Amritanjali Kiran[1], Maria Jose Ruiz Alvarez[2], Khine Swe Swe Han[3], Abhishek Kumar[4], Dipanjan Chakraborty[5], Lawrence Mugisha[6], Hana Elbadawi[7], Yara Mohsen[8], Aparna Panicker[9], Sajini Souda[10], Maarten Van Dongen[11]

The first author and all co-authors are members of the global Network of AMR Insights Ambassadors. For more information about this network: ***www.amr-insights.eu/about-us/ambassadors***

The notorious case of *Campylobacter* spp.: an increasingly antimicrobial-resistant foodborne pathogen

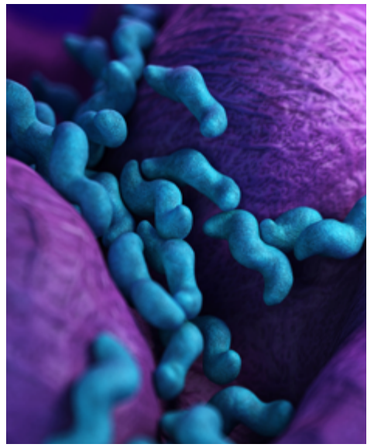
With *Campylobacter* infections on the rise and treatment options disappearing because of growing antimicrobial resistance, how should the food industry respond?

Why *Campylobacter*?

Campylobacter is one of the major foodborne pathogens of concern. *C. jejuni* and C. *coli* are the major causative agents, with *C. jejuni* accounting for approximately 90% of cases of *Campylobacter*-associated illness around the world (Tresse et al. 2017; Tam et al. 2003; Wieczorek et al. 2018). Campylobacteriosis caused by *C. jejuni* commonly causes severe diarrhoea, abdominal pain, fever, headache, nausea, and vomiting with some extreme cases resulting in Guillain–Barré syndrome (GBS) and acute flaccid paralysis (Havelaar et al. 2015; Ramos et al. 2021). Symptoms are severe in children below 5 years of age, the elderly, and immunocompromised individual. The infection is usually sporadic and self-limiting and thus does not require medication. Mortality due to *Campylobacter* infection is low (Mearelli et al. 2017). *Campylobacter* infection in humans is due to consumption of contaminated food and water (Tam et al. 2003).

