *C. concisus* strains was the zonula occludens toxin (*zot*) gene found in BAA-1475, which was inserted within a prophage. Zot increases intestinal permeability by affecting the tight junctions, a phenotype that is characteristic of IBD. Hence, a defect in the primary intestinal barrier caused by *C. concisus* Zot could be a mechanism by which this species may be related to the development of IBD. Also, UNSWCD and BAA-1457 were dissimilar in their flagellin glycosylation pathways, suggesting that genomic variability may also determine differential immune responses against genetically distinct *C. concisus* strains. Together, these preliminary differences found between these two strains, mainly in their repertory of virulence-associated genes, may explain the distinct pathogenic phenotypes found among *C. concisus* strains and set the basis for future studies involving pathogenomic analyses in this species.

A more comprehensive genomic comparison that involved 36 *C. concisus* strains from patients with gastroenteritis (isolated from intestinal biopsy specimens) or IBD (isolated from the oral cavity) deepened the characterization of virulence factor repertories and provided some clues about the relationship between intraspecific diversity and pathogenicity (47). This study analyzed for the first time multiple *C. concisus* genomes from the two main genomospecies, suggesting that the phylogenetic structure (genomospecies) was not linked to oral or intestinal origin, hence supporting a previous hypothesis that proposed that oral strains were the causative agents of gastroenteritis after translocating to the intestinal tract (39, 48). The same study reported several genetic differences between the genomospecies. For example, phosphate transport genes *pstS*, *pstA*, and *pstC* were specific to genomospecies 1, suggesting that different *C. concisus* genotypes may differ in their phosphate transport capacity. Also, genomospecies 2 encoded an aquaporin Z gene that functions to maintain intracellular osmotic pressure and that may be involved in *C. concisus* adaptation to environments with fluctuating osmolarity. Another important difference was the uneven distribution of CRISPR/Cas genes that were exclusively found in genomospecies 2. Even though CRISPR/Cas systems prevent the incorporation of foreign DNA, like plasmids and phages (49), no correlation was found between the presence/absence of CRISPR/Cas and the prophage that contains the zonula occludens toxin gene. Indeed, *zot* was detected in *C. concisus* strains from both genomospecies.

Another important discovery in this study was the presence of two genomic islands coding for type IV secretion systems (T4SS) and protein effectors similar to those found in pathogens like *Legionella pneumophila* and *Helicobacter pylori* (50). These islands were differentially prevalent in oral or intestinal strains, and even though the data suggested that they may preferably integrate into enteric *C. concisus* strains, analysis of a higher number of strains would be necessary to determine if these differences are statistically significant. These kinds of genomic features that have been identified can be used to guide phenotypic assays that could shed light on the pathogenicity potential between oral cavity- and intestinal biopsy specimen-derived strains or be used as genotypic markers for source tracking. Accordingly, a follow-up study from the same group focused on studying potential genetic factors discriminating commensal

from IBD-associated *C. concisus* strains by examining 86 genomes. This study found a novel gene in *C. concisus* that codes for the protein Csep1, which is homologous to enterotoxin B in *Staphylococcus aureus*. Enterotoxin B is involved in *S. aureus* pathogenesis by inducing diarrhea and activating T cells which produce large amounts of proinflammatory cytokines (51). Interestingly, the gene coding for Csep1 in *C. concisus* presented a 6-bp insertion (*csep1-6bpi*) in most strains isolated from CD patients in comparison with the gene from healthy controls (52). Based on this result, the authors suggested the use of *csep1-6bpi* as a molecular marker for CD-associated *C. concisus* strains. Beyond its potential application in the development of molecular methods to detect pathogenic CD-associated *C. concisus* strains, the fact that Csep1 is a secreted protein opens new opportunities to elucidate the molecular mechanisms by which *C. concisus* is implied in CD pathogenesis.

The last pathogenomic analysis of *C. concisus* constitutes the largest sequencing effort so far, which produced 104 *C. concisus* genomes from strains isolated from saliva, feces, and intestinal biopsy specimens. This analysis found no association between the genomospecies and IBD, diarrhea, or healthy controls, since strains were unevenly distributed in both phylogenetic lineages. However, when assessing the anatomical site of collection, genomospecies 2 was predominant in gut mucosal samples, while genomospecies 1 was predominant in oral samples. These differences were also reflected in the pangenomes of both genomospecies, given that genomospecies 2 harbored a bigger accessory genome than genomospecies 1, so this extensive genomic variation between *C. concisus* genomospecies could be related to functional variation and adaptation to different sites within the host. Indeed, several genes whose prevalence increases or decreases in association with the anatomical descent from the oral cavity to mucosal biopsy specimens to feces were identified, supporting the suggestion that the genetic heterogeneity of *C. concisus* is related to the source of isolation more than to the clinical phenotype (53).

Despite recent efforts that have significantly enlarged the number of available genomes from different sources and clinical conditions, the role of *C. concisus* in human disease remains elusive, with several studies arriving at contradictory results. Furthermore, most of these studies lack experimental evidence associated with the observed genomic variation which could help to infer mechanistic aspects of *C. concisus* pathogenicity. A couple of recent works have advanced the understanding of phenotypic variation by identifying signals of differential adaptation of genomospecies to the intestinal tract (54) and differential growth in response to specific carbon sources (55). This kind of phenotypic information, coupled with future global-scale sequencing surveys of *C. concisus*, can provide the conditions to apply genomewide association studies, an increasingly useful approach to discover genotype-phenotype associations in bacterial populations.

Campylobacter fetus

Campylobacter fetus is the type species of the genus and has been historically recognized as a livestock pathogen causing reproductive problems, mainly in cattle, and more recently as an increasingly reported opportunistic pathogen in humans. Currently, *C. fetus* is divided into three subspecies: *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* are primarily isolated from humans and cattle, respectively, and have traditionally been defined on the basis of two biochemical tests (growth in 1% glycine and H_2S production). *C. fetus* subsp. *venerealis* bv. *intermedius* is also described as a biochemical variant at the intrasubspecific level. *C. fetus* subsp. *testudinum* is genetically divergent from the others and is mostly isolated from reptiles but is also isolated from ill humans.

