Antimicrobial resistance of thermophilic Campylobacter

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Review article

Antimicrobial resistance of thermophilic *Campylobacter*

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Abstract – *Campylobacter* has become the leading cause of zoonotic enteric infections in developed and developing countries world-wide. Antimicrobial resistance has emerged among *Campylobacter* mainly as a consequence of the use of antimicrobial agents in food animal production. Resistance to drugs of choice for the treatment of infections, macrolides and fluoroquinolones has emerged as a clinical problem and interventions to reduce this are recommended. Resistance to fluoroquinolones and macrolides is mediated by chromosomal mutations. Resistance to other relevant antimicrobial agents, mediated by acquired resistance genes, has not become widespread so far. However, resistance genes originating from both Gram-positive and Gram-negative bacterial species have been found, showing the potential for acquired resistance to emerge in *Campylobacter*.

***Campylobacter* / resistance / susceptibility testing / trends / gene**

Résumé – Résistance aux antimicrobiens chez *Campylobacter* thermophile. *Campylobacter* est devenu la cause principale de zoonoses entériques infectieuses dans les pays développés et en voie de développement à travers le monde. La résistance aux antibiotiques a émergé chez *Campylobacter*, principalement à cause de l'utilisation d'antibiotiques chez les animaux entrant dans la chaîne alimentaire. La résistance aux antibiotiques principalement utilisés dans le traitement des infections, les macrolides et les fluoroquinolones, a émergé en tant que problème clinique, et les interventions visant à réduire cette résistance sont recommandées. La résistance aux fluoroquinolones et aux macrolides est due à des mutations chromosomiques. La résistance aux autres antibiotiques utilisables, qui est due à l'acquisition de gènes de résistance, ne s'est jusqu'à maintenant pas propagée. Cependant, le fait qu'il ait été trouvé des gènes de résistance ayant pour origine des espèces bactériennes à Gram-positif et à Gram-négatif, montre la possibilité d'émergence de résistance acquise chez *Campylobacter*.

***Campylobacter* / résistance / test de sensibilité / tendance / gène**

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1. INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are among the most common causes of bacterial diarrhoea in man world-wide [35]. Among these, *C. jejuni* accounts for the vast majority of infections. Infection with thermophilic *Campylobacter* spp. usually leads to an episode of acute gastroenteritis, which resolves within a few days to a few weeks. Most cases of *Campylobacter* enteritis do not require antimicrobial treatment, as they are of short duration, clinically mild and self-limiting. However, antimicrobial treatment is necessary for systemic *Campylobacter* infections and for severe or long-lasting cases of *Campylobacter* enteritis. Macrolides are normally considered the drug of choice for *Campylobacter* enteritis, but fluoroquinolones are also recommended [7, 21, 41, 51, 56]. In many cases fluoroquinolones are preferred if an infection with *Shigella* is suspected. Intravenous aminoglycosides is the treatment of choice for serious *Campylobacter* bacteraemia and other systemic infections. However, increases in the occurrence of *Campylobacter*, causing infections in man, that are resistant to macrolides and fluoroquinolones, have been reported in several countries [13, 16, 24, 45, 46, 52, 55, 57]. In these cases other antimicrobial agents may be used for treatment.

As food animals are considered one of the most important sources of *Campylobacter* causing infections in man, the development of antimicrobial resistance in *Campylobacter* spp., due to the use of antimicrobial agents in food animals, is a matter of concern. Several studies have reported a frequent and, in many cases, increasing occurrence of resistance to macrolides, fluoroquinolones and other antimicrobial agents among *Campylobacter* from food animals [11, 13, 23, 28, 50, 73].

This review provides a description of the occurrence and trends of antimicrobial resistance among *Campylobacter* from food animals and of the mechanisms of resistance in *Campylobacter*.

