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Occurrence of Antimicrobial Resistance in Foodborne Bacteria (*Campylobacter* and *E. coli*): A Food Safety Issue and Public Health Hazard

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Abstract

Campylobacter and *Escherichia coli* (*E. coli*) are prominent bacterial causes of human gastroenteritis in developing countries and the emergence of antibiotic resistance of these bacteria has been widely reported to be on the increase, particularly because of the increase in the number of resistant *Campylobacter* and *E. coli* isolated from human infections. The widespread use of antimicrobial drugs for therapeutic, prophylactic, and preventive purposes in modern food animal husbandry is a great concern in terms of the development of antibiotic resistance in many bacteria, and, therefore, a potential public health threat. This review simply describes the occurrence, spreading and public health significance of antimicrobial resistance in *Campylobacter* and *E. coli*.

Keywords: Antimicrobial resistance; *Campylobacter*; *Escherichia coli*;

Abbreviations: AMR: Antimicrobial resistance; *E. coli*: *Escherichia coli*; *C. jejuni*: *Campylobacter jejuni*; ESBL: Extended-spectrum beta-lactamase C. coli: Campylobacter coli.

Introduction

Antimicrobial resistance (AMR) is an emerging global threat. According to one estimate, unless appropriate action is taken, the annual number of deaths due to antimicrobial resistance may reach 10 million by 2050 [1]. This estimate, however, is considered high and has been questioned by others. Nevertheless, problems associated with antimicrobial resistance and the urgency to act are recognized by all. The presence of antimicrobial resistant bacteria and related resistant genes in the environment is well recognized for their role in the spread of antimicrobial resistance [2]. Numerous resistant genes associated with human diseases have an environmental origin [3]. Recent studies related to AMR have focused on the monitoring and treatment of resistant genes in different environmental matrices [4,5]. A focus on environmental occurrence is essential not only from the perspective of water and environmental safety but also to prevent the spread of AMR. Resistant genes in wastewater treatment plants, for example, are being studied from multiple perspectives due to the reuse of wastewater for agricultural purposes often without extensive treatment [6,7]. Globally, the increase and spread of antibiotic resistance, especially to foodborne zoonotic bacteria with their reservoirs in healthy food animals, such as poultry, pigs and cattle, have become a public health concern [8]. In developed countries, there is an increasing number of scientific reports regarding the widespread usage of antibiotics in food animal production, which leads to the development of resistant pathogenic organisms in the food chain [9,10].

The resistant elements reduce the efficiency of antibiotic therapy, which results in an increased morbidity and mortality associated with disease outbreaks [8]. It has been reported by Schwarz and Chaslus-Dancla [11] in 2001, that an antibiotic therapy with a specific agent has been either accompanied or followed shortly by the occurrence of resistant bacteria, and, for the past twenty years, the effect of antibiotics on the development of resistance has gained much attention. Poultry farms are regarded as the major source of resistant genetic elements for foodborne pathogens such as *Campylobacter* and *E. coli* [12-14], and poultry products are considered as vehicle of these pathogens to human. Once a broiler chicken becomes infected, *Campylobacter* spread rapidly to other broiler chickens in that flock, and up to slaughter age, or, at thinning, the chickens remain colonized. Almost all (100%) of the broiler chickens brought to the slaughterhouses were reported to be colonized with *Campylobacter* [15], and the contaminated chicken meat acts as a probable risk of human *campylobacter*iosis. The detection of *C. jejuni*, C. coli and *E. coli* on the carcasses is mainly due to contamination from the gastrointestinal contents of slaughtered healthy animals during processing as well as at retail [16].

Campylobacter and *E. coli* have been reported to develop resistance to a number of antibiotics including ciprofloxacin and other fluoroquinolones, macrolides and licosamides, chloramphenicols, aminoglycosides, tetracyclines, and ampicillins, as well as β-lactams, cotrimoxazole, and tylosin [17-19]. Wild birds are important with regard to antibiotic resistance in several different ways: 1) As sentinels, mirroring human activity and its impact on the environment because of the diverse ecological niches of birds, and as they easily pick up human and environmental bacteria. 2) As a reservoir and melting pot of antibiotic-resistant bacteria and resistant genes. 3) As potential spreaders of antibiotic resistance through the ability to migrate long distances in short periods of time. 4) As a possible source of antibiotic resistant bacteria colonizing and/or infecting human beings. The first antibiotic resistant bacteria noted in wildlife were in fact from wild bird strains of *E. coli*, which were resistant to multiple antibiotics; for example, chloramphenicol was isolated in pigeons around 1975 [20]. Many bird species have been found to carry antibiotic resistant bacteria. Resistant *E. coli* has been isolated from ducks and geese [21,22], cormorants [23], birds of prey [24], gulls [25], doves, and passerines [26].

