A Review on Antimicrobial Drug Resistance of Campylobacter

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Abstract: This seminar paper was done with the main objective of describing the mechanisms of Campylobacter that result in resistance to antibiotics. Campylobacter microorganisms are small, spirally curved and motile Gram-negative bacteria that are primarily present in the intestinal tract of domestic and wild animals. There are around 21 species that have been identified and among them, Campylobacter jejuni and Campylobacter coli are recognized as the most common causative agents of bacterial gastroenteritis in the world. Campylobacteriosis is one of the most common zoonotic bacterial diseases caused by the members of genus Campylobacter and it is normally a self limiting disease. Humans most often become infected by ingesting contaminated food, especially undercooked chicken, through other sources of bacteria have been described. Campylobacter is increasingly resistant to clinically important antibiotics and this has become a major concern for public health. 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 fluoroquinolone, as growth promoters in food animals and the veterinary industry is accelerating this trend. The occurrence of antimicrobial resistance to antimicrobials used in human therapy is increasing in human pathogenic Campylobacter from animals. Modern food animal production system depend on the use of large amounts of antibiotics for disease control. This provides favorable conditions for the spread and persistence of antimicrobial-resistant zoonotic bacteria such as Campylobacter. There is an urgent need to implement strategies for prudent use of antibiotics in food animal production system to prevent further increases in the occurrence of antimicrobial resistance in food-borne human pathogenic bacteria such as Campylobacter.

Key words: Antimicrobial Resistance • Campylobacter • Resistance Determinants

INTRODUCTION

Campylobacter microorganisms are small (0.2–0.9 µm wide and 0.2–5.0 µm long), spirally curved and motile Gram-negative bacteria that are primarily present in the intestinal tract of animals. There are around 21 species (spps) that have been identified up to now and among them Campylobacter jejuni (C. jejuni) and to some extent, Campylobacter coli (C. coli) are the most important species. As they are both microphilic, they are different from other pathogens related with food-borne diseases that makes them to be able to grow in atmosphere containing approximately 10% carbon dioxide and 5% oxygen [1]. Campylobacter is one of the most commonly reported bacterial causes of human food borne infections and increasing proportions of these pathogens become resistant to medically important antimicrobial agents and thus imposing a burden on public health [2].

Campylobacteriosis is a collective description for infectious diseases caused by members of the genus Campylobacter [3]. Poultry is generally considered to be the most important reservoir for Campylobacter spp. Many types of food animals including cattle, sheep and pigs also harbor this pathogen [4]. Campylobacteriosis is normally a self-limiting disease, but in some cases complications may occur, such as reactive arthritis and Guillain-Barré syndrome, a post infectious polyneuropathy that is a leading cause of paralysis in arm and leg muscle [5].

Antimicrobial treatment is needed only in patients with more severe disease and in those who are immunologically compromised. The most common antimicrobial agents used in the treatment of Campylobacter infections are fluoroquinolones (FQ), such as ciprofloxacin and macrolides, such as erythromycin. Tetracyclines have been suggested as an
alternative choice in the treatment of clinical campylobacteriosis, but in practice they are not often used [6].

Increasing antimicrobial resistance in both agriculture and medicine in Campylobacter is recognized by various national authorities including the World Health Organization (WHO) as a major emerging public health concern. The use of antimicrobial agents in animal production is one of the main factors influencing antimicrobial resistance, especially to FQ and macrolides. An increasing numbers of Campylobacter isolates have developed resistance to FQ, aminoglycosides, macrolides and beta lactams [8].

Campylobacter acquires resistance determinants by mutation and horizontal gene transfer. 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 [9]. The absence of national surveillance program, limited routine culture availability for the isolation of Campylobacter species at clinical and research settings and the need for selective media and unique growth atmosphere make it difficult to give an accurate picture of the burden of disease in Ethiopia. This fact indicates that Campylobacter as a zoonosis is not given appropriate attention and consideration [10]. Some researchers works on antimicrobial drug resistance of Campylobacter but the data is not much enough and there is little review in Ethiopia. Therefore, the objectives of this seminar paper were to review on the patterns of emerging resistance to the antimicrobial agents useful in the treatment of campylobacteriosis, to highlight the mechanisms of resistance to drugs in Campylobacter spp. and to indicate the persistence and fitness of antibiotic resistant Campylobacter.