Mammal-associated *C. fetus* strains. The first *C. fetus* genome was sequenced with the Sanger method and belongs to strain *C. fetus* subsp. *fetus* 82-40, which was isolated from an immunocompromised human patient in the United States. Three years after the release of this genome in 2006, the first *C. fetus* subsp. *venerealis* genome was reported from strain Azul-94, isolated from a bovine abortion in Argentina (56). An

initial comparison of these two genomes allowed identification of markers that were subsequently used for the molecular characterization of *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* (57) and to propose candidate virulence determinants that could potentially have explained clinical differences between these two subspecies. In particular, a genomic island harboring a type IV secretion system and putative plasmid genes was detected in the bovine strain *C. fetus* subsp. *venerealis* Azul-94 and was absent in the human-associated strain *C. fetus* subsp. *fetus* 82-40. However, further studies evidenced that this and other similar genomic islands coding type IV secretion systems were frequent in the chromosomes and plasmids of both subspecies (58). Whole-genome sequencing of individual *C. fetus* subsp. *venerealis* bv. *intermedius* strains isolated from cattle could not explain the subtle biochemical phenotype that distinguishes *C. fetus* subsp. *venerealis* bv. *intermedius* from *C. fetus* subsp. *venerealis* (59, 60), and no further effort has been dedicated to elucidate this peculiarity.

The first comprehensive analysis of *C. fetus* genomes involved the comparison of 24 strains, revealing a remarkable inconsistency between population structure (genomic characteristics) and the biochemical tests (phenotypic characteristics) traditionally applied to differentiate *C. fetus* subsp. *fetus* from *C. fetus* subsp. *venerealis* (61). This study showed the presence of two phylogenetic lineages based on the core genome; one of them exclusively conformed to phenotypically determined *C. fetus* subsp. *fetus*, and the other conformed to both phenotypically determined *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, supporting this lack of correlation between phenotypic and genomic information, which raises questions about the clinical relevance of *C. fetus* subspecies typing by phenotypic assays. Additionally, further inconsistencies were revealed even between multilocus sequence typing (MLST) and core genome phylogenies, indicating that some alleles can undergo homoplasy, confounding epidemiological conclusions based on traditional genotyping approaches (62). Afterwards, an extended study that involved the analysis of 42 strains provided a more accurate description of the population structure of mammal-associated *C. fetus* strains, concluding that *C. fetus* subsp. *venerealis* has derived from a *C. fetus* subsp. *fetus* ancestor and suggesting the deletion of a putative cysteine transporter as the reason for the H_2S-negative phenotype in *C. fetus* subsp. *venerealis* strains. Nevertheless, the overall conclusion of this study reinforces the notion of inconsistency between biochemical tests and genomics (63). Together, these results point to whole-genome analysis as the current standard approach for typing *C. fetus* strains from mammal origin. Accordingly, a recent study that analyzed the genomes of 182 *C. fetus* strains mainly isolated from cattle and humans revealed the presence of 8 discrete lineages that adapted as livestock pathogens or human intestinal pathobionts, as they were found in the gut metagenomes of healthy individuals (64). This study provided the basis to further investigate the evolution and transmission of *C. fetus*, since its presence in the human gut microbiota could facilitate alternative ways of contagion. Indeed, zoonotic transmission is currently the most widely accepted and documented route (65), but the recent identification of human-to-human transmission between men who have sex with men (66) reinforces the hypothesis that *C. fetus* behaves both as a zoonotic pathogen and as a pathobiont resident of the intestinal microbiota. In this sense, the upcoming challenge to completely understand the evolutionary landscape and epidemiology of mammal-associated *C. fetus* should be focused on the analysis of whole genomes from strains isolated from healthy individuals.

Reptile-associated *C. fetus* strains. Reptile-associated *C. fetus* isolates are genetically distant from mammal-associated strains, as originally observed by MLST, which evidenced two discrete clusters (sharing 90% of identity) enclosing reptile- and mammal-associated strains (67). These reptile-associated *C. fetus* strains have been found to cause infections in humans (68), and their genetic distance from mammal-associated strains motivated the whole-genome sequencing of its type strain, *C. fetus* subsp. *testudinum* 03-427, originally isolated from a human (69). This genome was released months before the official taxonomic revision that defined genetically distant, reptile-associated *C. fetus* strains as the novel subspecies *C. fetus* subsp. *testudinum* (15). The

subsequent whole-genome sequencing of strain *C. fetus* subsp. *testudinum* Pet-3, isolated from a reptile, revealed high genomic homogeneity between these two isolates from different host species (70).

The first comprehensive comparative study including reptile- and mammal-associated *C. fetus* strains (that analyzed 61 *C. fetus* subsp. *fetus*/*C. fetus* subsp. *venerealis* genomes and 18 *C. fetus* subsp. *testudinum* genomes) identified a recombinant locus that differentiates reptile- from human-derived *C. fetus* subsp. *testudinum* strains and that could be related to the invasive phenotype of human infections. These genomic comparisons also confirmed that mammal- and reptile-associated strains cluster in different phylogenetic lineages which are genetically isolated due to the existence of a barrier to lateral gene transfer or interlineage recombination. Consequently, several host-associated genomic features were found, including a tricarballylate catabolism pathway present in *C. fetus* subsp. *testudinum* but absent in *C. fetus* subsp. *fetus*/*C. fetus* subsp. *venerealis* which might explain adaptation to reptilian hosts (71). However, the recent identification of a *C. fetus* lineage that was isolated from reptiles but that was genetically closer to mammal strains and that showed strong signals of recombination with *C. fetus* subsp. *testudinum* evidenced that barriers to homologous recombination between divergent lineages of *C. fetus* occurring within the same host are not absolute (72). As the reptile gut seems to be a frequent niche for *C. fetus*, future work should couple metagenomics and *Campylobacter* selective media to uncover novel related taxa whose whole-genome sequencing and comparison could improve our understanding about the ecology and pathogenicity of this species.