The results from a study on *Campylobacter* by Chen et al. [27] showed that the vast majority of the isolates from birds appeared to be resistant to fluoroquinolones by more than 98%. In the same study, *Campylobacter* jejuni was resistant to gentamicin at 27.2%. Importantly, a previous study from the Netherlands revealed a substantial increase in fluoroquinolone resistance among human cases since the advent of enrofloxacin in veterinary medicine [28,29,30]. While, apart from poultry, other animals, such as pigs and cattle, also serve as reservoir hosts [31]. Resistant *Campylobacter* and *E. coli* are also found in various other domestic and wild animals, including goats, horses, cats, rodents, and dogs [32-34], and have been isolated from marine animals, such as shellfish and dolphins [35,36]. *Campylobacter* infection is normally self-limiting, but it may be associated with complications, such as Guillain-Barre Syndrome (neurological) and Reiter's Syndrome (reactive arthritis) [37]. *Escherichia coli* is part of the normal enteric microbial flora in humans, poultry, and other animals, and the pathogenic *E. coli* causes disease in both [17]. The pathogenic *E. coli* causes a number of diseases in both poultry and humans, which include hemorrhagic colitis, hemolytic uremic syndrome, acute and chronic endemic and epidemic diarrhea.

Diarrheagenic strains of *E. coli* can be divided into six main categories on the basis of distinct epidemiological and clinical features, specific virulence determinants, and association with certain serotypes: enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and Diffuse- Adhering *E. coli* (DAEC). They are transmitted from person to person via direct contact with animal carriers, feces, contaminated soil and water or via ingestion undercooked meat and other animal products, as well as contaminated vegetables and fruits. As a result of contamination from feces, they are often found in soil, water and food. Commensal *E. coli* flora can be regarded as a rich source of emergence and spreading of antibiotic resistance [38]. Chickens may also be infected with antibiotic resistant *E. coli* or may develop resistance to one or more antibiotics in the gut. Even though antibiotic consumption is a major contributor of the antibiotic resistance phenomenon different factors have enhanced the development and dissemination of antibiotic resistance [39]. For instance, population densities among humans have been identified as risk factors for the development and spread of antibiotic resistance [40].

Several antibiotics have been shown to give rise to antibiotic resistant bacteria, which, when shed in the environment, comprise a reservoir of these bacteria [41-43]. These resistance elements decrease the effectiveness of antibiotic therapy, and, thus, result in increased morbidity and mortality associated with disease outbreaks, and, later, are transferred or spread to other animals or humans [39,44]. This shows that an antibiotic therapy with a particular agent has been either conveyed or followed shortly by the appearance of resistant bacteria [11]. Clinically, based on antibiotic susceptibility tests, a bacterium is classified as susceptible, intermediate, or resistant. A susceptible bacterium is inhibited in vitro by a concentration of an antibiotic agent that is associated with high therapeutic success. When the outcome of treatment is indefinite as a result of the in vitro inhibition effect of the concentration of the antibiotic, the sensitivity of a bacterial strain to that antibiotic is said to be intermediate, and when the likelihood of therapeutic failure is significant or high due to in vitro inhibition by the concentration of the drug, a bacterial strain is referred to as resistant to that antibiotic [45].

There are several mechanisms through which bacteria can become resistant. An antibiotic (bactericidal or bacteriostatic) has an effect on microorganisms when it restricts within their site of action. The mechanisms of action include inhibition of cell wall synthesis, such as glycopeptides, vancomycin, and β-lactams; disruption of cell membrane function, such as polymixins, surfactants, polyenes, and polypeptides; inhibition of protein synthesis, such as aminoglycosides, tetracyclines, phenicols, and macrolides; inhibition of nucleic acid synthesis, such as fluroquinolones and metronidazole; action as antimetabolites, such as diaminopyrimidines, flucytosine, and sulfanilamide [46,47]. The mechanism of resistance can be either an acquired trait or an intrinsic activity of bacterial specie. Acquired resistance occurs due to mutation of the chromosomes (point mutations, deletions, inversions, insertions, etc.) or by the acquiring of genetic elements. Several bacterial strains have a natural resistance towards some types of antimicrobial agent. Intrinsic resistance

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covers whole bacterial specie and requires resistance without any addition of genetic elements or mutations. Some bacteria lack a cell wall, e.g., mycoplasma; this will be the effect of intrinsic resistance to β-lactams [48,49].