Highlight on Epidemiology of Campylobacteriosis in Animals: Campylobacter species especially C. jejuni is a leading causative agent of bacterial gastroenteritis with around 400 million human cases worldwide. Poultry is generally considered to be the most important reservoir for Campylobacter spp. Many types of food animals including cattle, sheep and pigs also harbor this pathogen [4].

In view of the complex epidemiology of Campylobacteriosis, a multi-tiered approach is needed to control it, taking into consideration the different reservoirs, pathways, exposures and risk factors [11]. The Hazard Analysis Critical Control Point System (HACCP) is the basis for requiring the implementation of food safety management systems in order to control Campylobacter spp. throughout the food chain. That is well-established principles and a structured systematic approach to achieving food safety which involves identifying potential hazards and measures for their control. In human, C. jejuni infections occur mainly from contaminated poultry or other animal meat, meat products, raw milk, milk products and surface water [12].

Animal food products are most commonly contaminated by this pathogen during slaughter and carcass dressing in abattoirs [13] and infections due to C. jejuni are generally self-limiting; with symptoms resolving in about three to five days though antibiotic therapy is required in immuno-compromised patients, in cases of bacteremia and in severe and long-lasting Campylobacter infections [10].

Mechanism of Antimicrobial Drug Resistance: An increasing numbers of Campylobacter isolates have developed resistance to FQ, aminoglycosides, macrolides and beta lactams. Furthermore, C. jejuni and C. coli has an intrinsic resistance against penicillin and most of the cephalosporins as well as trimethoprim, sulfamethoxazole, rifampicin and vancomycin [8]. 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 tet(O) and aphA3 are believed to have been acquired outside the genus Campylobacter from Gram positive cocci and have been incorporated into the Campylobacter genome by heterologous genetic exchange [9].

The genetic elements that underlie these mechanisms may be chromosomal or plasmid-borne and represent a combination of endogenous and acquired genes. In general, mechanisms of antibiotic resistance include: modification of the antibiotic’s target and/or its expression (i.e., DNA gyrase mutations), inability of the antibiotic to reach its target (i.e., expression of the major outer membrane protein or MOMP), efflux of the antibiotic (i.e., multidrug efflux pumps such as CmeABC) and Modification or inactivation of the antibiotic (i.e., â-lactamase production) [14].

Resistance to Tetracyclines: Resistance to tetracyclines in Campylobacter is conferred by tet(O) the gene, which is widely present in both C. jejuni and C. coli. The tet(O) gene, which encodes ribosomal protection proteins (RPPs), is located on a self-transmissible plasmid of a molecular size from 45 to 58 kb. This gene has been shown
to confer extremely high levels of tetracycline resistance (512?mg/L). Recent study demonstrates that this protein recognizes an open A site on the bacterial ribosome and binds it in such a manner that it induces a conformational change that results in the release of the bound tetracycline molecule [15].

Resistance to β-Lactam: Mechanisms of resistance to some β-lactams such as ampicillin and some of the expanded-spectrum cephalosporins are variable and not very clearly defined. 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 [10]. Beta lactam antimicrobials bind to penicillin binding proteins and disrupt peptidoglycan crosslinking during bacterial cell wall formation and leads to cell death. 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 [16].

Resistance to Fluoroquinolone (FQ): Fluoroquinolone resistance in most bacterial species is due to mutations in the DNA gyrase and DNA topoisomerase IV genes, although other mechanism including decreased outer membrane permeability and an efflux system have been described. In Campylobacter, fluoroquinolone resistance appears to be due mainly to mutations in the gyrA gene encoding part of the GyrA subunit of DNA gyrase [10].

The Development and Transmission of Antimicrobial Drug Resistance: Mutations play a major role in development of Campylobacter resistance. Several mechanisms have been reported to contribute to the emergence of these mutations [2]. Resistance to FQs in Campylobacter occurs spontaneously owing to mutations in target genes. Assessed in culture media, the frequencies of emergence of FQ-resistant mutants range from approximately 10^{-5} to 10^{-8}/cell/generation [17]. In Campylobacter, the elevated expression of cmeABC increases the frequency of emergence of FQ-resistant mutants. This enhancing effect on mutant emergence is probably attributable to the synergistic action of CmeABC and gyrA mutations in conferring FQ resistance, allowing more mutants to grow on antibiotic-containing plates [18].