Campylobacter hepaticus

Spotty liver disease (SLD) is an emerging infectious disease prevalent in Europe and Australia characterized by multifocal liver lesions with high mortality rates that particularly affects free-range and floor-raised chicken flocks (73). This infection is caused by *Campylobacter hepaticus*, a recently described species that was originally isolated from poultry with SLD and whose zoonotic risk and potential for transmission to other animals are yet unknown (19).

The whole genomes of four *C. hepaticus* strains were originally sequenced to support the description of *C. hepaticus* as a novel species (19), including its type strain, HV10. However, the authors did not detail the virulence genes or pathogenicity mechanisms encoded by the *C. hepaticus* genome. Subsequently, a more comprehensive study focused on the genomic characterization of this species sequenced 10 strains isolated from SLD cases in the United Kingdom (74). This work concluded that *C. hepaticus* represents a distinct genomic lineage, with the pathogenic *C. jejuni*/*C. coli* strains being its closest phylogenetic relatives. Also, this study revealed that *C. hepaticus* has experienced reductive genome evolution, as evidenced by a lower GC content and an average genome size reduction of ~140 kb with respect to those of *C. jejuni*. This reduction involved the loss of iron acquisition systems and the lack of many well-known *Campylobacter* virulence determinants, like adhesion factors and capsular polysaccharide biosynthesis or cytolethal distending toxin (CDT) genes. As in many other bacterial pathogens, genome reduction is typically associated with niche specialization, like the chicken liver, so the potential of this emerging pathogen to transmit and cause disease in other hosts (like humans) seems remote. However, the identification of a *C. jejuni* plasmid encoding tetracycline resistance present in the genomes of *C. hepaticus* strains evidenced that horizontal transfer between this species and other campylobacters can mediate the acquisition of antimicrobial resistance and pathogenicity-related determinants.

The availability of a larger, global *C. hepaticus* isolate collection is needed to explore the pangenome variability in this species and to determine its population structure, which will be helpful to better understand the impact of genome reduction and horizontal gene transfer in the evolution and epidemiological dynamics of this emerging pathogen. As well, uncovering which genotypes are circulating globally will provide

information that may be used to develop specific typing and detection tools for this emerging pathogen.

Campylobacter hyointestinalis

Campylobacter hyointestinalis was originally isolated from swine with proliferative enteritis and was described as a novel species in 1980 (21). Since then, it has been recovered mostly from healthy animals (like sheep, deer, hamsters, dogs, and cattle) but has also been sporadically isolated from livestock and human infections, pointing to this species as an emerging zoonotic pathogen (6). On the basis of genetic and phenotypic traits, *C. hyointestinalis* is currently divided into two subspecies: *C. hyointestinalis* subsp. *lawsonii* is mainly restricted to pigs, and *C. hyointestinalis* subsp. *hyointestinalis* has a broader host range (22, 75). First attempts aiming to assess the diversity of *C. hyointestinalis* were based on determining genetic and protein profiles, evidencing considerable intraspecies variability (76).

The whole genome of the species type strain, *C. hyointestinalis* subsp. *hyointestinalis* DSM 19053, originally isolated in 1985 from the intestine of a pig, was sequenced and released in 2014. However, no further analyses of this genome were performed. Recently, two closed genomes belonging to the human strain *C. hyointestinalis* subsp. *hyointestinalis* LMG 9260 and the porcine strain *C. hyointestinalis* subsp. *lawsonii* LMG 15993 were released (77). Comparison of these genomes revealed an average nucleotide identity between the two subspecies lower than the standard threshold (95%) used for bacterial species delimitation, indicating that *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii* could be reclassified as separate species within the genus *Campylobacter*. Indeed, a more recent study that performed whole-genome sequencing of 18 strains isolated from cattle, sheep, and deer from New Zealand confirmed the previously suggested genomewide plasticity of *C. hyointestinalis* (78). This work revealed high rates of gene gain/loss across *C. hyointestinalis* lineages, probably accounting for the effects of horizontal gene transfer. The presence of strains with an unusually high number of genes, multiple insertions of genomic islands, and a significant proportion of recombinant sites suggests that genomic introgression has been frequent along the evolutionary history of *C. hyointestinalis* subspecies. Indeed, pangenome estimations revealed that 67% of *C. hyointestinalis* genes belong to the core genome but only 5% of them correspond to the species clonal frame. Further variability has been reported in virulence-associated genes, like the gene for cytolethal distending toxin (CDT), for which new variants have been described in *C. hyointestinalis* (79, 80).

Comprehensive comparative genomic analyses including both subspecies have been limited because of the unavailability of *C. hyointestinalis* subsp. *lawsonii* genomes. However, the release of a set of nine *C. hyointestinalis* subsp. *lawsonii* genomes (81) has recently allowed proper comparison of the genetic diversity and evolutionary patterns distinguishing *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*. This study confirmed the phylogenetic separation of *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii* using genomewide information and identified that the two subspecies have separately evolved with null or extremely limited gene flow between lineages. This genetic isolation is probably driven by adaptation to distinct ecological niches, which has determined the fixation of genomic signatures in both subspecies. For example, the generalist *C. hyointestinalis* subsp. *hyointestinalis* enclosed a bigger and more diverse accessory genome than the specialist *C. hyointestinalis* subsp. *lawsonii*. This increased diversity is probably driven by a stronger incidence of genomewide recombination events in *C. hyointestinalis* subsp. *lawsonii* than in *C. hyointestinalis* subsp. *hyointestinalis*. Accordingly, the genomes of both subspecies encode distinct repertories of CRISPR/Cas and restriction-modification systems that play an important role in DNA recombination, repair, and integration (49). This has probably influenced genome plasticity and led to the observed differences in the accessory genomes of both subspecies (82).

