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Campylobacter ureolyticus
A portrait of the pathogen

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Herein, we provide a brief overview of the emerging bacterial pathogen *Campylobacter ureolyticus*. We describe the identification of the pathogen by molecular as opposed to classical culture based diagnostics and discuss candidate reservoirs of infection. We also review the available genomic data, outlining some of the major virulence factors, and discuss how these mechanisms likely contribute to pathogenesis of the organism.

Introduction

"If it is thus, I ask emphatically whence comes this thusness."
— James Joyce, *A Portrait of the Artist as a Young Man*

The genus *Campylobacter* incorporates bacteria that are gram-negative, non-spore forming, spiral shaped rods.[1] Cells are typically between 0.5 to 5 microns in length, 0.2 to 0.9 microns in width, and are motile, typically via a polar unsheathed flagellum at one or both ends. *Campylobacter* generally demonstrate microaerophilic metabolic characteristics, but there are also species that are capable of growth in both aerobic and anaerobic conditions.[2] The term campylobacteriosis is used to describe the infectious disease that arises as a result of *Campylobacter* infection.[3] *Campylobacter* are a leading bacterial cause of zoonotic disease worldwide and are considered a foodborne pathogen.[4] *Campylobacter* related illness affects approximately 1% of the European population per annum, causing infection in 13 in every 100 000 individuals in the US each year and, as such, are a major public health concern.[5] The incubation period associated with campylobacteriosis tends to last between one and seven days, followed by the onset of the symptoms which can last from three days to a week. Symptoms include abdominal cramping, fever and diarrhea, with or without blood in the stools and in most cases the infection is self-limiting.[6] *Campylobacter* related infections can also lead to the onset of post infection complications including Guillian Barré Syndrome (GBS) or Miller Fischer Syndrome (MFS). GBS is a self-limiting autoimmune disorder of the peripheral nervous system associated with weakness of the limbs, respiratory muscles, and areflexia. Similarly MFS is associated with ataxia, opthalmoplegia, areflexia, and is a subform of GBS.[7] It is estimated that the annual incidence of campylobacteriosis costs the US between $1.3 to 6.8 billion dollars and thus is a significant economic burden.[8] This is likely to be an underestimate as it does not consider indirect costs such as liabilities, both physical and psychological, that are associated with *Campylobacter*-induced GBS.[9] The two predominant causative agents of *Campylobacter* related infections are *C. jejuni* and *C. coli*.[10,11] However, recent studies have identified another species of *Campylobacter* that appears to have a much more significant role in human campylobacteriosis than previously realized.

Campylobacter ureolyticus has been identified by our group as an emerging gastrointestinal pathogen.[12-18] This organism, like other members of the species, is a gram-negative, anaerobic, non-sporing bacillus.[19] *C. ureolyticus* is aflagellate as opposed to some of the more virulent species including *C. jejuni,* which possess flagella.[20]

C. ureolyticus was first identified as *Bacteroides corrodens,* owing to its ability to produce "corroding" colonies, from isolates taken from buccal abscesses by Eiken.[21] In order to differentiate between the facultative and strictly anaerobic strains Jackson and Goodman[22,23] subsequently classified them as a separate genera reserving *B. corrodens* for the anaerobes. The authors recommended the name *B. ureolyticus* for the strict anaerobes to avoid any confusion with the facultative species which had been given the name *Eikenella corrodens*.[22,23] *B. ureolyticus* remained the commonly used name for the organism until Vandamme et al.[24] concluded that there was insufficient evidence to reclassify *B. ureolyticus* as a member of the *Campylobacter* genus. The organism thus remained a species incertae sedis and remained as such for a further 15 y.[24] In 2010 Vandamme again reviewed the situation; this time completing the reclassification to *C. ureolyticus* on the basis of an array of evidence, including a 16S rRNA sequence similarity within the range of 91–93% to the species of the genus *Campylobacter*.[25] The six *B. ureolyticus* strains investigated showed >99% similarity with respect to each other using the same phylogenetic anchor. This has led to the formal reclassification of *B. ureolyticus* as *C. ureolyticus*.[25]

Increasing Incidence of Campylobacteriosis

Campylobacter related gastroenteritis is a prominent cause of acute bacterial illness in both developed and developing