In addition, Mfd (Mutant Frequency Decline), a transcription repair coupling factor involved in strand specific DNA repair, promotes the emergence of FQ resistant mutants in Campylobacter. Inactivation of the mfd gene in Campylobacter resulted in a 100 fold reduction in the number of spontaneous mutants resistant to ciprofloxacin, while overexpression of mfd increased the mutant numbers. Given the fact that Mfd does not affect the MIC of FQ antibiotics in Campylobacter, the altered mutant number is likely to be a result of the direct effect of Mfd on mutation rates [19].

During antibiotic treatment, FQ-resistant mutants develop rapidly and the mutant population continues to persist even after removal of the selection pressure. Development of macrolide-resistant mutants involves a multistep process and requires prolonged exposure to the antibiotic [17]. Once the selection pressure is removed, macrolide resistant mutants cannot compete with macrolide susceptible Campylobacter and will decrease in number. In contrast to FQ resistance, the mutation frequency for macrolide resistance in Campylobacter is low (~10^{-9}/cell/generation) and is approximately 10,000 fold lower than that of FQ resistance [20].

Another unique feature of macrolide resistance in Campylobacter is the slow development of resistant mutants under antibiotic treatment. Using Campylobacter infected chickens, the therapeutic treatment of Campylobacter-infected birds with tylosin (administered in drinking water for three consecutive days) did not select for erythromycin-resistant Campylobacter, even after three treatments [20].

Campylobacter are also able to acquire resistance determinants by natural transformation, transduction, or conjugation, for example, conjugation of carrying plasmids [21]. Transfer of DNA between Campylobacter strains has been shown both in vitro in bacterial cultures and in vivo in chicken intestine [22]. Horizontal gene transfer (HGT) is mediated by natural transformation, conjugation and transduction, all of which can be found in Campylobacter. Conjugation plays a major role in the transfer of plasmid-mediated resistance, such as the tet(O) gene, while natural transformation may be a major mechanism for the transfer of chromosomally encoded resistance (e.g., FQ and macrolide resistance). Multiple plasmids have been reported in Campylobacter, some of which can be transmitted by conjugation. Many of the conjugal plasmids carry genes mediating resistance to tetracyclines and aminoglycosides. It was reported that the transfer of a conjugal plasmid carrying the tet (o) gene occurred between C. jejuni strains in the intestinal tract of chickens. Considering the high prevalence of conjugal tet(O) plasmids, it is possible that conjugation has contributed to the spread of tetracycline resistance in Campylobacter [23].
Factors Contributing to Antimicrobial Drug Resistance:
As campylobacteriosis is a zoonotic foodborne disease, the presence of resistant strains in the food chain also has an influence on human infections. The use of antimicrobial agents in animal production is one of the main factors influencing antimicrobial resistance, especially to FQ and macrolides. In the early 1990s, as soon as enrofloxacin was introduced into animal production in Asia and in Europe, the FQ resistance started to increase among human Campylobacter isolates. In areas, where there is low fluoroquinolone usage in animal production, the incidence of fluoroquinolone resistant strains has remained low or moderate. For instance in Australia, where there is prohibition of the application of FQ in animal production, Campylobacter strains isolated from pigs are mainly not ciprofloxacin-resistant [24].

In case of macrolides, one of an important factor in the selection of erythromycin-resistant Campylobacter strains is the use of these antimicrobials in animal production as therapeutic or growth-promoting agents. However, acquisition of erythromycin resistance in Campylobacter is a stepwise process and requires prolonged exposure in contrast to the rapidly evolving FQ resistance. Lin et al. [20] studied the frequency of spontaneous mutations to an erythromycin resistant phenotype and found that both C. jejuni and C. coli have extremely low rates of spontaneous mutations under in vitro culture conditions.