Together, the observed genomic variability of *C. hyointestinalis* may indicate a great potential to adapt to different ecological niches, underpinning its capacity to colonize a great variety of mammal species both as a commensal and as a disease-causing agent (78). Further comparisons between commensal and disease-associated strains may provide new insight into the genetic mechanisms underlying *C. hyointestinalis* pathogenicity.

Campylobacter lanienae

Campylobacter lanienae was first described in 2000 from the feces of healthy individuals during a hygiene survey of abattoir workers (26). Subsequently, it has been recovered from healthy cattle, sheep, and swine (83). In a single report, *C. lanienae* was isolated from symptomatic infections in lab chinchillas with gastric ulcer; however, these results were not fully conclusive about the pathogenic role of this species (84). The fact that *C. lanienae* has not been reported to cause symptomatic infections either in humans or in other animals indicates that this species could have limited pathogenic potential or be a nonpathogenic member of the genus *Campylobacter*.

In a recent study, the whole genomes of 26 *C. lanienae* strains and 50 strains from three putative novel *C. lanienae*-related taxa isolated from diverse hosts (including humans, swine, sheep, and goat) were sequenced (85). In this work, the authors compared these genomes with those from other sister species, such as *C. fetus*, *C. iguaniorum*, and *C. hyointestinalis*, evidencing that *C. lanienae* and its related taxa present a reduced gene content and distinct CRISPR/Cas loci. Additionally, the *C. lanienae* lineage presented a higher diversity of flagellin genes than other *Campylobacter* species and the absence of genes involved in selenium metabolism. Beyond the unknown consequences of these genetic distinctions in the biology of *C. lanienae*, a more in-depth (not yet reported) comparison of these genomes could shed light on the apparent nonpathogenic phenotype of *C. lanienae* strains, as they could be screened for genes that are well-known virulence determinants in major *Campylobacter* pathogens. Anyway, this work represents a good example of how the sequencing of species that are not relevant for human or animal health can improve our understanding of host adaptation and virulence evolution in the pathogenic members of the genus *Campylobacter*.

Campylobacter lari and Related Taxa

Campylobacter lari was originally isolated from gulls as nalidixic acid-resistant, thermophilic strains (NARTC). Subsequently, the urease-producing thermophilic group (UPTC), the nalidixic acid-susceptible group (NASC), and the urease-positive NASC were identified as phenotypic variants of the originally described *C. lari* strains. A more comprehensive inspection of these variants using molecular typing methods resulted in the taxonomic revision and reclassification of several strains as novel taxa, such as *C. peloridis* (27) and *C. volucris* (38). Additionally, other *C. lari*-like species have recently been described, including *C. insulaenigrae* (24), *C. subantarcticus* (34), and *C. ornithocola* (29). Currently, *C. lari* is divided into two subspecies: *C. lari* subsp. *lari* and *C. lari* subsp. *concheus*. Together, these phylogenetically related taxa comprise the *C. lari* group. These bacteria are typically isolated from coastal regions, marine environments, molluscs, and aquatic birds and mammals. However, the sporadic isolation of *C. lari* and related taxa from human infections (86–89) highlights their potential as emerging pathogens.

Indeed, the first member of this group whose whole-genome sequence became available was *C. lari* subsp. *lari* strain RM2100, isolated from a girl with watery diarrhea. The analysis of this genome allowed for the determination that many virulence and antibiotic resistance mechanisms present in the major pathogen *C. jejuni* were also conserved in *C. lari*. Additionally, this isolate harbored a megaplasmid similar to the conjugative plasmid pTet found in *C. jejuni*, coding for type IV secretion systems, invasins, and adhesins that may contribute to its pathogenic potential (90).

A subsequent comparative analysis aiming to expand the characterization of the *C. lari* group released the whole-genome sequences for several UPTC strains, *C. lari* subsp. *concheus*, and other related taxa, such as *C. peloridis*, *C. subantarcticus*, *C. volucris*, and *C. insulaenigrae*. This study revealed that the *C. lari* group is very homogeneous, with more than 70% of the genes identified in the previously sequenced *C. lari* subsp. *lari* RM2100 being conserved among its members. However, an important conclusion of this work is the absence of genes or pathways potentially implicated in the association of the *C. lari* group with marine environments and aquatic animals (91).

Future work focused on elucidating this and other ecological aspects of this group, like its potential pathogenicity and zoonotic risk, should aim to generate comprehensive sets of whole-genome sequences for many species that are currently represented by just a single sequenced strain (most except *C. lari*). This would allow for the application of pangenome analyses providing a more comprehensive insight into the intraspecific genomic variation of the *C. lari* group. This could result in the development of lineage-specific molecular characterization tools useful to improve the screening of species belonging to the *C. lari* group in diverse environments and hosts, including the identification of clinical strains causing emerging infections in humans.

Campylobacter ureolyticus

In 2010, a polyphasic analysis was applied over a diverse collection of 26 *Bacteroides ureolyticus* strains to reassess the taxonomic position of this species. This study demonstrated that *B. ureolyticus* should be more suitably allocated within the genus *Campylobacter*; hence, it was renamed *C. ureolyticus* (37). Subsequently, a retrospective study that screened more than 7,000 patients with diarrhea evidenced the presence of *C. ureolyticus* in 23.8% of *Campylobacter*-positive samples, representing the first report of *C. ureolyticus* in the feces of patients with gastroenteritis and suggesting the role of this species as an emerging enteric pathogen (92).