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countries.[26] Despite the high rate of reported infections, it is estimated that rates may actually exceed the reported incidence by several factors.[9,10,27] In the majority of cases the primary causative agents of campylobacteriosis have been identified as *C. jejuni* and *C. coli*. Indeed, these two species combined account for in excess of 95% of isolates in cases of human gastroenteritis using routine diagnostic culture methods.[10,1117]

Following a study by O'Leary et al.[28] it was reported that 28.6% of stool samples positive for *Campylobacter* spp., using a multiplex PCR method, failed to grow using routine *Campylobacter* culture methods. A complementary study by Bullman et al.[12] involved 7194 fecal samples collected over a 12 mo period from patients presenting with diarrhea that were examined using EntericBio®, a multiplex-PCR system. This study identified 373 samples which were *Campylobacter* positive, of which 72.4% were *C. jejuni,* 24.4% were *C. ureolyticus*, and 6.7% were *C. coli*.[12] Of the 373 samples tested, 33 contained mixed infections.

Based on this study, *C. ureolyticus* now appears to surpass *C. coli* as the second most common causative agent of *Campylobacter*-related gastroenteritis (in samples collected from southern Ireland, which in our opinion is likely to represent the trend nationally). In accordance with this study 51.8% of *Campylobacter* detections were distributed largely between two age groups, children <5 y (31.1%) and adults >70 y (20.7%). Furthermore, *C. ureolyticus* was identified as the sole *Campylobacter* species isolated from 16.7% of patient samples, the distribution of which was similar to that of general *Campylobacter*-related infections with most common detection in female patients (62.7%). In addition to this, 50% of such isolations in female patients were observed in the >70 y age group.[12]

C. ureolyticus also demonstrates a seasonal distribution that varies from the norm associated with the other commonly implicated species, with peak incidence in early spring, particularly the month of March, between one and two months earlier than when *C. jejuni*/*C. coli* infections are most commonly seen. The least number of *C. ureolyticus* related cases were reported in December, in contrast to *C. jejuni*/*C. coli,* declining numbers of infection from September which then remained low during the winter months[12,29]. This divergence in seasonal distribution is likely to result from differing animal host preference; *C. ureolyticus* eschewing avians in favor of bovines whose peak of March corresponds to that of humans.

Source Investigation

Human campylobacteriosis is regarded as a zoonotic disease with transfer being mediated through the fecal–oral route and associated organisms have been reported to be incapable of multiplication outside endothermic hosts.[30-32] In addition, campylobacteriosis is largely considered to be a foodborne pathogen and Stafford et al.[33] have estimated that 75% of cases can be attributed to the consumption of food products contaminated with *Campylobacter*. However, this was rebutted by Gillespie,[34] reporting that the role of chicken consumption in cases of *Campylobacter* outbreaks had been overestimated by a factor of 3.4. The actual number of foodborne cases remains unknown owing to the difficulty in determination of the source of notified cases and lack of reporting due to the self-limiting nature of infection.[35,36] The preferred environmental niche has been identified as the avian intestinal tract.[37] The most common source and vehicle of campylobacteriosis has been established as contaminated poultry meat.[38,39] Poultry may be exposed to a variety of environmental sources of *Campylobacter* both on the farm and at the processing plant. *Campylobacter* species can spread rapidly throughout the flock and in particular, hatchlings. Studies have demonstrated that the majority of the flock can be colonized within three days of contact with a single bird infected with *Campylobacter* species.[40] A number of factors have been implicated in the horizontal transmission of the agent including; contaminated water, fecal contact, litter, and vectors including farm personnel, rodents, and insects.[41,42] Poultry feed is not considered to be associated with the spread of campylobacteriosis as it is too dry to facilitate the survival of the agent.[43]

In the region of 20–30% of human campylobacteriosis cases have been attributed to handling, preparation and ingestion of broiler meat, while 50–80% can be credited to the chicken reservoir as a whole.[44] *C. ureolyticus* differs from this paradigm in that it is not routinely identified (either by culture or molecular based methods) in poultry samples as demonstrated by Koziel et al.,[15] a factor that may be associated with its non-thermophilic phenotype. Indeed, Koziel et al. suggest that cattle as opposed to poultry are a possible reservoir.[15] This correlates with the study by Wilson et al.[4] where molecular analysis of 87 bovine samples revealed no evidence of *C. ureolyticus* in urine samples; however, 1 fecal sample was positive as well as 6 milk samples.

