MINIREVIEW

Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*

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Abstract

Campylobacter jejuni and *Campylobacter coli* are recognized as the most common causative agents of bacterial gastroenteritis in the world and infections with these organisms occur more frequently than do infections due to *Salmonella* species, *Shigella* species, or *Escherichia coli* 0157:H7. The incidence of human *Campylobacter* infections has increased markedly in both developed and developing countries worldwide and, more significantly, so has the rapid emergence of antibiotic-resistant *Campylobacter* strains, with evidence suggesting that the use of antibiotics, in particular the fluoroquinolones, as growth promoters in food animals and the veterinary industry is accelerating this trend. In this minireview, the patterns of emerging resistance to the antimicrobial agents useful in treatment of the disease are presented and the mechanisms of resistance to these drugs in *Campylobacter* spp are discussed.

Introduction

Campylobacter species, from the δ–ε group of *Proteobacteria*, are microaerophilic, nonspore-forming Gram-negative curved or spiral bacilli that are motile by means of unipolar or bipolar flagellae (Parkhill *et al*., 2000; Allos, 2001; Padungton & Kaneene, 2003). Campylobacteriosis is a collective description for infectious diseases caused by members of the genus *Campylobacter* (Coker *et al*., 2002). Of the infectious diseases caused by members of the *Campylobacter* genus, *Campylobacter* gastroenteritis due to *Campylobacter jejuni* and *Campylobacter coli* is the only form of disease that is of major public health importance (Nachamkin & Blaser, 2000) and increasing antimicrobial resistance in both medicine and agriculture in *Campylobacter* is recognized by various national authorities including the World Health Organization (WHO) as a major emerging public health concern (Moore *et al*., 2006). Although *C. jejuni* and *C. coli* have both been implicated as causes of diarrhoeal disease, including the most common cause of diarrhoea in travellers from developed nations (Allos, 2001), *C. jejuni* is the species most frequently isolated in cases of human infection (Padungton & Kaneene, 2003). In the United States, $>99\%$ of reported infections with *Campylobacter* are with *C. jejuni* (Friedman *et al*., 2000).

In different developed countries, incidences of *Campylobacter* infection are estimated at between 27 and 880 notifications/100 000 population (Friedman *et al*., 2000; Blumer *et al*., 2003; Gallay *et al*., 2003; Takkinen *et al*., 2003; CDC, 2004; Unicomb *et al*., 2006), and in tropical developing countries where *Campylobacter* infection is hyperendemic among young children (Allos, 2001), community-based studies estimate incidences of *Campylobacter* infection rates for children <5 years of age at between 40 000 and 60 000 notifications/100 000 population (Oberhelman & Taylor, 2000; Coker *et al*., 2002). In both industrialized and developing countries, *Campylobacter* remains one of the most common bacterial causes of diarrhoea.

Emerging resistance to the drugs of choice for treatment of *C. jejuni* infections

Macrolides and fluoroquinolones are normally considered for treatment of *Campylobacter* enteritis (Aarestrup & Engberg, 2001; Allos, 2001; Coker *et al*., 2002); tetracyclines would rarely be used. Intravenous aminoglycosides are

considered the treatment of choice for serious bacteraemia and other systemic infections due to *Campylobacter* (Aarestrup & Engberg, 2001). Until recently, however, if antimicrobial therapy was indicated for *Campylobacter* infection, fluoroquinolones were generally considered as the first choice. The fluoroquinolones were also considered useful for the prophylaxis of travellers' diarrhoea (Taylor *et al*., 1991; Petruccelli *et al*., 1992). This approach was adopted because the symptoms of *Campylobacter* enteritis essentially mimic those of bacterial gastroenteritis caused by other enteric pathogens, such as *Salmonella* and *Shigella* spp. Because of the susceptibility of these pathogens to fluoroquinolones, empirical treatment for severe gastroenteritis with these drugs could be used without waiting for culture results (Allos, 2001).