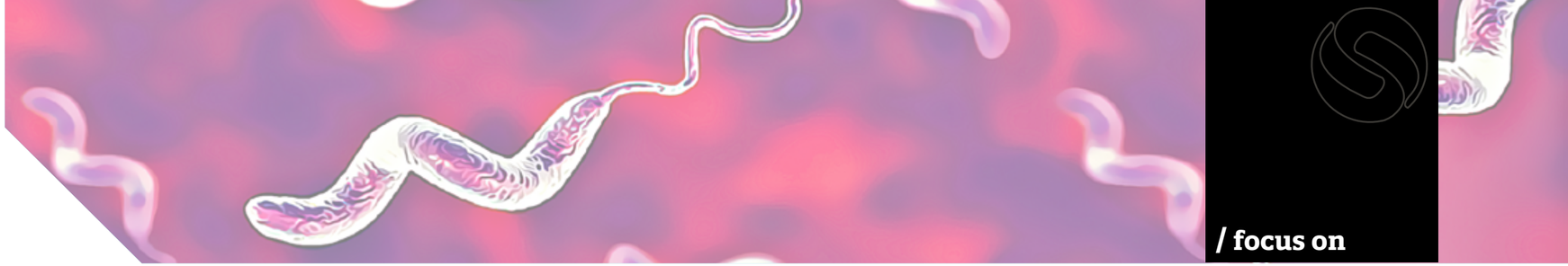
Epidemiology of *Campylobacter*

Annually, approximately 800 000 cases occur in the Unites States of America alone, and the incidence in high-income countries varies from 4.4 to 9.3 per 1 000 people (Havelaar et al. 2015). In 2014, Europe reported a total of 240 379 confirmed cases from a total of 28 countries – an increase of 13% over the previous year (European Centre for Disease Control and Prevention 2017). In the Oceania region, Campylobacteriosis was the most reported foodborne infection in Australia in 2010, with 16 968 cases (Kaakoush et al. 2015). Although surveillance data from developing countries is greatly lacking, Campylobacteriosis is endemic in certain parts of Africa, Asia, and the Middle East, especially in children under 2 years of age, who often have repeated or chronic infections (Kaakoush et al. 2015; Pascoe et al. 2020). *Campylobacter* infections rarely cause mortality, though occasional deaths occur in the elderly, immunocompromised, or paediatric populations (Kaakoush et al. 2015; Havelaar et al. 2015).



Geographical impact of *Campylobacter*

Campylobacter infection is zoonotic and it is present in domestic as well as wild birds and animals all around the world (Hald et al. 2016; Truccollo et al. 2021). Infection rates spike around summer in Nordic countries like Sweden and Norway (Kuhn et al. 2020). However, infection reports from Australia do not indicate an increase in the number of infections reported during the summer (Bi et al. 2008). The influence of weather and temperature on Campylobacteriosis is considered to be indirect as countries with high average temperature ranges like Nigeria (18 °C [65 °F] to 37 °C [98 °F] and countries with low average temperature ranges like Iceland (10 °C [50 °F] to 15°C [59 °F]) have reported cases of Campylobacteriosis, indicating that there is no correlation between temperature and the growth and spread of the *Campylobacter* species (Callicott et al. 2008). There are not many studies reporting the geographic, climate, and temperature impact on *Campylobacter* infections and the available studies do not show any signifi-



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... its genetic flexibility... promote(s) positive selection in *Campylobacter* for adaptability to colonize hosts and to develop antimicrobial resistance

cant correlation between geographical location and *Campylobacter* infection (Gwimi et al. 2015).

Resistance mechanisms in the context of *Campylobacter* food poisoning

Campylobacter's dynamic adaptation is attributed to its genetic flexibility, which benefits the organism with rapid evolution. The innate error-reading mechanisms, the genome's vulnerability to mutation, and the exposed cells that make it possible to receive horizontal genes collectively promote positive selection in *Campylobacter* for adaptability to colonize hosts and to develop antimicrobial resistance (Costa and Iraola 2019). As the bacteria passes through various host communities, the cost of bacterial fitness increases and *Campylobacter* infections in humans are commonly associated with antibiotic resistance (Costa and Iraola 2019).

A study of diarrhoeal cases caused by *C. jejuni* to determine the antibiogram associated with molecular resistance mechanisms shows that resistance to fluoroquinolones (55.8%) and tetracyclines (49.7%) was high (Elhadidy et al. 2020). Previous studies show that resistance to ciprofloxacin and tetracycline has exponentially increased (Wieczorek et al. 2018) with a growing trend of treatment complications in infants who are commonly treated with antibiotics for non-self-limiting diarrhoea and systemic Campylobacteriosis (Schiaffino et al. 2019; Dai et al. 2020). DNA gyrase mutation C257T in the *gyrA* gene and the tetracycline resistance *tetO* gene is detected in the majority of the clinical isolates, but few isolates seem to have also developed physiologic resistance. The tetracycline resistance *tetO gene* is transferred horizontally from poultry sources (Avrain et al. 2004). Approximately 2% of *Campylobacyter* isolates from diarrhoeal patients were resistant to macrolides and contained *ermB* coding for efflux pump, *rplD*, and *rplV* genes in the 23s rRNA mutation of 50S ribosomal subunit resistance genes (Elhadidy et al. 2020). This highlights the need for future exploration of physiologically resistant populations of *Campylobacter* spp. in human infections. Thermotolerant communities of *Campylobacter* with higher antibiotic resistance were encountered in poultry and bovine meat (Di Giannatale et al. 2019). Gastrointestinal pathogens require thermotolerance and other physiological resistance to acids or salts to survive and to flow between hosts along the food chain. Such tolerance mechanisms may be attributed to the phenotypic resistance to antibiotics, which requires further study.