Furthermore, the ingestion of unpasteurized/raw milk and raw red meat has been implicated as sources of foodborne *Campylobacter* infections.[45,46] The association with milk may be as a result of udder infection such as *Campylobacter*-related mastitis or fecal contamination.[46] The first instance where unpasteurized milk was associated with *Campylobacter* infection was in Los Angeles in 1978 when four cases of *C. fetus* infection were identified in a hospital within a three week period. Three of these patients had consumed large amounts of an identical brand of commercially available certified raw milk from which *C. fetus* subspecies *jejuni* was isolated from the patients' blood.[47] This correlates with the findings of Koziel et al. which implicates bovine milk samples as a potential source of *C. ureolyticus* infection.[15]

A recent study by Taylor et al. found that 5% (12/262) of outbreaks of *Campylobacter* infection in the US between 1997 and 2008 were as a result of the consumption of contaminated game, beef or pork.[48] There is increasing evidence to suggest that cattle serve as a reservoir for *Campylobacter* species that are associated with gastroenteritis in humans.[49] The gastrointestinal tract of clinically normal cattle has been identified as a significant reservoir for a number of species of *Campylobacter*.[50] Calves have been identified by Stanley and Jones[49] as having greater rates of bacterial shedding than adult cattle. Inglis et al.[45] conducted a study involving 300 faecal extractions for *Campylobacter* species using PCR based methodology. Campylobacters were detected in 89.6% (n = 268) of samples with a consistent frequency among

the five sample times studied (28 d intervals). It was noted that all animals shed *Campylobacter* on at least one of the sample times. It was found that most (66.1%) of the animals tested shed campylobacters at all sample times (over 4 mo), in addition it was also noted that 20.3% of animals were found to be positive at 4 out of 5 of the sample times.[45] Depending on the rates at which cattle shed *Campylobacter* they may be regarded as a "permanent" or "chronic shedder", to limit and control transmission to other cattle, Stanley and Jones[49] propose culling as a potential means of doing so.[49] Eliminating high shedders may limit the contamination of carcasses and equipment within the abattoir as well as preventing transmission within the herd.[45] At the time this study was conducted *C. ureolyticus* had not yet been reclassified and the presence of this organism may have been overlooked. Hanninen et al.[51] implicated contaminated water as a means of *Campylobacter* transmission among cattle.

The incidence of *C. ureolyticus* in cattle at different stages of the year may be associated with the seasonal distribution of *C. ureolyticus*-related gastroenteritis.[12] More extensive research will be required to investigate the prevalence of this pathogen in cattle samples, its zoonotic potential as well as to determine the likelihood of bovine milk as the true source of *C. ureolyticus* in patients presenting with related gastroenteritis.

Strain Heterogeneity

Comparative genomic studies between related species and strains of bacterial pathogens has demonstrated that a "one size fits all" approach cannot be applied to the evolutionary dynamics of bacterial pathogenomics, giving rise to the concept of genomospecies. These are associated with a degree of variation of the genome of a particular species of bacteria.[52-54]

An important consideration in the emergence of genomospecies is the "eco–evo" perspective on host-pathogen interactions. It is becoming increasingly evident that a single strain rarely typifies an entire species. This is based on genomic evidence that commonly used laboratory strains have undergone extensive changes, producing genotypic and phenotypic variants during their descent from the environmental, free living agent.[55,56] A key point in the "eco–evo" perspective is the identification of the selective advantages of virulence factors in the pathogens lifestyle as well as the consideration that what, at first, appear to be virulence factors are also encoded in the genomes of non-pathogenic organisms.[57] There are a number of potential explanations for this; first, that pathogenic, commensal, and symbiotic microorganisms rely on similar strategies and molecular systems in their interaction with eukaryotic hosts.[57] In addition, it has been demonstrated that many pathogens produce virulence factors that provided an advantage in a previous, now non-existent, niche. Finally, many bacterial infections occur incidentally in humans and produce virulence factors that are active against non-mammalian antagonists. It is therefore evident that many bacterial virulence factors have been influenced by a range of evolutionary forces, not limited to the context of human pathogen interactions. The emergence of human infections can be understood and predicted by studying these influences and may yet prove an important consideration in the emergence of *C. ureolyticus* as a gastrointestinal pathogen.[57]