2. SUSCEPTIBILITY TESTING OF *CAMPYLOBACTER*

In general there are two different methods for testing the susceptibility of different bacterial species to antimicrobial agents, dilution and diffusion methods. Several

variations of both methods are used worldwide. Standardised procedures are available for susceptibility testing of a wide range of organisms and in general the guidelines provided by NCCLS are the most widely used [36]. *Campylobacter* require microaerobic conditions for growth and standardised procedures for susceptibility testing are not currently available. Consequently, a number of different diffusion (disk, tablets and E-test) and dilution methods (macro- and microbroth dilution and agar dilution) have been used [14, 35]. Owing to the lack of international standards for susceptibility testing, the procedures have to be managed locally. Currently, we recommend susceptibility testing using agar dilution or diffusion on plates supplemented with 5% blood and cultivated under microaerobic conditions [35]. In order to monitor the antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. isolated from food animals, food of animal origin, and humans in different laboratories, standardised methods should be applied, and comparative studies on the performance of testing procedures are required [14, 35].

3. RESISTANCE OCCURRENCE AND TRENDS

Campylobacter can be isolated from a wide variety of wild and domestic animals [71]. Among food producing animals, *C. jejuni* predominates among cattle and broilers, whereas *C. coli* is the most commonly found species among pigs [2, 11, 34]. The occurrence of resistance is in general higher among *C. coli* compared to *C. jejuni* [2, 11, 50]. This is especially the case for macrolide resistance and *C. coli* from pigs [2, 11, 50]. Resistance to macrolides and fluoroquinolones has been high in several studies, while resistance to other antimicrobials agents including tetracycline, aminoglycoside and chloramphenicol is generally low. Resistance to beta-lactam antimicrobials is in general high and most isolates are resistant to trimethoprim and sulphonamides [35, 67].

Macrolides and fluoroquinolones are normally regarded as the drugs of choice for the treatment of infections with *Campylobacter*, and resistance to these two classes is among the most commonly reported for *Campylobacter*.

Several studies have documented an increase in the occurrence of resistance to fluoroquinolones among *Campylobacter* from food animals following the introduction of fluoroquinolones for the treatment of infections in animals [15, 58].

The first study that documented a link between the veterinary use of fluoroquinolones and the occurrence of quinolone-resistant *Campylobacter* among both food animals and humans was from The Netherlands [13]. The fluoroquinolone enrofloxacin was introduced for veterinary use in The Netherlands in 1987. No fluoroquinolone-resistant *Campylobacter* isolates were found in poultry products from 1982 to 1983 or in humans from 1982 to 1983 or 1985. The percentage of fluoroquinolone-resistant isolates in poultry products increased to 8.4% in 1987/1988 and 14% in 1989 [13]. In 1992 and 1993 the percentage of resistant isolates from broilers was 29% [26]. This emergence and increase of resistance among poultry products and broilers has been closely followed by an emergence and subsequent increase in resistance among isolates causing infections in humans. The percentage of resistance was 8% in 1988, 11% in 1989 and 29% in 1997 [13, 63]. Similar trends of quinolone resistance in humans have been observed in Austria, Denmark, Finland, France, Italy, Spain, Thailand, the United Kingdom, and the United States [15, 58]. Thus there is compelling evidence today that quinolone resistance emerged and increased among food animals as a consequence of the use of fluoroquinolones in animal production and then spread to and caused infections in man [15, 58]. There is almost no evidence for changes in the occurrence of fluoroquinolone resistance following a more limited usage of fluoroquinolones for food animals. However, preliminary data

from the Danish monitoring of antimicrobial resistance indicate that the occurrence of resistance is decreasing following more limited usage since 1998 [4].

High frequencies of macrolide resistance have been reported among *C. coli* from pigs in several studies [2, 9, 11, 50, 72]. Several studies have also shown an increase in the occurrence of macrolide resistance among *Campylobacter* causing infections in man [24, 46, 52]. The reason for the high frequency of macrolide resistance among *C. coli* from pigs has not been finally determined. It could be related to a higher frequency of mutations to resistance among *C. coli* or a higher selective pressure induced by the use of antimicrobial agents. However, the macrolide tylosin has for several years been used in large amounts for growth promotion and therapy in the pig industry world-wide and it is likely that the occurrence of resistance in *C. coli* is related to its usage [1]. In 1998, farmers in Denmark decided voluntarily to stop the use of antimicrobial agents for growth promotion in slaughter pigs above 35 kg from June 1998 and in all pigs from the end of 1999. This has had a major impact, especially on the consumption of the macrolide growth promoter tylosin in Denmark. Tylosin is still used for the treatment of infections in pigs, but in much lower quantities. Preliminary data from the Danish monitoring of antimicrobial resistance indicate that the frequency of macrolide resistance among *C. coli* from pigs has decreased since the more limited usage of tylosin for growth promotion ([4], Aarestrup et al., unpublished results).