Morphophysiological characteristics of *Campylobacter*

Campylobacter bacteria are small, gram-negative, non-spore-forming, oxygen-sensitive, highly mobile curved or spiral-shaped rods that grow best in micro-aerobic conditions. They have a polar flagellum at one or both ends of the cell, they are catalase- and oxidase-positive, and they are urease-negative. They are fastidious organisms which belong to a distinct group, designated "rRNA superfamily VI", and have been reported to change into coccoid forms upon exposure to adverse conditions, especially oxidation. In general, these bacteria are fragile and easily destroyed by heat, acidity, desiccation, and disinfectants.

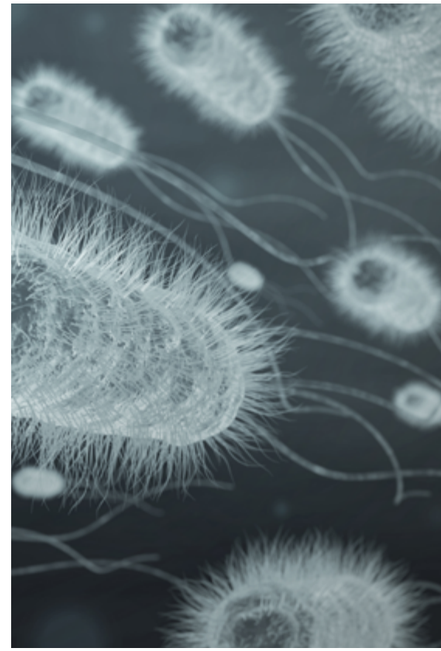
There is an overwhelming increase in *Campylobacter's* antimicrobial resistance, especially to fluoroquinolones...

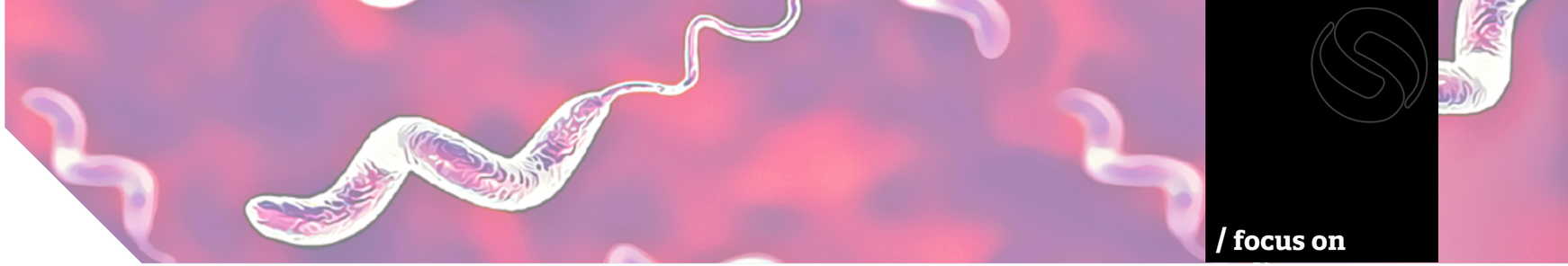
Campylobacter virulence factors

Enteric *Campylobacters* likely express several virulence factors when colonising the intestines, allowing for their survival against food processing and resistance to physiological stress (Bolton 2015). The different virulence-related mechanisms include invasive properties (facilitating binding and entry into host cells), bacterial adherence to intestinal mucosa, oxidative stress defence, heat shock, toxin production (e.g. cytotoxins and cytolethal distending toxin that cause cell death), iron acquisition (for nutrition), and the ability to remain in a viable but non-culturable (VBNC) state (Backert et al. 2013). Other *Campylobacter* virulence factors entail secretion of certain sets of proteins, translocation capabilities, chemotaxis (to traverse chemical gradients), and flagella-mediated motility (enabling movement into the mucous layer) (Biswas et al. 2011).