The first whole-genome analysis of *C. ureolyticus* strains was published in 2013 and was based on the comparison of two genomes. The species type strain, DSM 20703 (originally isolated in 1978 from amniotic fluid), was sequenced in that study, and strain ACS-301-Sch-V-3b (isolated from the vaginal tract of a woman) had been previously sequenced as part of the Human Microbiome Project (93). This work uncovered the virulence gene repertories of *C. ureolyticus*, which resembled those present in other *Campylobacter* pathogens, including genes for adhesion and colonization (*cadF*, *PEB1*, *icmF*, and *flpA*), invasion (*ciaB*, type IV secretion systems), and toxin production (S layer, RTX, and Zot). Additionally, the study revealed that the two strains shared only 83% of their genes, suggesting considerable intraspecific heterogeneity within *C. ureolyticus*. Since then, only two additional genomes have been sequenced: that of strain CIT007, which was originally isolated from stools from an elderly woman presenting with diarrheal illness and end-stage chronic renal disease (94), and that of strain RIGS9880, which was isolated from an immunocompromised patient with diarrhea (95). Both strains presented very similar virulence repertories in comparison with the previously sequenced genomes, proposing that these genes are conserved features of *C. ureolyticus* that may define its pathogenic potential.

Considering the increasing clinical awareness of *C. ureolyticus* as an emerging pathogen causing human gastrointestinal disease, future research should focus on the generation of extensive whole-genome sequencing data from a representative collection of strains. This will allow the population structure, accessory gene dynamics, and selective pressures shaping the genomes of this pathogen to be uncovered. This information can be useful to seek genotype-phenotype associations, dissect its epidemiological behavior, and identify transmission patterns, which are largely unknown.

Campylobacter upsaliensis: a Salient Example of a Not (Enough) Sequenced Pathogen

For many nonclassical *Campylobacter* species reported to be causative agents of infections in humans and other animals, sequencing efforts have been extremely

limited, often including just single genomes of type strains. This reflects the existing bias toward *Campylobacter* species that are more frequently or more easily isolated. Despite this general bias in bacterial genomics, the effort of sequencing nonclassical, clinically, or economically nonrelevant species deserves to be claimed as a way to improve our understanding of the mechanisms directing the evolution of bacterial pathogenicity.

C. upsaliensis represents a salient example of this situation. This species belongs to the thermophilic campylobacters and is phylogenetically close to *C. jejuni*. Despite *C. upsaliensis* being highlighted as an important emerging gastrointestinal pathogen more than 2 decades ago, its clinical underestimation has been mainly explained by the fact that it is sensitive to the antibiotics routinely used in selective media for the isolation of *C. jejuni* (96). Nevertheless, many studies carried out in different geographic areas have increasingly reported high prevalences of this species in human infections (6). Also, companion animals, such as dogs and cats, have been identified to be possible reservoirs of *C. upsaliensis* (97). Indeed, a pioneering study that applied amplified fragment length polymorphism (AFLP) to characterize *C. upsaliensis* strains isolated from humans and dogs evidenced two main genomic clusters: one exclusively composed of human strains and the other comprising both human and dog strains (98). This work revealed that, despite dogs being a possible source for human infections, other routes of transmission would explain most cases of *C. upsaliensis* in humans. Undoubtedly, whole-genome sequencing of *C. upsaliensis* populations isolated from humans, dogs, and other animals could provide an enhanced understanding of its epidemiology, host-associated evolution, and virulence mechanisms.

EVOLUTIONARY MECHANISMS IN EMERGING *CAMPYLOBACTER* SPECIES

The evolution of bacterial populations is directed by the incidence of two main mechanisms: DNA damage or replication errors which generate deletions, rearrangements, and point mutations and the horizontal exchange of genetic material, through which genes are externally acquired and eventually incorporated through recombination. The impact of these diversification mechanisms in the structuring of bacterial populations can be explained under entirely neutral evolutionary models, where microorganisms do not differ in their fitness and all members of the population can be tracked back to the most recent common ancestor using the coalescent framework. Though neutral models can explain the population structure, bacteria are probably under selection pressures for adaptive traits which influences their fitness, specifically, in host-adapted species which are exposed to particular conditions within hosts. Some *Campylobacter* species can survive in the environment, but they are mainly found in association with vertebrate hosts; hence, they are presumably subjected to selective pressures whose traces can be observed as genomic signatures. Here, we summarize and discuss how pathogenomic studies have contributed to unveil the main evolutionary mechanisms in emerging *Campylobacter* species and their clinical relevance as drivers of new pathogenic phenotypes.

Host-Associated Population Structure and Adaptation

Emerging *Campylobacter* species are mainly host adapted and colonize a wide variety of niches within birds, mammalian, and reptilian hosts. Accordingly, the observation of coexisting lineages associated with different hosts suggests that natural selection acts to maintain that given population structure, for example, human-associated and cattle-associated *C. fetus* strains that represent different phylogenetic lineages that exhibit host-specific core gene repertories under positive selection (64). In particular, strong positive selection signals in the *flgD* gene (coding for the flagellar hook cap protein FlgD) were found in human-adapted lineages. Interestingly, diversifying alleles of this gene have been found to be a defining feature of hyperinvasive *C. jejuni* strains (99). In cattle-associated lineages, the enterobactin uptake receptor *cfrA* has been identified to be the most diversifying gene. The expression of the CfrA protein is induced under iron-restricted conditions and plays an important role in iron scavenging and colonization in *C. jejuni* (100), suggesting that selection acting on *cfrA* could

be associated with niche adaptation and virulence in *C. fetus*. Another example of host adaptation is observed in mammal- and reptile-associated *C. fetus* strains that represent phylogenetically distinct lineages. Remarkably, the *tcuRABC* operon, which directs the catabolism of tricarballylate, allowing its utilization as a carbon and energy source, is present in the reptile-associated lineage consisting of *C. fetus* subsp. *testudinum* strains but absent in the mammal-associated lineage consisting of *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* (71). This genomic signature reflects functional adaptation to different ecological niches within mammal and reptile hosts.