Antimicrobial resistance has become a major public health concern in both developed and developing countries in recent years (Isenbarger *et al*., 2002; Nachamkin *et al*., 2002). *Campylobacter* with resistance to ciprofloxacin or other fluoroquinolones, macrolides and lincosamides, chloramphenicol, aminoglycosides, tetracycline, ampicillin and other β-lactams, cotrimoxazole, and tylosin have been reported (Padungton & Kaneene, 2003; Moore *et al*., 2006). In the past decade, a rapidly increasing proportion of *Campylobacter* strains world-wide have developed resistance to the fluoroquinolones. In 1995, the incidence of fluoroquinolone resistance in *Campylobacter* isolates from Thailand was reported as 84% and, in 1997–1998, the incidence of fluoroquinolone resistance in Spain was reported as 72%. Incidence of resistance to the fluoroquinolones has also increased in the United States, United Kingdom, and the Netherlands. In 1998–1999, the proportion of *Campylobacter* isolates resistant to fluoroquinolones was reported as 10%, 18%, and 29%, respectively (Allos, 2001).

Fluoroquinolone-resistant *C. jejuni* were recognized during the late 1980s in Europe, where researchers suggested that such resistance was due, in part, to acquisition of fluoroquinolone-resistant strains from animal sources (Engberg *et al*., 2001). Several studies have linked the use of antimicrobial agents, in particular the fluoroquinolones, in the agricultural industry and veterinary medicine, with the emergence and spread of resistance among *Campylobacter* strains, with potentially serious effects on food safety and both veterinary and human health (Endtz *et al*., 1991; Jiminez *et al*., 1994; Khachatourians, 1998; McDermott *et al*., 2002; van Boven *et al*., 2003; Griggs *et al*., 2005; Humphrey *et al*., 2005). The initiation of the administration of the fluoroquinolone, enrofloxacin, to food animals began in the early 1990s in Asia and in European countries such as Sweden, the Netherlands and Spain coincided with primary resistance to fluoroquinolone therapy in humans in those countries (Endtz *et al*., 1991). Similarly, in the United Kingdom, resistance to fluoroquinolones in *Campylobacter* isolates was observed after the approval of the use of the fluoroquinolones as growth promoters in veterinary animals (Sam *et al*., 1999). In the United States, the introduction of sarafloxacin and enrofloxacin in the mid-1990s for use as growth promoters in poultry flocks also contributed to fluoroquinolone resistance, with resistance among *Campylobacter* isolates from humans increasing from 1.3% in 1992 to 10.2% in 1998 (Nachamkin *et al*., 2002).

In the Netherlands, resistance to fluoroquinolones in *Campylobacter* isolates from food animals has been observed since the early 1990s. Because then, there have been several reports of antimicrobial resistance in *Campylobacter* spp. isolated from food animals in both developed and in developing countries (Padungton & Kaneene, 2003). High-level resistance to the fluoroquinolones was reported in the Netherlands (Jacob-Reitsma *et al*., 1994), Spain (Saenz *et al*., 2000), Germany (Luber *et al*., 2003), and Taiwan (Li *et al*., 1998). Comparatively, the proportion of human isolates of *Campylobacter* resistant to fluoroquinolones in Spain and Taiwan was 72% and 52%, respectively, while 99% of *Campylobacter* isolated from broilers and pigs in Spain were resistant to fluoroquinolones, and 92% of isolates from chickens in Taiwan were resistant to the same agent (Li *et al*., 1998; Saenz *et al*., 2000). In 2001, in Germany, the proportion of human *Campylobacter* isolates resistant to ciprofloxacin was 41–46%, while 42% and 71% of chicken isolates of *C. jejuni* and *C. coli*, respectively, were resistant to ciprofloxacin (Luber *et al*., 2003). In Denmark, the incidence of resistance to several antimicrobial agents, including tylosin, virginiamycin, and erythromycin, by the *Enterococci*, reduced after these agents were banned from the market (Aarestrup *et al*., 2001). These observations indicated a significant association between antimicrobial use as growth promoters in food animals and the prevalence of bacteria resistant to the same antimicrobial agents.