Impact of *Campylobacter* Antimicrobial Resistance

There is an overwhelming increase in *Campylobacter*'s antimicrobial resistance, especially to fluoroquinolones; this is perhaps unsurprising given the alarming increase in resistance to fluoroquinolones on a global level. This limits the usefulness of fluoro-





Campylobacter bacteraemia has a very low detection rate, accounting for less than 1% of European patients with gastroenteritis.

Ingestion of 500–800 bacteria can result in human disease.

quinolone as a treatment option for human infection; however, there is relatively low resistance to macrolides (Ruiz-Palacios 2007). Because Campylobacteriosis is a foodborne zoonotic disease, the presence of resistant strains in the food chain will have an impact on human health (Wieczorek et al. 2018). Infection with quinolone- or erythromycin-resistant strains of *Campylobacter* species is associated with increased adverse drug reactions when compared with infection with antimicrobial-susceptible species and is associated with long duration of illness, narrowed treatment options for cases requiring antibiotics, and an increased likelihood of treatment failure for diarrhoeal disease (Ruiz-Palacios 2007).

It is extremely important to tackle rising *Campylobacter* antimicrobial resistance because high-risk populations such as the elderly or immunocompromised are likely to be prescribed antibiotics to prevent bacteraemia and other complications. In some cases, *Campylobacter* can even disseminate in the periphery and cause profound disease (Yang et al. 2019).

Campylobacter bacteraemia has a very low detection rate, accounting for less than 1% of European patients with gastroenteritis. This can be attributed to many factors such as under-diagnosis and the lack of routine blood cultures for gastroenteritis patients (Mearelli et al. 2017). *Campylobacter* bacteraemia is very serious in patients with humoral immunodeficiency (mainly AIDS), gammaglobulinemia, diabetes mellitus, cirrhosis, and complement system disease, as well as in those who are receiving corticosteroid therapy.

Campylobacter jejuni is the most frequently isolated *Campylobacter* species in patients with sepsis (Meyrieux et al. 1996). A fatal case of septic shock with multiple organ failure due to *Campylobacter jejuni* has been reported in the literature, and the culture in that case showed resistance to cephalothin, amoxicillin, amoxicillin/clavulanate, aminoglycosides, erythromycin, and pefloxacin (Meyrieux et al. 1996). Another case in the literature describes a patient with septic shock and multiple organ failure due to fluoroquinolone-resistant *Campylobacter jejuni* that was also sensitive to macrolides (Mearelli et al. 2017). Prevention and control measures to address antimicrobial resistance are essential for improving the management and treatment of patients with *Campylobacter*.

Source and transmission pathways in *Campylobacter*

The colonization of different animal reservoirs by *Campylobacter* poses an important risk to humans through shedding of the pathogen in livestock waste and contamination of water sources, the environment, and food (Igwaran and Okoh 2019). *Campylobacter* bacteria colonize the gastrointestinal and reproductive tracts of a wide variety of wild and domestic food animals including poultry, cattle, pigs, sheep, dogs and cats, ostriches, deer, and shell fish (Liu et al. 2018; Buchanan et al. 2017). Human infections may be acquired via infected animals and their food products through various means, including through the consumption of unpasteurized milk, untreated water, or undercooked poultry or red meat (Kenyon et al. 2020; Kaakoush et al. 2015).