Similarly, other emerging species possess a structured population. An example is *C. hyointestinalis*, which is subdivided into *C. hyointestinalis* subsp. *hyointestinalis* (a generalist colonizing several mammalian species) and *C. hyointestinalis* subsp. *lawsonii* (a specialist adapted to pigs), which represent clearly divergent phylogenetic lineages (82). The genomes of these host-adapted subspecies are characterized by distinct accessory gene patterns which reflect dissimilarities in their functional repertories. Specifically, genes involved in DNA replication, recombination, and repair, such as those coding for CRISPR/Cas and restriction-modification systems, are unevenly distributed among subspecies. These genes may modulate the generation of sequence diversity that is the source for natural selection and the subsequent adaptation of lineages to ecological niches, like different host species. These observations are reminiscent of the major pathogen *C. jejuni*, where the differential host tropism of lineages has been well documented. For example, differences in the vitamin B_5 biosynthesis pathway have been identified between cattle-associated and chicken-associated clonal complexes, suggesting adaptation to the host diet (101).

As hosts represent a complex combination of selective pressures given by distinct immune responses along tissues or fluctuating concentrations of metabolites and cell by-products, different *Campylobacter* genotypes could adapt to distinct ecological subniches within the same host organism and eventually exhibit distinct virulent phenotypes. Indeed, this kind of adaptation to subniches within the same host has been proposed in *C. jejuni*, where up to 10 different clonal complexes have been reported coexisting in a single chicken flock (102). Interestingly, this has been reported in emerging species, such as *C. fetus* subsp. *testudinum*, which also shows signatures of genomic adaptation to different subniches within the same host. Specifically, a recombination event in the *iamA* gene has been identified among strains causing invasive disease in humans (recovered from blood, bile, hematoma, or pleural fluid) but absent in strains isolated from stool samples. The *iamA* gene belongs to an ABC transporter system that is considered a virulence factor associated with invasion in *C. jejuni* (103). These examples evidence that genomic traces in host-adapted lineages within structured populations can be identified at different levels of complexity and could be related to the pathogenic potential of emerging *Campylobacter* species.

Barriers to Homologous Recombination

Recombination occurs between individual microorganisms, so it can be detected when comparative analyses are performed at the population level through the identification of genomic mosaicisms. This evidences gene flow occurring between physically close cells, hence, the absence of an ecological barrier between them. However, different types of barriers to homologous recombination can be implicated in a maintaining population structure, particularly in host-associated lineages, where strains colonizing a certain host are typically not in contact with those colonizing a different host. For example, the *C. hyointestinalis* subspecies found in different mammalian species are separated by a strong recombination barrier (82). Interestingly, these subspecies present a borderline average nucleotide identity indicating an underlying speciation process driven by genetic and ecological isolation. Also, in *C. fetus* a barrier to homologous recombination between mammal-adapted and reptile-adapted strains reflects that these lineages have been evolving separately for a long time in association with different host species (71).

This phenomenon can have adaptive explanations, since bacteria sharing similar niches will require certain combinations of genes that confer a fitness advantage in that

environment. Recombination can be the underlying mechanism by which adaptive genetic variants can be incorporated and selected. A clear example of recombination barriers and differential gene flow between closely related *Campylobacter* lineages can be observed in *C. coli*. Barriers to homologous recombination have been identified in *C. coli* clade 1 strains, which mainly represent clinical and farm animal isolates, with respect to clade 2 and 3 strains, which are more abundant in watercourses and riparian environments. On the contrary, substantial genome introgression from *C. jejuni* has been detected in *C. coli* clade 1 after a long period of independent evolution (104).

These examples evidence that limited gene flow between *Campylobacter* populations may be a mechanism driving genomic diversification and possible adaptation to new hosts in emerging species. Some similarities can be observed in emerging and major *Campylobacter* pathogens, where recombination barriers and gene flow have shaped their genome dynamics. From the clinical perspective, understanding how genomic admixture underpins the adaptation of pathogens to new environments and/or hosts is relevant, since this information can help to provide an understanding of phenotypic variation associated with pathogenicity potential, identify new reservoirs for zoonotic species, and predict potential host jumps that can lead to emerging infections.

Horizontal Gene Transfer

Horizontal gene transfer is a main driver of bacterial evolution, and its role in genome plasticity is being understood more precisely as new genomic information becomes available for different bacterial lineages. The impact of horizontal gene transfer in *Campylobacter* evolution has been well-documented in *C. jejuni*, where it has played an important role in the acquisition of antimicrobial resistance and virulence. Additionally, horizontal gene transfer, including the incorporation of plasmids and the integration of genomic islands, has been described in several emerging *Campylobacter* species. Here, we summarize the most salient examples of horizontal gene transfer events in emerging campylobacters and discuss how this can impact antimicrobial resistance and virulence.

Role in the acquisition of antimicrobial resistance. Antimicrobial resistance conferred by horizontal gene transfer has been largely documented mainly in *C. jejuni* and *C. coli* (105). Indeed, as *Campylobacter* species possess genetic mechanisms for natural transformation and conjugation, antimicrobial resistance genes could be rapidly transferred between strains (106). For example, *C. jejuni* isolates carrying a plasmid coding for a *cfr*(C) gene that confers multidrug resistance were recently described. Interestingly, this genetic mechanism was also found in *C. coli* primary isolates recovered from cattle (107). Considering the widespread distribution of several emerging *Campylobacter* species in the farm environment, this constitutes a risk for the appearance of new *Campylobacter* lineages that could incorporate this plasmid. The fact that this plasmid can also be successfully transferred *in vitro* between *C. jejuni* and *C. coli* suggests that it could disseminate to other species coexisting in the same niche. Indeed, the analysis of *C. hepaticus* and *C. lari* genomes, among other emerging *Campylobacter* genomes, has recently revealed the presence of plasmids coding for the tetracycline resistance gene *tetO* and other antimicrobial resistance determinants which are widespread in mobile elements found in *C. jejuni* and *C. coli* (74, 90). Additionally, other mechanisms of horizontal gene transfer seem to be important in emerging *Campylobacter* species, as evidenced by the detection of tetracycline and aminoglycoside resistance genes within a transferable genomic island found in *C. fetus* (108). This suggests that the intra- and interspecies transfer of mobile elements coding for antimicrobial resistance is possible between *Campylobacter* species. Importantly, as most *Campylobacter* infections in humans are caused by the ingestion of contaminated animal products or contact with animals, the dissemination and fixation of antimicrobial resistance mechanisms in emerging campylobacters that are mainly adapted to the farm environment constitute a possible vehicle for the appearance of emerging pathogens with extended antimicrobial resistance repertories. This is particularly relevant, since the same classes

of antimicrobials are basically being used in food-producing animals and in human medicine, generating similar selective pressures in both environments.