Further, the selective pressure of therapeutic fluoroquinolone administration for clinically relevant infections in poultry flocks has been demonstrated unequivocally to select for ciprofloxacin-resistant campylobacters in commercially- reared poultry that enters the food chain (Griggs *et al*., 2005; Humphrey *et al*., 2005). Resistance was not as a result of the spread of a single resistant clone, but that numerous clones were selected by fluoroquinolone treatment (Humphrey *et al*., 2005). The *gyrA* mutations identified among the poultry isolates were identical to those described in human clinical isolates, the majority conferring high-level ciprofloxacin resistance (Griggs *et al*., 2005).

In contrast, quinolone resistance among strains of *C. jejuni* and *C. coli* in Australia remains low and this is attributed to the infrequent use of antibacterials for the treatment of diarrhoea and the regulatory prohibition on the use of fluoroquinolones in food-producing animals in

Australia (Huysmans & Turnidge, 1997; Unicomb *et al*., 2003). Supporting this premise, *Campylobacter* species isolated from pigs in Australia have been shown to be uniformly susceptible to ciprofloxacin (Hart *et al*., 2004), although other data regarding antimicrobial susceptibility among *Campylobacter* isolates infecting Australian animals are limited.

In addition, induction of fluoroquinolone resistance during treatment is also well recognized and has been reported (Adler-Mosca *et al*., 1991; Ellis-Pegler *et al*., 1995). A predicted 10% of patients treated with a fluoroquinolone for *Campylobacter* enteritis reportedly harbour quinolone-resistant *Campylobacter* strains (Endtz *et al*., 1991) and development of resistance has been reported within 24 h of treatment with fluoroquinolones, but prolonged therapy, especially in the immunocompromised, is also a risk factor (Engberg *et al*., 2001). A natural resistance to the fluoroquinolone ciprofloxacin in bacterial populations isolated within city-area soil of Vancouver was also reported (Waters & Davies, 1997). DNA sequencing revealed a high degree of variation in DNA gyrase, the target of the fluoroquinolones, and therefore, without any selective pressure, these bacteria showed the same alterations in the target sequence as those isolated from clinical environments (Khachatourians, 1998).

The macrolides are now generally considered to be the optimal drug for treatment of *Campylobacter* infections; however, resistance to macrolides in human isolates in some countries is becoming a major public health concern. Although macrolide resistance is infrequent and stable in most countries, resistance rates of *C. jejuni* to the macrolides of 10% and 11% have been reported in Taiwan and Spain, respectively (Gaudreau & Michaud, 2003), while higher rates of resistance of 31%, 51%, and 79% have been reported in Bulgaria, Singapore, and Nigeria, respectively (Gibreel & Taylor, 2006); resistance to macrolides is found to be more prevalent in *C. coli* than *C. jejuni* (Padungton & Kaneene, 2003). Despite decades of use, however, trends over time for erythromycin resistance of human *Campylobacter* isolates show stable and low rates in countries including Sweden, Finland, and Japan (Allos, 2001; Padungton & Kaneene, 2003). The use of antimicrobial agents, including macrolide derivatives in food animals, creates selective pressure for the emergence and dissemination of resistance among human pathogens that have food-animal reservoirs (Gibreel & Taylor, 2006). Spiramycin and erythromycin, which are used to treat bovine mastitis, and tylosin, which is used in feedlot cattle to prevent hepatic abscesses and in swine production as a growth promoter, may also select for erythromycin resistance.

Induction of macrolide resistance during treatment of *C. jejuni* enterocolitis is rare but has been reported most frequently from among patients with HIV infection (Gaudreau & Michaud, 2003). The newer macrolides, azithromycin and clarithromycin, also have activity against *C. jejuni* infections; however, their cost prohibits their use in most instances as they provide no clinical advantage over erythromycin (Allos, 2001). Clarithromycin MIC_{90}s have been reported as only twofold higher than those for erythromycin (Hardy *et al*., 1988) and compared with erythromycin, azithromycin does not show increased efficacy against *Campylobacter* sp. (Gibreel & Taylor, 2006). The emergence of *Campylobacter* isolates resistant to both quinolones and azithromycin in Thailand has been reported (Hoge *et al*., 1998) and threatens their usefulness in this demographic region.