Faecal contamination of meats, particularly of poultry origin, often occurs during slaughtering, resulting in cross-contaminated food products. *Campylobacter* infections can also be acquired through direct contact with infected pets within the family environment (Javid 2019; Asuming-Bediako et al. 2019; Igwaran and Okoh 2019). A review of the literature published between 2007 and 2013 shows that a majority of *Campylobacter* outbreak cases were associated with poultry products (50-70%) and contaminated water, with colonised animals becoming lifelong carriers. Cross-contamination in the kitchen from contaminated meat to items that will be eaten raw, such as salads, is also considered a major pathway for transmission (Centers for Disease Control and Prevention 2019; Newell and Fearnley 2003).

Clinical manifestations of *Campylobacter* infection in humans

Ingestion of 500–800 bacteria can result in human disease. However, there are reports of 100 *Campylobacter* cells or fewer causing infections in humans (Frirdich et al. 2017). Acute diarrhoea (including traveller's diarrhoea and children's diarrhoea) is the major symptom caused by a *Campylobacter* infection. Watery or bloody diarrhoea, accompanied by fever, stomach cramps, abdominal pain, nausea, and vomiting are common. Bloating, headache, and muscle pain may also occur in some patients.

Symptoms of Campylobacteriosis usually manifest within two to five days of exposure, but symptoms can also begin in as little as one day or after as many ten days. Symptoms generally last three to six days, although occasionally they may last longer (Gharst et al. 2013; Scallan et al. 2015). *Campylobacter* has been reported as one of the pathogens responsible for benign convulsions associated with mild gastroenteritis (BCWG), especially in summer and autumn (Chen et al. 2019). Childhood Campylobacteriosis has been reported to manifest as a single course of gastroenteritis in a previously healthy young child or as recurrent episodes in an older, immune-compromised child, usually without gastrointestinal symptoms (Bi et al. 2008).

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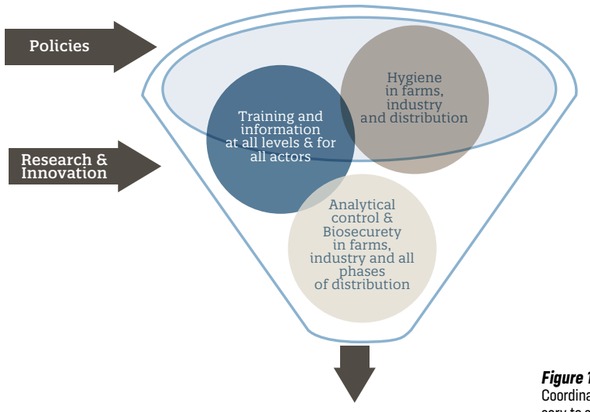

Figure 1:
Coordinated strategies are necessary to solve the global *Campylobacter* issue.

Current guidelines to tackle the *Campylobacter* and their drawbacks

The epidemiology of Campylobacteriosis is consequential for public health, not only because of increasing Campylobacteriosis prevalence but also because of the increasing number of human infections where antimicrobial-resistant *Campylobacter* has been identified. In humans, *Campylobacter* spp. accounted for 50% of all reports of foodborne illness (220 682 confirmed cases) in the most recent annual report on zoonoses, zoonotic agents, and epidemic outbreaks of foodborne illness from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (2019 data from 28 EU Member States and 8 European non-EU countries) (EFSA/ECDC).

The most effective strategies for preventing *Campylobacter* infections are based on interactive epidemiological information from surveillance systems, such as the Foodborne Diseases Active Surveillance Network (FoodNet) that identifies food infections caused by *Campylobacter* and other pathogens. FoodNet monitors more than 650 laboratories in 10 US states for a surveillance area that covers nearly 50 million people. In Europe, the lines of control are established according to EFSA research and Commission Regulation (EU) 2017/1495 of August 23, 2017, that amended Commission Regulation (EC) No 273/2005 of 17 February 2005. The 2011 EFSA opinion on *Campylobacter* was updated using more recent scientific data and reviewed the control options for *Campylobacter* in broiler chickens at primary production sites.