Role in the acquisition of virulence mechanisms. Mobile elements are important for virulence in major pathogens like *C. jejuni*. Particularly, this is mainly caused by the presence of plasmids containing genes homologous to the genes for type IV secretion systems (T4SS), which are macromolecular machineries used to exchange DNA between bacteria but also to inject protein effectors into host cells, which can lead to functional impairment (109). Indeed, experimental mutations to inactivate T4SS genes caused reduced adherence and invasion in *C. jejuni* (110). Interestingly, these systems have subsequently been described in other *Campylobacter* species, like *C. fetus*, where diverse T4SS clusters are present in their genomes and are contained within pathogenicity islands that can be found both integrated into the chromosome and in plasmids. Even though the effector proteins that could be delivered by these T4SS and cause damage to the host cell have not been experimentally determined, T4SS-containing pathogenicity islands in *C. fetus* also code for filamentation induced by cAMP (FIC) domain proteins. These proteins could be potential effectors for virulence since in other bacteria they have critical roles in cellular processes, including disruption of host cell signaling pathways leading to cytotoxic effects (111).

Phylogenetic characterization of T4SS genes in *C. fetus* revealed multiple evolutionary origins and acquisition from different *Campylobacter* donors. These genes are also conserved in other emerging species, like *C. ureolyticus*, *C. upsaliensis*, and *C. lari*, indicating that horizontal gene transfer of plasmids and other genetic elements harboring T4SS has been an important evolutionary force shaping the repertory of virulence genes in *Campylobacter* species. The exploration of T4SS diversity in other *Campylobacter* genomes, together with the development of experimental approaches in emerging species, can provide important information about the role of these secretion systems in the virulence of *Campylobacter* species. Importantly, the identification of genes coding for effector proteins that modulate virulence could lead to the development of straightforward molecular typing tools targeting these genes, as they have been developed, for example, to characterize *cagA*-positive *Helicobacter pylori* strains, which are known to increase the risk of developing gastric cancer (112, 113).

Genome Reduction

Genome reduction has been documented in diverse bacterial lineages and is typically associated with functional specialization, host association, and/or increased pathogenicity in some species (114, 115), since niche adaptation requires selection for traits that optimize pathogen fitness in the new environment. Reduced genomes are also characterized by diminutive gene sets with a loss of metabolic functions and a low genomic GC content. The genome size in the genus *Campylobacter* varies from ~1.4 Mb to ~2.5 Mb, and the GC content ranges from very low values of about 28% up to 45% (116). The most relevant species for human and animal health, *C. jejuni* and *C. coli*, have genomes smaller than those of most of the emerging species and are typically associated with the gastrointestinal niche. This highlights the relevance of genome reduction during the evolution of pathogenicity in the genus *Campylobacter*. However, the most salient example of genome reduction is the recently described species *C. hepaticus*, which presents one of the lowest GC values within the genus (~28%) and a smaller genome than its closest relative, *C. jejuni*. The genes lost in this species include genes for pathways for iron acquisition and metabolism, which is consistent with adaptation to an iron-rich environment, such as the chicken liver, which constitutes its reservoir. Additionally, *C. hepaticus* genomes code for a very reduced repertory of virulence-associated genes in comparison to that in *C. jejuni*, with the *C. hepaticus* genome lacking many well-known genetic factors important during *Campylobacter* infection, like those coding for the cytolethal distending toxin (CDT) and capsular and extracellular polysaccharides (74). This reduced set of virulence genes may be the consequence of the evolution of attenuated virulence in *C. hepaticus*, which has been documented in other bacteria and which occurs as a result of immune evasion within the host (117). Beyond being beneficial for the pathogen due to the establishment of a

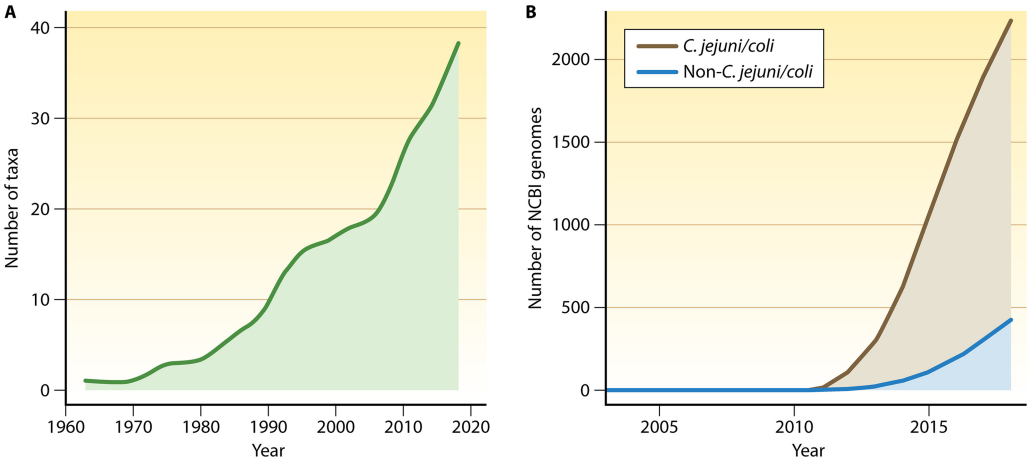

FIG 2 *Campylobacter* taxa and available genomes through time. (A) Graph showing the number of described *Campylobacter* species through time since the official description of the genus in 1973. (B) Graph showing the number of available whole-genome sequences through time for *C. jejuni*/*C. coli* and the rest of the *Campylobacter* members, including those emerging species discussed in this review.

long-term chronic infection, this may constitute a limited capacity for transmission within hosts or survival in the environment. Consequently, further work is needed to determine the impact of genome reduction in *C. hepaticus* and how this correlates with virulent phenotypes, which would constitute a cornerstone for studying this evolutionary mechanism in this and other emerging *Campylobacter* species.