Recent studies suggest that *C. ureolyticus* may in fact be made up of a number of genomospecies similar to those observed in *C. concisus*.[58,59] Similarly, in *C. jejuni* the existence of several tens of homopolymeric base repeat sequence can result in slippage during the process of DNA replication, giving rise to a plethora of structures exposed on the cell surface.[60] While phenotypically an isolate may appear to be similar or even identical to another isolate of the same species, it is possible that it may demonstrate a large degree of heterogeneity at the genomic level.[61,62] This concept of intra-species variation provides a logical compromise to a recent differing of opinion as to the true pathogenic nature of *C. ureolyticus*.[18,63] Cornelius et al.[63] reporting the identification of *C. ureolyticus* in 12 (24.5%) of 49 healthy volunteers[12] concluded that *C. ureolyticus* species "are unlikely causes of diarrhea". While variations in diagnostic methods likely account for a certain degree of variability, a detection rate of 24.5% in healthy volunteers (overall detection rate 14.7%) as compared with our reported rate of 1.15%[12] is most likely due to the existence of subtypes exhibiting varying degrees of virulence potential.

A recent study by Bullman et al.[14] found that 75–79.5% of proteins were highly conserved (70% identity) between the two *C. ureolyticus* strains whose genomes are currently available on the databases (i.e., DSMZ 20703 and ACS-301-V-Sch3b). Using the same parameters with *C. jejuni* NCTC11168 set as the reference genome, individual and multiple comparisons to 3 other *C. jejuni* strains, revealed that 92% and 87% of proteins respectively are highly conserved. Such data suggest that substantial variation exists within these two *C. ureolyticus* genomes. Interestingly, average percentage identities for all homologs revealed that *C. ureolyticus* strains had a higher variation when compared with the phylogenetically related species *C. jejuni* (94% vs. 98%, respectively). Furthermore, whole genome comparison of the protein encoding sequences of the two *C. ureolyticus* strains (DSMZ 20703 and ACS-301-V-Sch3b) against other members of the same genus exhibit conservation across the different species of *Campylobacter* with 9–22% of gene products conserved. The largest number of conserved protein homologs were found in *C. concisus* with the lowest identified within *C. upsaliensis.*

There were 128 protein coding sequences identified as being highly conserved across all species of *Campylobacter* when DSMZ 20703 was used as the reference genome, with functions ranging from membrane bound transporters, respiration, and metabolism of macromolecules as well as stress response mechanisms. In comparison, *C. upsaliensis* RM3195 and *C. lari* RM2100 shared the lowest percentage of conserved protein encoding sequences, with up to 40% of *C. ureolyticus* proteins lacking homologs within the genomes of these species. The genomes of DSMZ 20703 and ACS-301-V-Sch3b have a similar estimated size, 1.74 Mb and 1.66 Mb respectively; however analysis indicates that only 18.8% (341/1810) and 17.1% (290/1700) of the protein coding sequence of these strains are unique (i.e., not found in the other).[14]

In accordance with the observations of Goris et al.[64] the variation between strains demonstrated by Bullman et al.[14] is

suggestive of species delineation with a cut-off value for species of 85% conserved genes for two strains.[64] It is entirely likely that there will be fundamental functional differences between the two strains with regard to both constitutive and virulence functions due to the genomic heterogeneity.[14]

In addition to the significant degree of variation observed between the genomes of DSMZ 20703 and ACS-301-V-Sch3b strains, protein profiles of a further 6 *C. ureolyticus* isolates, in which strains are clustered in accordance with banding patterns, contributes to further demonstrating the high degree of heterogeneity between strains. Bullman et al.[14] conducted whole genome analysis of at least 12 *C. ureolyticus* strains (CIT01–CIT13) which were isolated from animal reservoirs, asymptomatic patients, and patients with diarrheal illness, the initial results of which suggest substantial heterogeneity between different *C. ureolyticus* strains, supported by comparisons of whole genome coding sequences between these strains. It was demonstrated that 2–20% of their proteins are unique based on paired genome comparison of the coding sequences of 14 *C. ureolyticus* strains. Additionally, the individual comparison of the protein coding sequences of the 12 *C. ureolyticus* isolates with ACS-301-Sch-V-3b and DSM 20703 demonstrated that 9–16% and 13–19% of proteins respectively are unique.[14]