4. MECHANISMS OF RESISTANCE (Tab. I)

4.1. Aminoglycoside resistance

Resistance to aminoglycosides is normally mediated by enzymes that modify the drugs. These enzymes are divided into three different groups based on the reaction they mediate [53]. The enzymes are aminoglycoside phosphotransferases (APH), aminoglycoside adenyltransferases (AAD or ANT) and aminoglycoside acetyltransferases (AAC) [53]. A large number of enzymes have been found to mediate aminoglycoside resistance and the nomenclature for both the genes and the enzymes are complex [53]. In the following, the names of the genes are given in italics and the names of enzymes in capital letters. In *Campylobacter*, kanamycin resistance has been found to be encoded by *aph(3')IIIa* (APH (3') III) and *aph(3')IVa* (APH (3') IV) [47, 64, 66]. These genes are also found among Gram-positive bacterial species [53]. A number of different *aph(3')I* genes have been found to encode the enzyme APH(3')I. This enzyme mediates kanamycin resistance, is believed to have its origin in *Enterobacteriaeceae*, and has also been found in *Campylobacter* [39]. The *ant(3')-Ia* and *ant(6)-Ia* genes encode streptomycin resistance [42, 53]. The *ant(3')-Ia* gene (ANT(3')-I) is commonly found in Gram-negative bacterial species, whereas the *ant(6)-Ia* gene (ANT(6)-I) has mainly been found in staphylococci [25, 42, 53]. The *sat4* gene encoding resistance to streptothricins has been observed in *C. coli* of different animal and clinical sources in Germany [6, 8]. The different aminoglycoside resistance genes have also been found in other bacterial species, mainly Gram-positive. This could indicate that *Campylobacter* mainly acquire horizontally transferred genes from Gram-positive bacteria. However, the presence of *ant(3')-Ia* indicates that genes may also be acquired from Gram-negative bacterial species.

4.2. Beta-lactam resistance

With the exception of imipenem, the majority of *C. jejuni/coli* strains are resistant to β-lactam agents, i.e. principally penicillins and cephalosporins. However, they are moderately susceptible to cefotaxime,

Table I. Antimicrobial resistance mechanisms in *Campylobacter*.

Antimicrobial	Resistance mechanisms	Resistance genes or mutations	Transmissible	Host-range	Reference
Aminoglycosides*	Inactivation of the drug by				
Kanamycin, neomycin, gentamicin B	chemical modification	*aph(3')-Ia*	+	*Enterobacteriaceae* Gram-positive cocci	[39, 42, 53]
Kanamycin, neomycin, gentamicin B		*aph(3')-III*	+	Gram-positive cocci	[12, 53]
Kanamycin, neomycin		*aph(3')-IV*	+	Gram-positive cocci	[46, 53]
Streptomycin		*ant(6)-Ia*	+	Gram-positive cocci	[12, 42, 53]
Streptomycin, spectinomycin		*ant(3')-Ia*	+	Gram-negative bacteria	[42, 53]
Streptothricins		*sat4*	+	Gram-positive cocci	[12, 33]
Beta-lactam	Production of beta-lactamases or low permeability	ND	ND	ND	[29, 30]
Chloramphenicol	Inactivation of the drug by chemical modification	*cat*	ND	ND	[74]
Fluoroquinolones	Mutations in the drug-sensitive target	Ala-70 to Thr in *gyrA* Thr-86 to Ile in *gyrA* Asp-90 to Ala in *gyrA* Arg-139 to Gln in *parC*	–	–	[20, 75, 81]
Macrolides	Mutations in the drug-sensitive target	A to G at position 2230 in the 23S rDNA gene	–	–	[27, 67]
Sulphonamides	Mutations in the drug-sensitive target	Mutations in *folP* gene	–	–	[18]
Tetracyclines	Protection of the target	*tet*(O)	+	Gram-positive cocci	[10, 31, 80]
Trimethoprim	Replacement of the sensitive target by a new enzyme	*dfr1* *dfr9*	ND ND	*Enterobacteriaceae*	[17, 19]