Public Health significance of antibiotic resistance

In humans, most of the antibiotic resistance problems originate from the misuse of these agents in human medicine, and there is evidence that antimicrobial resistant enteric bacteria can transfer from animals to humans, and thus create a reservoir of resistant bacteria [50,51]. The transfer of resistant bacteria between animals and human via food products has been recognized and could pose a threat to public health [51,52]. The transfer pathway is diverse and complex, and bacteria can transfer from food animals to humans by direct contact through which farmers, abattoir workers and veterinarians are particularly at risk [53], and the bacteria can be carried between farms via the food chain through meat, eggs, and milk [54-56], as well as via the environment as animals produce a large amount of waste that can be used for manure and can contaminate water, soil, and crops [17,57]. Moreover, the situation is made more complex by the transfer of bacteria from humans to animals and the environment; from pets to humans and vice versa; from feed to production animals; and from the environment to wildlife to production animals. The main concern is over the risk and dissemination of resistant zoonotic bacteria, such as *Salmonella* spp or *Campylobacter* spp and resistant commensals bacteria, such as *E. coli*, which can spread their resistance gene onto the human gut flora and possible human pathogens. There is broad scientific agreement that the use of antibiotics in food animals has harmful effects on human health. According to [58], the use of the antibiotic avoparcin as a growth promoter in food animals in Europe resulted in the development and amplification of vancomycin-resistant enterococci (VRE) and the subsequent colonization of a significant percentage of these VRE in the human population via the food chain (between 2% and 17%).

When the use of avoparcin in food animals in the European Union was banned, there was a marked reduction in the percentage of the general population carrying VRE in their bowels. In Australia, vancomycin-resistance is a cause for concern because vancomycin is used as a last- line antibiotic for some hospital-acquired infections of enterococci and staphylococci that have become resistant to the more commonly used antibiotics. Collignon [59] also reported that the use of the antibiotic enrofloxacin has been approved for use in food production animals in many countries. The use of this antibiotic in food animals has led to the development of ciprofloxacin-resistant strains of *Salmonella* and *Campylobacter*. The use of antibiotic tylosin in the animal feed as a supplement has led to the development of erythromycin-resistant streptococci and staphylococci not only in the animals but also in their caretakers. It has been reported that there is a relationship between the approval of fluoroquinolones for therapeutic use in food producing animals and the development of fluoroquinolone resistance in *Campylobacter* in animals and humans after the approval of fluoroquinolones for use in food-producing animals [60]. The approval of these drugs in food- producing animals led to an increase in the resistance in the *Campylobacter* isolates from treated animals and ill humans in the Netherlands, Spain, and the United States.

Campylobacter

The aim of using antibiotics in both human and veterinary practice was to acquire health benefits for humans and animals, however, resistance to certain classes of antibiotics has been discovered that could have a public health impact [60]. Consequently, the imprudent use of antibiotics would facilitate the persistence of these emerging resistant bacteria, which could subsequently be transmitted to humans and cause untreatable illnesses [18]. *Campylobacter* is an important foodborne zoonotic disease. However, the highly resistant strains of this bacterium, which can acquire resistance in response to antibiotic selection pressures in animal food production, can cause severe economic losses for humans, especially when these antibiotics are used as the drugs of choice for treating infections in humans where the resistance is acquired [61]. Resistance to antibiotics has developed into a serious problem worldwide, and the rate of *Campylobacter* resistance to multiple antibiotics continues to rise. *Campylobacter* resistant strains can successively transpire because of the administration of antibiotics for the treatment of bacterial infections in animals, which may possibly contaminate human food [62].

The ability of *Campylobacter* resistance to antibiotics poses a major problem and highlights the need for further research, especially in livestock production [63,64]. Several studies implied that livestock-associated *Campylobacter* are gradually becoming resistant to numerous antibiotics that are of high importance for the treatment of human infection [64-67], and, consequently, poses a threat to public health. Furthermore, there is a clear link in the antibiotic resistance among the pathogens that are isolated from dairy cattle and the use of antibiotics in dairy cows and other livestock to significantly increased antibiotic resistance [68]. In a study by Sato et al. [69], there was no significant distinction in the occurrence of *Campylobacter* species in organic and conventional farms. In the United States, resistance to *Campylobacter* isolates to fluoroquinolones (FQ) in humans is consistently growing with the widespread use of the same drug in animals and poultry [70,71]. Of specific interest is the increased resistance to fluoroquinolone and macrolides by the *Campylobacter* isolates from food animals, which has been significantly established, as these are the drugs of choice for the treatment of *Campylobacter* infection in humans, especially when clinical treatment is required [71,72].