Secretome and Virulence Factors/Toxins

The bacterial secretome is defined as the totality of the secreted proteins and is characterized by a dynamic nature, undergoing variations and adjustments to meet that required by the established environmental conditions.[65] In *C. ureolyticus* the function of many of the secreted proteins remains putative pending, experimental determination; however molecular methods give a reliable indication of what these proteins are and their potential functions based on comparison with other *Campylobacter* species such as *C. jejuni*. While there is an extensive list of such proteins we are only going to discuss those which may be key virulence factors in the pathogenesis of *C. ureolyticus*. Bullman et al.[14] identified a total of 288 proteins which were estimated to be released by DSMZ 20703 with 25 being associated with putative virulence functionalities. In comparison, ACS-301-V-Sch3b was predicted to express 269 protein products, 28 of which are proven to have a role in virulence of the organism. In the same study, 13 hemolytic cytotoxins and cytolysin-related proteins were identified (8 of which were predicted to be secreted). These pore-forming toxins may represent an important component of the virulence potential of *C. ureolyticus* as they function to increase the availability of iron during infection and as such help to promote human disease.[66] Within the group of emerging *Campylobacter* species including *C. ureolyticus*, 8 repeat-in-toxin (RTX) related proteins were identified, 6 of which were predicted to be secreted. The use of a type I secretion system to allow protein export across the bacterial envelope is a characteristic feature of these exoproteins. The functionality of these proteins is regulated by the availability of Ca^{2+} ions which sequesters activity until outside of the cell.[67] Further to this, 3 of the secreted RTX hemolysins show evidence of iron regulation with homology to the FrpC RTX protein of *Neisseria meningitidis*. The detection of antibodies directed against the FrpC protein in the serum of patients infected with the pathogen demonstrates that this protein is produced in vivo during the infection cycle; however its exact role remains unclear.[68-70] The release of high levels of toxins causes cells to lyse as pores formed by the toxin allow cytoplasmic contents to leak out of the cell.[71] In relation to this, an S-layer RTX protein was shown to be secreted by the *C. ureolyticus* strain UNSWCD, which had been isolated from an intestinal biopsy of a young Crohn disease patient.[72] These proteins are typically associated with complement resistance as well as providing structures that enable bacterial adherence to the host cell.[73]

C. ureolyticus also encodes a number of adhesins which are proteins that play an important role in host cell interaction and aid in the initial establishment of infection.[74] There have been 2 genes identified, unique to DSMZ 20703, that code for the production of the HecA protein, a member of the filamentous hemagglutinin adhesin (FHA) family. The *hecB* locus has been identified directly upstream of the *hecA* genes and codes for a hemolysin activation protein.[14] The protein products of these genes form a two-partner secretion (TPS) system, in which a TpsA family exoprotein (with a specific conserved secretion signal) is recognized by the associated TpsB family channel-forming transporter permitting passage through the membrane.[75] The *hec*A gene within the HecA/B operon has a G+C content that is 17% lower than the remainder of the *C. ureolyticus* genome sequence (G–C content 29%). In line with the "eco–evo" theory outlined above, and documented lateral gene transfer of *hec*A in other organisms, it is possible that this adhesin may have been acquired from a bacteria outside the *Campylobacter* genus, possibly during occupation of the same ecological niche.[57,76,77] One potential source of this gene may be the *Fusobacterium* species, which like *C. ureolyticus*, has been implicated in gastroenteritis and periodontitis.[19,78-80]

Bullman et al.[14] identified a number of genes that code for proteins involved in sialic acid metabolism.[14] This includes *Sia*A (NeuC superfamily), *sia*B (CMP-Neu5Ac-synthase), and *sia*C (NeuB superfamily), which are involved in de novo sialic acid synthesis. These genes are homologous to those of *N. meningitidis* with 50–76% identity and 96–100% coverage over the entire amino acid sequences. These are important virulence factors in *N. meningitidis*; studding the surface of the capsule polysaccharide component and LOS with sialic acid enables the bacteria to adhere, colonize, endure, and evade host immune mechanisms and cause the onset of disease in mammals.[81,82]