The guidelines for controlling *Campylobacter* are divided into three pillars: farms, the food industry, and distribution. In the food industry, the most important factors determining the presence of *Campylobacter* are sanitization practices that incorporate sufficiently strong biocides (a reduction > 5 log CFU/mL of *Campylobacter jejuni* is required); the capacity to form mono- and multispecies biofilms, both in aerobic and anaerobic conditions; and increased resistance to environmental conditions and increased cell transfer capacity. Solving these issues is neither simple nor easy and it will be necessary to harmonize control methods along the entire food chain and promote new research and new exchanges of information (***Figure 1***).

The most important challenges are standardizing validation measures, implementing improved biocontrol methodologies in the poultry sector (principally, mitigating the source of *Campylobacter* transmission to humans), and promoting innovation and efficacy among all ecosystem actors. These actions must occur in parallel with policies for control and contamination notification and they must increase consumer protection (Kaakoush et al. 2015).

Recently, scientists have started looking at alternatives to antibiotics to address multidrug-resistant Campylobacter.

Recent interventions

Multiple strategies have been implemented to reduce *Campylobacter* colonization in the food chain. Recently, scientists have started looking at alternatives to antibiotics to address multidrug-resistant *Campylobacter*. Scientists have also explored prebiotics as a supplement to prevent *Campylobacter* colonization in the guts of animals, though the findings were inconsistent (Dai et al. 2020). Researchers have also focused on the use of chemical-based antimicrobials such as trisodium phosphate (TSP), peracetic acid (PAA), acidified sodium chlorite (ASC), and cetylpyridinium chloride (CPC). These chemicals are usually applied as spray or surface sanitizers in poultry farms and in the sites where broiler chickens are processed (Johnson et al. 2017).

Major investigations are currently underway to improve antimicrobial intervention methods in poultry processing. Processing plants cannot rely on the integrity of cold transportation to retailers and, therefore, must prioritize the advancement of antimicrobial interventions. There are many factors driving industrial changes but one of the most prominent factors is pressure from the public for safer poultry (MacRitchie et al. 2014). Producers are exploring alternative and novel methods to kill these microbes, including electrostatic spraying, cold plasma treatment, and bacteriophage-based methods (Soro et al. 2020).

Conclusions

Campylobacter is notorious for its rapid spread and colonization in animals and humans through food and excreta. There is debate regarding the administration of antimicrobial treatments in the management of uncomplicated *Campylobacter* infections because these infections are usually self-limited and most patients recover without the need for antibiotics. Antibiotics should be reserved for severe cases of *C. jejuni*, which include symptoms such as bloody diarrhoea, fever, worsening symptoms, or a large number of stools (Ruiz-Palacios 2007). According to the CDC, antibiotics should be reserved for elderly people (65 years or older), pregnant women, and the immunocompromised. Government bodies need to focus more attention on how *Campylobacter* spreads. A coordinated strategy and a push for novel interventions might provide new perspectives and a better chance to mitigate the rise in antimicrobial-resistant *Campylobacter* infections.

The AMR Insights Ambassador Network consists of an integrated global and cross-professional community discussing, devising, and driving actions to combat AMR. The Network aims to inspire, connect, and empower our Ambassadors to take individual and collective actions to curb AMR.

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Author affiliations:
[1]Centre for Cellular and Molecular Platforms, Bengaluru, India
[2]Research Coordination and Support Service, Istituto Superiore di Sanità, Rome, Italy
[3]Department of Medical Microbiology, National Health Laboratory Service, Durban, South Africa
[4]Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India
[5]ZeiniX Life Sciences, Bengaluru, India
[6]College of Veterinary Medicine, Makerere University, Kampala, Uganda
[7]Microbiology and Parasitology Department, University of Khartoum, Sudan
[8]Department of Epidemiology, Alexandria University, Alexandria, Egypt
[9]Ramniranjan Jhunjhunwala College, Mumbai, India
[10]Faculty of Medicine, University of Botswana, Gaborone, Botswana
[11]AMR Insights, Amsterdam, The Netherlands

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