* Only the most commonly used for therapy.

ceftazidime and cefpirone [70]. Resistance to beta-lactam antibiotics is, in most bacterial species, caused by the production of beta-lactamases that break the beta-lactam ring of the antibiotics. However, in some bacteria, changes in the penicillin-binding-proteins or lack of penetration of the drug into the bacteria are the main mechanisms of resistance. A large proportion of *C. coli* and *C. jejuni* produce beta-lactamases [29, 30, 50, 62]. However, the β-lactamase of *C. jejuni/coli* seems to play a role only in resistance to amoxicillin, ampicillin and ticarcillin. With penicillin G, piperacillin and cephalosporins, the mechanism of resistance in *C. jejuni/coli* is primarily considered to be dependent on their limited ability to bind to penicillin-binding proteins and their low permeability [29, 62, 68].

4.3. Chloramphenicol resistance

Chloramphenicol resistance is mainly due to the production of enzymes that acetylate chloramphenicol and thereby prevent the binding to the ribosome, referred to as the chloramphenicol acetyltransferase (cat) genes [54]. In *C. coli* a single *cat*-gene has been identified [74]. Resistance to chloramphenicol has not become widespread among *Campylobacter.*

4.4. Fluoroquinolone resistance

Fluoroquinolones inhibit the activity of DNA gyrase and in most bacterial species resistance is due to mutations in the gyrase or topoisomorase genes. Moreover, in *Campylobacter* fluoroquinolone, resistance appears to be due mainly to mutations in the *gyrA* gene encoding part of the A subunits of DNA gyrase. Cloning and sequencing of the *C. jejuni gyr*A gene has demonstrated that mutations in *gyr*A at positions Thr-86, Asp-90 and Ala-70 can be responsible for resistance [75]. The most common mechanism of resistance among wild type isolates is a mutation at position threonine-76. This mutation (A**C**T → A**T**T) causes an amino acid change to isoleucine [20, 49, 75, 81]. Ruiz et al. [49] found a Thr-86-to-Lys substitution in a single clinical isolate of *C. jejuni*. The substitutions at position Asp-90 to Asn and Ala-70 to Thr have only been found in laboratory mutants and did not confer the same level of resistance as the substitution at Thr-86 [75]. Gibreel et al. [20] reported that high-level fluoroquinolone resistance was also caused by simultaneous substitutions at position Thr-86 to Ile in gyrA and at Arg-139 to Gln in the *parC* gene (topoisomerase IV).

4.5. Macrolide resistance

Resistance to macrolides can be based on different mechanisms: target modification by point mutation or methylation of 23S rRNA, thereby inhibiting the binding of macrolides [76], hydrolysis of the lactone ring in the macrolide [5], and efflux pumps removing the macrolide from the bacteria [60]. In *Campylobacter* it has been shown that resistance is not consistent with the presence of rRNA methylase, with modification of the antibiotic or with efflux [79]. In a closely related bacterium, *Helicobacter pylori*, resistance to clarithromycin has been shown to be due to the alteration of one of two adenine residues in the 23 rRNA at the erythromycin-binding site [65]. The sequencing of 23S rRNA genes from erythromycin-resistant and susceptible *C. coli* and *C. jejuni* has identified mutations at these same sites, indicating that this is the mechanism of resistance [27, 67]. Thus, resistance to macrolides in *Campylobacter* will spread with the bacteria and not be transferable to other bacteria.

4.6. Sulphonamide resistance

Sulphonamides are structural analogues of p-aminobenzoic acid (PABA) and

compete with PABA for the enzyme dihydropteroate synthetase (DHPS), thereby preventing PABA from becoming incorporated into folic acid. Resistance to sulphonamides in Gram-negative bacteria is normally due to the acquisition of horizontally transferable drug-resistant variants of DHPS [44]. In Gram-positive bacteria the most common mechanisms are mutations in the gene encoding DHPS [22, 32, 61]. Gibreel and Sköld [18] found that sulphonamide resistance in *C. jejuni* was associated with the mutational substitution of four amino acid residues in DHPS resulting in a reduced affinity for sulphonamides. Other mechanisms of sulphonamide resistance have not been found so far in *Campylobacter*.