E. coli

In studying the levels of resistance in bacteria, *E. coli* is considered to be the organism of choice; this is because the strains

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are capable of transmitting genetic material not only to each other but also other enteric pathogens (Lambrecht et al., 2019). A study in northern Georgia, USA 73. Zhao et al. [73] tested 95 APEC isolates and found that 92% of the isolates were resistant to three or more antibiotics. A study by Yuan et al. [74] in China reported that 80% of 71 *E. coli* isolates from the livers of chickens that died in 10 poultry farms were resistant to eight or more antibiotics. Similarly, between 2004 and 2005, Li et al. [75] found high levels of antibiotic resistant *E. coli* isolates from diseased chickens in China; the isolates showed 100% resistance to tetracycline and trimethoprim/sulfonamide, and 79–83% resistance to chloramphenicol, ampicillin, ciprofloxacin, and enrofloxacin. Antibiotic resistance in commensal strains of *E. coli* could play an essential role in the ecology of resistance and infectious diseases. European data in France, the UK, and the Netherlands show that there is a moderate resistance pattern to ampicillin, streptomycin, tetracycline, and trimethoprim/ sulfonamide; low resistance to gentamicin, chloramphenicol, and ciprofloxacin; and no resistance to cephalosporins [76]. There is 100% and 42% resistance of the isolates to nalidixic acid and ciprofloxacin, respectively, in 181 *E. coli* from broiler chickens based on the study by Moniri and Dastehgoli [77]. In addition to fluoroquinolone resistance, there is also a need to know the resistance to beta-lactams, especially extended spectrum beta-lactams. In Belgium, five-broiler chicken farms revealed 295 ceftiofur resistant *E. coli* from cloacal swabs [78].

In another study in the US, 194 *E. coli* recovered from egg shells showed that 73% of the isolates were susceptible to all seven antibiotics used [79]; the most resistance was to tetracycline at 17%. A study in Lithuania to evaluate the occurrence of the antibiotic resistance of *E. coli* isolated from the livers of chickens sold in the retail market showed that 42% of the isolates were resistant to the most frequently used tetracyclines, quinolones, and aminopenicillins. Chambers et al. [80] reported a resistance to 3rd generation cephalosporins, which are important in human medicine. In Japan, in a study on retailed chicken meat, twenty-eight (28) out of 69 *E. coli* isolates showed a multidrug resistance profile [81], and the most frequently reported resistance pattern was against trimethoprim/sulfamethoxazole, streptomycin, ampicillin, ciprofloxacin, spectinomycin, kanamycin, nalidixic acid, and cephalosporins. In a study in Kenya on the antibiotic resistance in *E. coli* isolated from feces and the carcasses of pigs, chicken, and cattle, the highest was 74%, which was for the chicken isolates that also showed a significantly high multidrug resistance [82]. Extended spectrum beta lactamase producing *E. coli* have also been reported in chickens. In poultry practice, with the widely used beta lactam antibiotics, e.g., amoxicillin and cephalosporins (particularly extended spectrum cephalosporins), Extended-spectrum beta-lactamase (ESBL) induced resistance in gram-negative bacilli has become critical and the treatment options for such infections are becoming inadequate [83]. A study by Yuan et al. [74] indicated that 60.8% of the isolates tested were ESBL-producing *E. coli*. Another survey in China by Lee et al. [84] showed that the occurrence of ESBL producing animal-associated bacteria was 30%.

Conclusion

The use of antibiotics in human therapy, aquaculture, and food animal production contributes to the emergence and development of antibiotic resistant pathogens due to selective pressure. The surveillance of antibiotic resistance in commensal, zoonotic and pathogenic bacteria from humans, animals, and food is a basic source of information when planning actions to increase food safety and identify new problems and potential outbreaks. Surveillance data are also crucial to monitor policy decision-making and to evaluate and validate their results. A combined approach is essential in order to be able to use and interpret these data, and this type of approach involves numerous sectors: healthcare organizations and public health, animal food production, food processing, and distribution.

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