Mechanisms of antimicrobial resistance in *Campylobacter*

Campylobacter species have been shown to possess the genetic mechanisms for natural transformation and conjugation, indicating that if antibiotic resistance genes were acquired, the trait would be rapidly transferred between strains. Indeed, antibiotic-resistance determinants *tetO* and *aphA-3* are believed to have been acquired outside the genus *Campylobacter* from Gram-positive cocci (Taylor & Courvalin, 1988; Zilhao *et al*., 1988) and have been incorporated into the *Campylobacter* genome by heterologous genetic exchange (Taylor, 1992).

Aminoglycoside resistance

Aminoglycoside–aminocyclitol resistance genes are present in many bacterial species and commonly encode proteins that modify these antimicrobials. The enzymes are divided into three different groups (aminoglycoside phosphotransferases, aminoglycoside adenyltranferases, and aminoglycoside acetyltransferases) based on the reaction they mediate; however, all three act via a similar mechanism: the production of a 3′-*O*-aminoglycoside phosphotransferase (Taylor, 1992; Aarestrup & Engberg, 2001). The aminoglycoside phosphotransferases are widely distributed in nature and have been reported in both Gram-positive and Gram-negative organisms (Tenover *et al*., 1989). The *Campylobacter*-like organism BM2196 contains a chromosomally located *aphA-1* gene that shares high identity with the kanamycin-resistance determinant originally derived from *Escherichia coli*, suggesting that this gene was acquired by *Campylobacter* from a member of the *Enterobacteriaceae* (Ouellette *et al*., 1987). In contrast, the *aphA-3* gene, found previously only in Gram-positive cocci (Trieu-Cuot *et al*., 1985), was identified on a 48-kb *C. coli* plasmid pIP1433 Taylor, 1992) and, recently, from large plasmids identified in strains of *C. jejuni* (Gibreel *et al*., 2004). In the *C. jejuni* plasmids, the *apha-3* gene was located downstream of an apparent insertion sequence, IS607*, or as part of a

resistance cluster, *aadE-sat4-aphA-3*, in which the genetic organization suggests that it has been acquired by *C. jejuni* from a Gram-positive organism (Gibreel *et al*, 2004). The *aadE-sat4-aphA-3* gene cluster was originally described as part of a transposon structure, Tn*5405*, from staphylococci (Derbise *et al*., 1997). More recently, a 25.7-kb *C. jejuni* plasmid, pCG8245, was described, which contained 10 ORFs that encoded copies of aminoglycoside-inactivating enzymes from both Gram-negative and Gram-positive sources (Nirdnoy *et al*., 2005). In pCG8245, the *aadE-sat4-aphA-3* gene cluster mapped next to hybrids of two *Helicobacter pylori* transposons; IS*Hp608* and IS*606* (Nirdnoy *et al*., 2005).

A third kanamycin-resistance phosphotransferase gene, *apha-7*, was also identified on a 14-kb *C. jejuni* plasmid, pS1178 (Tenover *et al*., 1989). The DNA sequence of the *apha-7* gene demonstrated 55% identity with the *apha-3* gene from streptococci; however, it showed a %G+C ratio of 32.8%, consistent with the chromosomal content of *C. jejuni*, suggesting that the *apha-7* gene may be endemic to the *Campylobacter* genus (Tenover *et al*., 1989). Kanamycin resistance is often mediated by a plasmid that also encodes tetracycline resistance (Taylor & Courvalin, 1988) and has been reported to be transferred along with tetracycline resistance, by conjugation, from representative *C. jejuni* strains to a recipient strain of *C. jejuni* (Gibreel *et al*., 2004).