4.7. Tetracycline resistance

Tetracycline resistance can be mediated by four different mechanisms: efflux, modification of tetracycline, protection of the ribosomal binding site of tetracycline and mutations in 16S rDNA [48]. In *C. coli* and *C. jejuni*, tetracycline resistance has been found to be located on self-transmissible plasmids. The genes encoding resistance have been identified as a ribosomal protection gene and designated *tet*(O) [31]. The *tet*(O) gene is widespread in *Campylobacter* and has since been found in different Gram-positive bacterial species including enterococci and streptococci [3, 48, 59, 80], suggesting the Gram-positive origin of this gene. A high frequency of resistance to tetracycline (72%) has recently been reported among *C. jejuni* in Spain [43].

4.8. Trimethoprim resistance

Trimethoprim acts by binding to and inhibiting the activity of the enzyme dihydrofolate reductase (*dfr*). Resistance is due to the acquisition of horizontally transferred *dfr*-genes that are not inhibited by trimethoprim. In *Campylobacter* two different genes (*dfr1* and *dfr9*) have been found to mediate resistance [17, 19]. The genes have been found on the chromosome in transposons or integrons [17, 19]. These two dihydrofolate reductases are also found in Gram negative bacterial species, mainly *Enterobacteriaceae*, indicating that *Campylobacter* may also acquire genes from this group [17, 19].

5. DISCUSSION

Modern food animal production provides favourable conditions for the emergence and spread of zoonotic bacteria such as *Campylobacter*. Furthermore, large amounts of antimicrobial agents are used in these production systems to control infections. This usage will select for resistance in the zoonotic bacteria and thereby pose a risk for human health. In the case of *Campylobacter*, the drugs of choice for the treatment of infections in man are macrolides and fluoroquinolones. Unfortunately, the occurrence of resistance to these antimicrobials has become widespread and seems to be increasing [15, 58]. Studying the transmission of antimicrobial resistance from animals to man is difficult, especially from poultry to man, because the chain of transmission is often complex. However, several studies have shown that food animals can serve as an important source of infection in humans and that the same sero- and genotypes can be isolated from humans and food animals [37, 38, 40]. Since human-to-human transmission of *C. jejuni*/*coli* is rare, patients infected with resistant *Campylobacter* are not an important source of resistant *Campylobacter* for other humans. The most likely reason for the increased resistance is the use of antimicrobial agents in food animal production. Interventions reducing the reservoir of resistant *Campylobacter* among food animals may prolong the lifetime of macrolides and fluoroquinolones for human use. For these reasons, international public health organisations such as the World

Health Organization have recommended to limit or suspend the use of antimicrobial agents that are of importance for human health [77, 78].

With the exception of macrolides, fluoroquinolones and tetracycline, antimicrobial resistance does not seem to have become widespread among *Campylobacter*. Thus resistance to aminoglycosides and chloramphenicol is in general still low. In addition, resistance to macrolides and fluoroquinolones is mediated by chromosomal mutations and not by horizontally acquired genes. This could indicate that so far, *Campylobacter* have, not to a large extent, acquired resistance genes from other bacterial species. However, acquired resistance genes have been observed in *Campylobacter*. In most cases, these are genes that are also found in Gram-positive bacterial species, indicating that *Campylobacter* generally share genes with Gram-positive species. However, studies have shown that resistance genes may have a very broad host spectrum and spread between both Gram-positive and negative species [69]. This is also the case for *Campylobacter* that have also acquired genes believed to be of Gram-negative origin [17, 42].

Campylobacter have become the leading cause of zoonotic enteric infections in developed and developing countries and their incidence is increasing. More knowledge is required on this group of bacteria to enable targeted interventions to reduce this increase. There is still a lack of knowledge regarding the genetics of *Campylobacter* and their ability to adapt to and colonise new niches, maybe as a consequence of horizontally acquired traits. In the case of resistance to fluoroquinolones and macrolides it seems to be possible to control the increase in resistance by a more restricted or suspended use of these antimicrobial agents in food animal production. In order to protect human health it is recommended that such interventions be implemented on a worldwide scale.

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