FUTURE DIRECTIONS

Beyond the fact that *C. jejuni* and *C. coli* still remain the main causes of bacterial gastroenteritis worldwide, we now identify most *Campylobacter* species to be clinically relevant pathogens. Among them, *C. fetus*, *C. concisus*, *C. ureolyticus*, and *C. upsaliensis* are being systematically detected from humans, although the increasingly frequent report of infections caused by other related species still has not motivated a unified effort to understand the genomic variability of emerging *Campylobacter* species. On the contrary, these efforts have been mostly isolated, causing some important species, such as *C. upsaliensis*, to remain neglected. Accordingly, our understanding about host adaptation, transmission, and pathogenicity evolution in *Campylobacter* species could be enhanced by coordinating sequencing efforts among international groups, working as a consortium to fill the gap in genomic information for emerging campylobacters that exists today. As for many major pathogens, it is clear that the availability of genomic data and the information derived from subsequent comparative analyses constitutes a major opportunity for an improved understanding of the mechanisms driving *Campylobacter* evolution. In particular, this could facilitate the development of high-resolution tools for typing emerging species. For example, the development of core genome multilocus sequence typing (cgMLST) schemes like those currently available for *C. jejuni* and *C. coli* requires comprehensive genomic data sets that represent global isolate collections. Once cgMLST schemes, among other tools, become available for species like *C. fetus*, *C. ureolyticus*, *C. concisus*, or *C. upsaliensis*, understanding the genetic variation that underpins ecological and epidemiological patterns will be straightforward, enabling the rapid identification and more efficient tracking of emerging campylobacters, which will eventually result in more effective interventions. In parallel, the identification of genetic features associated with virulence traits will guide the development of new therapeutic procedures to prevent or control infections caused by species different from *C. jejuni* and *C. coli*, contributing to limiting the emergence and spread of new clinically relevant genetic variants.

The description of novel *Campylobacter* species has accelerated since 2009 (Fig. 2), mainly due to improved culturing conditions for *Campylobacter* and the exploration of new hosts and environments. Also, the recent application of culture-free methods, like 16S rRNA

amplicon sequencing, to study the microbiome of wild animals has allowed the identification of novel species, like *C. pinnipediorum* (30). This approach seems promising to discover new species but also to monitor the presence of emerging pathogens without the need for bacterial isolation, which can provide useful epidemiological information to guide more comprehensive sequencing efforts. Also, metagenomics can be applied to have a general overview of the human microbiome composition at the population level by analyzing samples from urban environments (118). For example, as the urban sewage microbiome has been shown to recapitulate the human gut microbiome, metagenomic sequencing of wastewater samples can provide information about the circulation of pathogens and antimicrobial-resistant genotypes in the population (119), including unappreciated *Campylobacter* species. Furthermore, as many emerging campylobacters (like *C. concisus*) have been found in healthy individuals, the reanalysis of thousands of public gut metagenomic data from healthy humans can help to recover genomic information from *Campylobacter* lineages that are being carried asymptomatically. This approach has proved useful to identify and quantify *C. fetus* lineages in the intestinal microbiota of healthy humans (64) and could be easily extended to analyze other species. Together, the application of integrative approaches that consider improved culture conditions, whole-genome sequencing, and microbiome analysis can set the basis for future research toward the elucidation of the epidemiological behavior and real clinical impact of emerging *Campylobacter* species.

CONCLUSIONS

Not every infectious disease is subject to epidemiological research. Hence, the resultant lack of information can lead to the unappreciated spread of pathogens which constitute public health emergencies. Indeed, this originally happened with *C. jejuni*, whose importance in human gastroenteritis was unseen until methodological improvements for isolation and identification became available. In this sense, emerging *Campylobacter* species that have been reported at a high frequency in certain geographic regions, like *C. upsaliensis*, are known to be underestimated because they are susceptible to some antibiotic combinations used in selective media for the isolation of *C. jejuni* and *C. coli*. This represents an important challenge, since the application of high-resolution tools to characterize these infections, like those based in comparative genomics, require the availability of bacterial isolate collections and clinical information. However, we envisage that constant improvements in clinical microbiology will gradually uncover the real burden of many emerging *Campylobacter* species. Indeed, this has already happened for some species, like *C. fetus*, which is currently recognized as the most frequent *Campylobacter* species recovered from human blood infections, after being largely underestimated and considered an infrequent pathogen. Thus, this underpinned the recent development of pathogenomic analyses that resulted in a better understanding of its epidemiology, evolution, and transmission.

Upcoming efforts focused on understanding the biology of emerging campylobacters will require the extensive use of pathogenomic approaches to comprehensively address a set of key unpostponable aspects, including the genomic screening of global collections of main emerging species, like *C. upsaliensis*, *C. ureolyticus*, or *C. concisus*, and the incorporation of microbiome data analysis to recover asymptomatically carried lineages or to evaluate the incidence of *Campylobacter* infection in the host microbiota composition. The outcomes of these studies will assist with the rational selection of genomic markers for the design of novel diagnostic assays, the development of tools for molecular epidemiology, and the identification of phenotype-genotype associations that can at last have an impact on improving the surveillance, control, and treatment of infections caused by emerging *Campylobacter* species.

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