Tetracycline resistance

Mechanisms of tetracycline resistance include efflux-based mechanisms found in Gram-positive and Gram-negative bacteria (Chopra & Roberts, 2001), the protection of the ribosomal binding site of tetracycline by ribosomal protection proteins (RPPs) (Chopra & Roberts, 2001), the enzymatic degradation of tetracyclines found in *Bacteroides* (Speer & Salyers, 1989), and, rarely, mutations in the 16S rRNA gene found in *Propionibacterium acnes* and *H. pylori* (Ross *et al*., 1998; Gerrits *et al*., 2002; Trieber & Taylor, 2002). In both *C. jejuni* and *C. coli*, resistance to tetracycline was found to be located on a self-transmissable plasmid encoding an RPP gene, designated *tet*(*O*) (Taylor & Courvalin, 1988; Taylor & Chau, 1996; Aarestrup & Engberg, 2001; Padungton & Kaneene, 2003). The *tet*(*O*) genes demonstrated 75–76% identity with the *tet*(*M*) gene of *Streptococcus pneumoniae* and have a %G+C ratio of 40%, which is close to that of *tet*(*M*) but significantly higher than those of *C. jejuni* and *C. coli* chromosomal and plasmid DNAs (Taylor & Courvalin, 1988).

Tetracyclines can be divided into two groups; the atypical tetracyclines (e.g. anhydrotetracycline and 6-thiatetracycline) and typical tetracyclines (e.g. tetracycline, chlortetracycline, and minocycline) (Connell *et al*., 2003). The atypical tetracyclines function by disrupting bacterial membranes (Rasmussen *et al*., 1991; Oliva *et al*., 1992), whereas the typical tetracyclines, which are the subject of RPP-mediated resistance, including Tet(O) and Tet(M), bind to the ribosome and inhibit accommodation of the aminoacyl-tRNA (aa-tRNA) into the ribosomal A site and, therefore, prevent the elongation phase of protein synthesis (Chopra, 1985). Tet(O) was first cloned from a transferable plasmid pUA466 in *C. jejuni* (Taylor, 1986) but, like the other RPPs, may have originated in *Streptomyces rimosus*, which harbours *otrA*, an RPP determinant (Doyle *et al*., 1991). Similarly, many of the RPP determinants are located on mobile genetic elements, which may have facilitated their spread throughout the eubacteria via lateral genetic exchange (Connell *et al*., 2003).

The RPPs are grouped into the translation factor superfamily of GTPases (Leipe *et al*., 2002) and accordingly bind and hydrolyse GTP (Taylor *et al*., 1995). Tet(O) confers resistance by displacing tetracycline from its primary binding site on the ribosome (Trieber *et al*., 1998; Connell *et al*., 2003), an ability that is strictly dependent on the presence of GTP (Trieber *et al*., 1998), thereby freeing the ribosome from the inhibitory effects of the drug, such that the aa-tRNA can bind to the ribosomal A site and protein synthesis can continue (Connell *et al*., 2003).

Plasmid content in human isolates of *C. jejuni* has been reported to be between 13% and 52%, with the majority being resistance plasmids (Gibreel *et al*., 2004). Plasmids carrying *tet*(*O*) have been reported to range in size from 45 to 58 kb (Taylor & Courvalin, 1988) and have been shown recently to confer extremely high levels of tetracycline resistance (512 mg L^{-1}), by conjugation, in some recipient *Campylobacter* spp. (Gibreel *et al*., 2004). Recently, two large tetracycline-resistance plasmids, pTet (45.2 kb) from *C. jejuni* strain 81–176 (Bacon *et al*., 2000), and pCC31 (44.7 kb) from *C. coli* strain CC31, have been sequenced (Batchelor *et al*., 2004). Interestingly, the two plasmid sequences revealed a high level of sequence identity (94.3%) and genomic organization although they were isolated 20 years apart and on different continents (Batchelor *et al*., 2004). Sequence analysis of the two plasmids revealed genes encoding orthologues of a putative type IV secretion system (T4SS) that has been shown to be involved in bacterial conjugation, DNA export, and protein secretion (Cao & Saier, 2001). Apart from 30 ORFs of unknown function, all the genes present in pTet and pCC31 were predicted to be involved in plasmid replication and conjugative transfer, and pTet and pCC31 were demonstrated to be self-mobilizable and capable of conjugative transfer between *C. jejuni* and *C. coli* (Batchelor *et al*., 2004); however, conjugative transfer to *E. coli* was not possible the suggesting that the host range was restricted to *Campylobacter* spp.