A PEB1 homolog has also been identified in *C. ureolyticus* strains which have been demonstrated to have a role in virulence and colonization by *C. jejuni*.[83] Mutations in the associated gene results in a 50- to 100-fold decreased adherence to epithelial cells maintained in cell culture and 15-fold fewer invasions of such cells.[84] The intracellular multiplication factor, IcmF, has also been identified in *C. ureolyticus*. This protein is a constituent of a Type VI secretion system (T6SS) which has recently been identified as having a role in bacterial virulence within eukaryotic host cells and may contribute to human pathogenesis.[85,86] For a

comprehensive list of secreted proteins in *C. ureolyticus* we direct the reader to the paper by Bullman et al.[14]

GI Pathogenesis

A number of research groups have investigated the ability of *C. ureolyticus* to cause GI related illness. *C. ureolyticus* demonstrated minimal attachment to the colorectal cell lines, Caco-2 and HT-29 as shown in a study conducted by Man et al.[82] However, in cases where the bacterium was able to adhere to cells, it used a flagellum-independent mechanism of attachment. This non-flagellate sticky end mechanism attracts nearby microvilli to the bacterial cell surface, which may be mediated by the presence of surface adhesions.[87] Attachment by *C. ureolyticus* caused the destruction of the filamentous microvilli structure causing Caco-2 cell lines to lack highly dense accumulations of microvilli on the apical surface when compared with a negative control sample.[88] This finding was supported by Fontaine et al.[89] who showed that *C. ureolyticus* was capable of causing loss of ciliary action from the epithelial cells of the mucosal surface of human fallopian tubes by causing the disruption and sloughing of cells. Ganan et al.[90] demonstrated that *C. jejuni* strain 118 adhered to Caco-2 cells at a rate of 2.56% (at an exponential phase of 18 h). In comparison Burgos-Portugal et al.[72] showed that *C. ureolyticus* UNSWCD attached to cells at a rate of 5.4%,[72] supporting the pathogenic potential of *C. ureolyticus* by demonstrating greater adherence than the principally associated *Campylobacter* species.[90]

A number of proteins have been identified that may mediate *C. ureolyticus* attachment, including the surface antigen CjaA protein. This is a surface exposed protein with homology to ABC transport systems and is also found in *C. jejuni* where it is immunodominant.[91] In addition, two proteins, fibronectin like protein A (FlpA) and a *Campylobacter* adhesion to fibronectin (CadF) homolog, an outer membrane protein which is widely known to mediate cellular adhesion by binding fibronectin has been observed to be secreted by *C. ureolyticus*.[92]

It has been shown that *C. ureolyticus* does not invade Caco-2 cells using gentamicin protection assays, this finding correlated with the results of scanning electron microscopy.[87] *C. ureolyticus* has been shown to be capable of trans-locating across the cell monolayer and invading paracellularly, suggesting that their pathogenic mechanisms differ from those of *C. jejuni,* which invades transcelluarly.[88] Evidence supporting this finding includes the presence of fibronectin-binding proteins and the presence of the zona occludins toxin (Zot). Zot induces a reversible opening of the tight junctions and leads to an increase in paracellular permeability between cells. This is a non-toxic virulence strategy commonly used by other pathogenic organisms such as *Vibrio cholerae* and *Neisseria meningitidis*.[93,94] It has been postulated by Bullman et al.[14] that *C. ureolyticus* targets the tight junctions of host cells, to invade via a paracellular route, by expressing Zot and then binds to fibronectin of the cellular basolateral surface via the secreted fibronectin binding proteins including CadF and FlpA. Furthermore, Bullman et al.[14] detected two further proteins that are likely to contribute to pathogenesis of this organism, *Campylobacter* invasive antigen (CiaB) and phospholipase A (PldA),[14] both of which have been linked to an invasive *C. jejuni* strain.[95]