Persistence and Fitness of Antibiotic Resistant Campylobacter: In the absence of antibiotic selection pressure, FQ resistance mediated by gyrA mutations can be stably maintained in Campylobacter [25]. FQ-resistant Campylobacter, carrying the T86I mutation in GyrA, colonized chickens persistently without losing the resistance-associated mutation and resistance phenotype. Both in vitro culturing and chicken colonization studies suggested that FQ resistant Campylobacter mutants do not carry a fitness burden. In fact, pairwise competition experiments indicate that FQ-resistant mutants outcompete FQ-susceptible strains in chickens, suggesting that, in fact, the FQ-resistant mutants possess an enhanced fitness. This fitness change is related to the T86I mutation and does not appear to be owing to a compensatory mutation, as transformation of FQ-susceptible C. jejuni strains with this mutation changed their fitness in chickens [18].

Determination of Minimum Inhibitory Concentration: Usually, antimicrobial susceptibility testing prior to treatment of Campylobacter infections is unnecessary; however, it may be useful, especially with the increase of resistant Campylobacter organisms. Several antimicrobial susceptibility testing methods, including agar dilution, broth micro dilution, epsilometer test (E-test) and disk diffusion test, have been used to measure antimicrobial resistance in Campylobacter species [26].

Resistance to multiple antibiotics was common among Campylobacter isolates found in approximately 95% of both C. jejuni and C. coli isolates. Resistance to FQ, ampicillin and trimethoprim/sulfamethoxazole was similar between the C. Jejuni and C. Coli isolate. FQ resistance was very common among the 57 Campylobacter isolates: 54 were resistant to nalidixic acid (95%) and 51 (89%) were resistant to ciprofloxacin (Table 1). One C. coli isolate was resistant to azithromycin.

Molecular and Biological Detection of Antimicrobial Drug Resistance Determinants: Extracted Campylobacter DNA from all samples and strains was used for molecular biological determination of selected antibiotic resistance determinants by PCR [28].

Detection of Erythromycin Resistance: Detection of mutations at positions 2074 and 2075 in domain V of the 23S rRNA gene, which mediates resistance to erythromycin, was carried out by MAMA–PCR and PCR–RFLP. Genes responsible for resistance of erythromycin and ciprofloxacin were tested at two loci using MAMA–PCR and PCR–RFL [28].

Detection of Ciprofloxacin Resistance: A single point mutation (Thr 86 Ile) in the quinolone resistance determining region (QRDR) of g-yrA was defined as source of high-level resistance to fluoroquinolones, MAMA–PCR for C. jejuni isolates was carried out, for C. coli a procedure according to Zirnstein et al. [29] was used.

Detection of Tetracycline Resistance: Primers DMT1 and DMT2 (Jena Bioscience GmbH) were used for the detection of the tet (O) gene which is strongly associated with tetracycline resistance in C. jejuni and C. coli. As a second gene locus associated with tetracycline resistance the presence of tet (A) was examined by PCR assay [30].

Status of Campylobacteriosis in Ethiopia: In developing countries, most of the infection is food borne commonly due to consumption of unpasteurized milk, contaminated water and meat especially poultry meat, rather than human to human transfer [31]. The few reported studies of
Table 1: Number and percent of Campylobacter isolates resistant to selected antibiotics in Kenya [27]

<table>
<thead>
<tr>
<th>Campylobacter spp.</th>
<th>No. of isolate</th>
<th>AM</th>
<th>AZM</th>
<th>CIP</th>
<th>NA</th>
<th>SXT</th>
<th>TE</th>
<th>&gt;=2 Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni</td>
<td>47</td>
<td>34</td>
<td>0</td>
<td>89</td>
<td>94</td>
<td>19</td>
<td>68</td>
<td>96</td>
</tr>
<tr>
<td>C. coli</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td>20</td>
<td>70</td>
<td>90</td>
</tr>
</tbody>
</table>

Key: AM ampicillin, AZM azithromycin, CIP ciprofloxacin, NA nalidixic acid, SXT trimethoprim/sulfamethoxazole, TE tetracycline

Campylobacter spp. as human enteric pathogens in Ethiopia showed isolation rates ranging from 13.6 to 16.7% [32] and 39.6% from apparently healthy food animals. In Ethiopia, studies have revealed that diarrhoeal diseases are major causes of infant and child mortality and morbidity. About 39, 000, 000 episodes of diarrhoea per year were estimated to occur in Ethiopia; out of which 230, 000 deaths occur in children below five years of age. The pediatric admission review at Jimma hospital showed that diarrhoea was the second leading cause of admission and hospital deaths and Campylobacter is one cause of diarrhoea in the area [33].