Location of the *tet*(*O*) gene on the chromosome has also been reported in 33% of tetracycline-resistant *C. jejuni* isolates from Alberta, Canada, lacking plasmids (Gibreel *et al*., 2004) and in 76% of tetracycline-resistant isolates from Australia, 26% of which did not harbour detectable plasmids (Pratt & Korolik, 2005). The presence of an insertion element IS*607**, similar to IS*607* found on the chromosome of *H. pylori*, has been reported on *tet*(*O*)-carrying plasmids (Gibreel *et al*., 2004) and therefore it is possible that mobile genetic elements other than transmissable plasmids may be involved in the acquisition and dissemination of *tet*(*O*).

Fluoroquinolone resistance

Fluoroquinolone resistance in most bacterial species is due to mutations in the DNA gyrase and DNA topoisomerase IV genes (Aarestrup & Engberg, 2001), although other mechanisms including decreased outer membrane permeability and an efflux system have been described (Charvalos *et al*., 1995). The corresponding gene products are large enzymatic quarternary structures consisting of two pairs of subunits, named GyrA and GyrB (DNA gyrase), and ParC and ParE (topoisomerase IV), respectively (Payot *et al*., 2006). Resistance to the fluoroquinolones arises from amino acid(s) substitution(s) in the quinolone resistance-determining region (QRDR) of the corresponding topoisomerase. QRDR is located within the DNA-binding domain on the surface of these enzymes. Reduction in susceptibility to the fluoroquinolones occurs after a first-step mutation, with additional mutations in *gyrA* and *gyrB* or *parC* further increasing the level of resistance (Payot *et al*., 2006).

In *Campylobacter*, fluoroquinolone resistance appears to be due mainly to mutations in the *gyrA* gene encoding part of the GyrA subunit of DNA gyrase (Aarestrup & Engberg, 2001). In *C. jejuni*, high-level resistance to ciprofloxacin is conferred by the point mutation Thr-86-Ile in *gyrA*, which is homologous to Ser-83-Leu in *E. coli* (Ge *et al*., 2005). Other reported mutations of *gyrA* in *C. jejuni* include Thr-86-Ala (high-level resistance to nalidixic acid and low-level resistance to ciprofloxacin), Ala-70-Thr, Thr-86-Lys, Asp-90-Asn, and Pro-104-Ser (Wang *et al*., 1993; Ge *et al*., 2005). Double point mutations of *gyrA* combining Thr-86-Ile and Asp-85-Tyr, or Asp-90-Asn, or Pro-104-Ser have also been reported (Ge *et al*., 2005). Mutations in the GyrB subunit in *Campylobacter* have not been described (Payot *et al*., 2006).

High-level fluoroquinolone resistance has also been reported to be caused by a point mutation Arg-139-Gln in *parC*, which encodes a topoisomerase (Gibreel *et al*., 1998); however, subsequent studies reported by other investigators failed to confirm a *parC* gene in *Campylobacter* (Parkhill *et al*., 2000; Bachoual *et al*., 2001; Luo *et al*., 2003; Piddock *et al*., 2003). In *C. jejuni and C. coli*, the absence of a secondary target for fluoroquinolones infers a situation whereby a unique modification in the GyrA subunit is sufficient to confer a fluoroquinolone-resistant phenotype (Payot *et al*., 2006).