Burgos-Portugal et al.[72] also identified that *C. ureolyticus* is capable of aggregating to inert material as well as living surfaces.[72] This behavior is commonly seen in organisms that are capable of forming biofilms in which the bacteria are surrounded by an exopolymeric matrix which forms a structured cellular community which adhere to one another and to the surface. This feature assists cell to cell communication and aids virulence and antibiotic resistance.[88] This study also demonstrated that the ability of *C. ureolyticus* to cause infection is not promoted by the existence of inflammation as shown by stimulating cell lines with pro inflammatory cytokines such as TNF-α and IFN-γ, which did not significantly affect the ability of cells to adhere to the cell monolayer.[72] This finding was confirmed by scanning electron microscopy, and is in contrast to other *Campylobacter* species including *C. concisus* UNSWCD where the presence of inflammation has been identified in promoting adherence and invasion.[87] Burgos-Portugal et al.[72] measured the level of IL-8 produced by cells exposed to TNF-α and IFN-γ in order to detect evidence of inflammation, it was found that exposure to these cytokines caused cells to produce significantly larger amounts of IL-8 and thus inflammation. Furthermore, cells that were infected with *C. ureolyticus* UNSWCD produced higher levels of IL-8 than the negative control. The addition of IFN-γ to *C. ureolyticus*-infected cells produced much larger amounts of IL-8 in comparison to non-infected cells exposed to IFN-γ. In contrast, when TNF-α was added to cells infected with *C. ureolyticus* it was noted that the cells produced IL-8 levels similar to non-infected cells that had been treated with TNF-α. These results indicate that this strain of *C. ureolyticus* can elicit a mild inflammatory response of the gastrointestinal epithelium and even more so on exposure to the cytokine, IFN-γ.[72]

Additional Pathology

Campylobacter ureolyticus has been implicated as a contributing species in many pathogenic conditions other than those related to the gastrointestinal tract. A study conducted by Duerden et al.[96] examined this from the perspective of skin and soft tissue infections (SSTIs) and studied 103 isolates from superficial necrotic or gangrenous lesions where the infection appeared to contribute to tissue damage. *C. ureolyticus* (or *Bacteroides ureolyticus* as it was known at the time of the study) was not routinely isolated in pure culture and was rarely the only pathogen present. It was reported as the sole isolate from only 5 of the 103 samples tested. This correlates with the work of Bullman et al.[13] in relation to gastrointestinal illness, where *C. ureolyticus* was found as the sole pathogenic agent in a relatively low number of cases yet with a higher incidence than in this study suggesting its greater affinity as a causative agent of gastrointestinal illness as well as its emerging nature.

The work performed by Duerden and colleagues[96] identified the most common region of infection to be the perianal area.

The study identified 27 isolates from the perineum and genitalia in addition to 15 samples identified from perianal abscesses. There was no apparent incidence variation based on gender in relation to the perianal and genital isolates, 14 in men and 13 in women. The cases identified as having *C. ureolyticus* as a contributing pathogen included; necrotic cellulitis, gangrene and abscesses on the scrotum, vulva, or penis, in addition to a single case of postpartum uterine infection. Infections were a mixture of primary type and complications of surgery. Isolates were also obtained from sebaceous cysts, pilonidal abscesses as well as axillary abscesses.[96] Research has often implicated anaerobic species including *C. ureolyticus* as a causative agent of decubitus and varicose ulcers.[97] However, many clinicians dispute this, believing instead that the primary lesion occurs as a result of vascular insufficiency and that bacterial infection has a much less significant role. The results compiled by Duerden et al.[96] correlated with those who believed that *Bacteroides* spp. (including *C. ureolyticus*) played a contributing role in the pathogenesis of decubitus and varicose ulcers, isolating 16 strains from ulcers or gangrenous lesions in the lower limbs of such patients. Furthermore, *C. ureolyticus* was implicated in a variety of other infections associated with tissue necrosis with or without abscess formation. Duerden's group[96] isolated this agent from oral abscesses including root canal infections and submandibular abscesses. This association with soft tissue pathology in the mouth corresponds with later studies by Deurden which identified the contribution of *C. ureolyticus* to adult periodontal disease, bacteria that are not usually associated with the gingival flora.[19]