The absence of national surveillance program, limited routine culture availability for the isolation of Campylobacter species at clinical and research settings and the need for selective media and unique growth atmosphere make it difficult to give an accurate picture of the burden of disease in Ethiopia. This fact indicates that Campylobacter as a zoonosis is not given appropriate weight and consideration. Treatment with antibiotics for uncomplicated campylobacter infection is rarely indicated. However, antimicrobial resistance to clinically important drugs used for treatment (especially macrolides and FQs) is increasingly reported for Campylobacters [10]. There is evidence that patients infected with antibiotic-resistant strains suffer worse outcomes (invasive illness or death) than those infected with sensitive strains [34].

Status of Antimicrobial Drug Resistance in Ethiopia:
In Ethiopia, a few publications have reported on the occurrence and susceptibility testing of Campylobacter strains to antimicrobials in food animals and foods of animal origin and antimicrobial susceptibility pattern on sheep carcasses. There is growing scientific evidence that the use of antibiotics in food animals, particularly in developed countries, leads to the development of resistant pathogenic bacteria that can reach humans through the food chain. This underlines the need to limit the use of antimicrobials in veterinary practice to limit the occurrence of resistance [35].

In Ethiopia, epidemiological data about the prevalence and antimicrobial susceptibility patterns of Campylobacter spp. are restricted to strains from clinical samples isolated from children with gastroenteritis. There is neither an official surveillance nor monitoring system for the presence of Campylobacter in animals, nor for the use of antimicrobials in veterinary medicine [32].

The increasing rate of human infections caused by antimicrobial resistance strains of Campylobacter makes clinical management of cases of campylobacteriosis more difficult. Antimicrobial resistance can prolong the illness and compromise treatment of patients with bacteraemia. The rate of antimicrobial resistant enteric infections was highest in the developing world, where the use of antimicrobial drugs in humans and animals are largely unrestricted [36].

Control Options of Antimicrobial Drug Resistance:
Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. Steps can be taken at all levels of society to reduce the impact and limit the spread of resistance [11, 37].

Individuals: To prevent and control the spread of antibiotic resistance, individuals can only use antibiotics when prescribed by a certified veterinary professional, never demand antibiotics if your health worker says you don’t need them, always follow your health worker’s advice when using antibiotics, never share or use leftover antibiotics, prevent infections by regularly washing hands, preparing food hygienically and avoiding close contact with sick people, Prepare food hygienically, following the WHO Five Keys to Safer Food (keep clean, separate raw and cooked, cook thoroughly, keep food at safe temperatures, use safe water and raw materials) and choose foods that have been produced without the use of antibiotics for growth promotion or disease prevention in healthy animals [37].

Agriculture Sector: To prevent and control the spread of antibiotic resistance, the agriculture sector can only give antibiotics to animals under veterinary supervision and not use antibiotics for growth promotion or to prevent diseases in healthy animals, vaccinate animals to reduce the need for antibiotics and use alternatives to antibiotics when available and improve biosecurity on farms and prevent infections through improved hygiene and animal welfare [37, 38].
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. The absence of national surveillance program, limited routine culture availability for the isolation of *Campylobacter* species at clinical and research settings and the need for selective media and unique growth atmosphere make it difficult to give an accurate picture of the burden of disease in Ethiopia. This fact indicates that *Campylobacter* as a zoonosis is not given appropriate weight and consideration.

Based on the above conclusion, the following recommendations are listed out:

- Efforts to establish at least a National Laboratory with facilities for performing phenotyping and genotyping methods, reduce the limitation of routine culture availability is highly recommended.
- Controlled and careful use of antimicrobials, both in veterinary and human treatment regimens and further wider investigation of antimicrobial resistance pattern for well-targeted use of antimicrobials.
- Collaboration between veterinary and medical institutions should be established.

**REFERENCES**