Macrolide resistance

Macrolide resistance can be based on mechanisms including target modification by point mutation or methylation of 23S rRNA gene, hydrolysis of the drug, and efflux pumps (Aarestrup & Engberg, 2001). Modification of antibiotics through the activity of esterases and/or phosphotransferases has only been reported in staphylococci (Leclercq, 2002). High-level macrolide resistance [minimum inhibition concentration (MIC) $> 128\,\mu g\,mL^{-1}$] in *C. jejuni* and *C. coli* has been attributed to nucleotide mutations at positions 2074 and 2075 in the peptidyl transferase region in domain V of the 23S rRNA target gene (Gibreel *et al*., 2005; Gibreel & Taylor, 2006; Payot *et al*., 2006). The resistance-associated mutations in the majority of *C. jejuni* isolates have been identified in three copies of the target 23S rRNA gene (Gibreel & Taylor, 2006; Payot *et al*., 2006); however, at least two mutated copies are necessary to confer macrolide resistance (Gibreel *et al*., 2005). Additionally, a genetic assortment of wild-type and mutated copies has also been described (Jensen & Aarestrup, 2001; Payot *et al*., 2004; Gibreel *et al*., 2005). The predominant mutation detected among *C. jejuni* and *C. coli* is the transition mutation A2075G, which is usually associated with high-level resistance to erythromycin, although a transversion mutation at position 2074 (A2074C or A2074T) of the 23S rRNA gene as well as a double mutation A2074C/A2075G in one isolate has also been described (Vacher *et al*., 2005). An A2074G transition has also been found in one isolate of *C. jejuni*; however, the mutation was observed to be relatively unstable in the absence of erythromycin selection pressure and was demonstrated to have a negative effect on the growth rate of its host (Gibreel *et al*., 2005).

Mutations affecting macrolide binding have also been identified in the ribosomal proteins L4 and L22, both of which form portions of the polypeptide exit tunnel within the bacterial 70S ribosome, and have been described in several bacteria (Payot *et al*., 2006). Recently, 13 isolates of *C. jejuni* and *C. coli* were described, all of which possessed the characteristic A2075G mutation as well as one or more amino acid substitutions in the L4 protein and two or more substitutions in the L22 protein. A unique A103V substitution was identified in the L22 protein in each of two isolates of *C. jejuni* and *C. coli* demonstrating a high-level-erythromycin-resistant phenotype (Corcoran *et al*., 2006), possibly linking this substitution to the resistant phenotype. Moreover, macrolide-resistant mutants of *C. jejuni* and *C. coli*

have been recently described as exhibiting modifications in the ribosomal proteins L4 (G74D) and L22 (insertions at positions 86 or 98). As synergy between the efflux pump CmeABC and these modifications in conferring resistance to the macrolides was observed (Cagliero *et al.*, 2006).

Sulphonamides and multidrug resistance

Sulphonamide resistance in *C. jejuni* is also chromosomally mediated through mutational substitution of four amino acid residues in the enzyme dihydropteroate synthetase (DHPS), resulting in a reduced affinity for sulphonamides. Sulphonamides compete with PABA for DHPS, thereby preventing PABA from being incorporated into folic acid (Aarestrup & Engberg, 2001).

Integrons, carried by transposons, are a major vehicle for the spread of multiple-antibiotic resistance (Liebert *et al.*, 1999) and have a broad distribution among Gram-negative faecal bacteria of animal origin (Lee *et al.*, 2002). The roles of class 1 integrons and gene cassettes in the acquisition and spread of antibiotic resistance genes are well established and over 75 gene cassettes that harbour genes encoding antibiotic resistance have been reported (Partridge & Hall, 2003). Lucey *et al.* (2000) reported that integron-like structures in *Campylobacter* spp. may account for the presence of sulphonamide resistance and Gibreel & Skold (1998, 2000) reported that trimethoprim resistance in *C. jejuni* was due to the acquisition of horizontally transferred *dfr*genes found on the chromosome in transposons or integrons. Class 1 integron-associated tobramycin and gentamicin resistance, due to the aminoglycoside resistance gene, *aacA4*, has recently been reported in five per cent of *C. jejuni* isolated from broiler chickens; 21% of isolates possessed class 1 integrases (Lee *et al.*, 2002). Further, in a large study of unrelated Irish thermophilic *Campylobacter* spp., 16% of isolates including both *C. jejuni* and *C. coli* were found to possess a complete class 1 integron with a recombined gene cassette containing the aminoglycoside-resistance gene, *aadA2* (O'Halloran *et al.*, 2004).