An interesting finding of Duerden et al.[96] was the association of *C. ureolyticus* with anaerobic gram-positive cocci which were isolated together with *C. ureolyticus* from over half the lesions. The association of anaerobic bacteria with superficial gangrene and other related lesions has been recognized for many years. Anaerobic cocci have also been implicated among other bacteria in conditions called synergistic bacterial gangrene.[98] The pathology associated with *C. ureolyticus* differs from that which is typical for the most common group of anaerobe-related infections including: peritonitis, intra-abdominal abscesses, postoperative wound infections, and diseases of the large intestine and the appendix. *C. ureolyticus* has also been implicated in cases of non-gonnococcal urethritis, bacterial vaginosis, and lesions of the female genital tract.[99,100] In recent years the majority of studies in relation to the pathogenic potential of *C. ureolyticus* have focused on its implications in gastrointestinal pathology and as such much of the information in relation to other disease processes has not been re-evaluated in a number of years.

Recent studies have suggested that *C. ureolyticus* may play a significant role in ulcerative colitis (UC). Mukhopdhya et al.[78] identified this agent in 15 out of 69 (21.7%) *Campylobacter* positive samples as opposed to 2 out of 65 controls (3.08%). Overall the incidence of *Campylobacter* in UC was 73.9% in comparison to 23.1% prevalence in controls.[78] In addition, the most commonly found combination of *Campylobacter* species in adult patients with UC was *C. hominis* and *C. ureolyticus* which was evident in 8.7% of all cases. As *C. ureolyticus* has only been recently implicated as a cause of diarrheal illness[12,13] the significant association documented with UC may suggest patient susceptibility to *Campylobacter* colonization with genus specificity as opposed to species-related factors. This reasoning is further supported by the increased number of mixed infections including relatively rare members of the *Campylobacter* genus such as *C. gracilis*, *C. curvus*, as well as *C. showae* in adult patients affected by UC in comparison to controls.[78] In comparison, *C. ureolyticus* was found to play a less significant role in patients with Crohn disease with a prevalence of 13% (2 of 15 cases). In contrast, the control group showed a prevalence of 6% (2 of 33). This association with Crohn was not investigated further and as such no definitive conclusion can be made as to whether or not *C. ureolyticus* has an influence on the pathology of this condition. Furthermore, *C. ureolyticus* was found in significantly fewer cases than the most prevalent *Campylobacter* species; *C. concisus* (67%, 10 of 15 cases) similar to the results indicated for cases of UC.[78,101]

For both UC and Crohn, cause-and-effect is still unclear; it is entirely likely that the increased incidence of *C. ureolyticus* in both of these conditions may be the result of a more favorable intestinal environment for the pathogen, rather than a definitive indication that the bacterium plays an initiating role in the disease or contributes to its causation.

Conclusion

C. ureolyticus is an important emerging, gastrointestinal pathogen. Many routine laboratory *Campylobacter*-culturing techniques fail to identify this organism and a molecular approach of detection is the best for diagnosis. Its incidence may be associated with patients who have immune disorders with cases being identified in patients who are immunocompromised and have diabetes mellitus or HIV.[12,13] It is becoming increasingly more important and has surpassed *C. coli* as the second most common cause of campylobacter-associated gastroenteritis. The majority of research into *C. ureolyticus* is relatively novel due to its recent reclassification and identification as an emerging pathogenic species. Its genome has recently been sequenced and research is ongoing to identify its virulence proteins (106 potential virulence related factors have been identified by Bullman et al.[14]).

While an efficient means of treating infection has not yet been established, we argue that the focus should be on prevention, particularly relating to consumable products from cattle such as meat and milk which have been implicated as a source of *Campylobacter* species and *C. ureolyticus* respectively. Potential intervention protocols include the enforcement of strict on-farm biosecurity measures including the disinfecting of water supplies and farm premises, restricting access of livestock to essential personnel and taking measures to ensure that food supplies are not subject to bacterial contamination.[4] Investigation into the use of phage to control *Campylobacter* species in poultry has been explored and has become increasingly attractive as a means of preventing the emergence of antibiotic resistant strains of *Campylobacter* spp. Preliminary studies have been promising but requires further investigation and development.[102] A potential problem in this line of treatment is the observation that in vitro

results do not always correlate with those obtained from in vivo studies.[103]

In summary, further research is warranted to fully elucidate the organism's pathogenesis as well as the development of effective treatment regimens. While many questions relating *C. ureolyticus* remain unanswered; at least they have begun to be asked.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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