Resistance to more than one group of antimicrobial agents in *C. jejuni* may be the result of self-transmissable plasmids or efflux mechanisms (Padungton & Kaneene, 2003). An energy-dependent efflux system with a broad specificity was reported previously to be responsible for the multidrug resistance profile (resistance to β-lactams, erythromycin, tetracycline, chloramphenicol, and quinolones) in a laboratory mutant of *C. jejuni* (Charvalos *et al.*, 1995). More recently, the multidrug efflux pumps, CmeABC and CmeDEF, have been identified and described in *C. jejuni* (Lin *et al.*, 2002; Pumbwe & Piddock, 2002; Pumbwe *et al.*, 2005), conferring resistance to multiple antibiotics including fluoroquinolones, erythromycin, tetracycline, chloramphenicol, and ampicillin, as well as detergents and dyes (including ethidium bromide), bile salts, and heavy metals (Lin *et al.*, 2002). The *cmeABC* operon is widely distributed in *C. jejuni* and *C. coli* strains and is constitutively expressed in wild-type strains (Payot *et al.*, 2006). The efflux pump, CmeF, belonging to the CmeDEF system, has also been identified and confers multidrug resistance, but apparently does not transport cipropfloxacin (Pumbwe *et al.*, 2005). Although CmeDEF is expressed at a low level, it acts interactively with CmeABC in conferring resistance to antimicrobials and toxic compounds and probably functions as a secondary efflux mechanism (Akiba *et al.*, 2006).

β-Lactam resistance

Mechanisms of resistance to some β-lactams such as ampicillin and some of the expanded-spectrum cephalosporins are variable and not very clearly defined (Lachance *et al.*, 1991; Reina *et al.*, 1994; Tajada *et al.*, 1996). With the exception of the carbapenems imipenem and meropenem, the majority of *C. jejuni* and *C. coli* strains are resistant to a large number of β-lactam antimicrobial agents (Lariviere *et al.*, 1986; Aarestrup & Engberg, 2001). In general, *C. jejuni* and *C. coli* isolates show intrinsic resistance to penicillin G and narrow-spectrum cephalosporins related to their slight binding to PBPs present in the bacteria (Tajada *et al.*, 1996). In addition, other acquired resistance mechanisms such as the production of β-lactamase may also be found (Taylor & Courvalin, 1988; Lachance *et al.*, 1991). Most recently, a novel class D β-lactamase gene, $bla_{OXA\text{-}61}$, from an Australian isolate of *C. jejuni* was described (Alfredson & Korolik, 2005), which conferred resistance to the β-lactams ampicillin, piperacillin, and carbenicillin in a β-lactam susceptible strain of *C. jejuni*.

Conclusions

The incidence of *Campylobacter* infection is increasing worldwide and trends in antimicrobial resistance have shown a clear association between use of antibiotics in the veterinary industry and resistant isolates of *Campylobacter* in humans. *Campylobacter jejuni* has also been able to acquire resistance-determinants from outside of its genus, in particular, from Gram-positive organisms and genes have been able to be incorporated into plasmids or into the chromosome via insertion sequences on transposons or integrons. The spread of these resistance determinants both within and outside of the *Campylobacter* genus is likely.

Globally, the incidences of resistance to several key antibiotics useful in the treatment of *Campylobacter* disease are increasing and multiple resistance patterns to several classes of antibiotics are emerging. In many countries, resistance in *Campylobacter* to the fluoroquinolones has limited its usefulness as a drug of choice in the treatment of the disease, although in countries such as Australia, the

fluoroquinolones remain an effective antibiotic. Similarly, resistance to erythromycin is increasing in some countries, particularly in *C. coli*; however, the incidence of erythromycin resistance in human *Campylobacter* isolates is still relatively low and stable and currently erythromycin should be regarded as the drug of choice in the treatment of *Campylobacter* disease. Gentamicin also remains effective against campylobacters, although it would only normally be considered for serious *Campylobacter* infections.

With the global concern with the increasing prevalence of resistance among clinically important bacterial pathogens, monitoring and surveillance of antimicrobial resistance of *Campylobacter* isolates, infection prevention, and strategies for regulatory control of antibiotic use will enable effective management of the progressive increase in antimicrobial resistance among campylobacters in the human global population.

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