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Major *Campylobacter jejuni* in chicken carcasses are more multidrug-resistant to different antibiotics than the standard strain.

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Abstract

Chickens are major reservoirs for *Campylobacter jejuni*. In this study, we assessed the multidrug resistance (MDR) of *C. jejuni* found in chicken carcass obtained from different companies in Riyadh city, Saudi Arabia and compared it with that of the standard strain of *C. jejuni*, ATCC 22931. Of the 30 strains tested, *C. jejuni subsp doylei* H1 exhibited the highest resistance to the tested antibiotics, with a ratio of resistance of 92.86%. Moderate resistance to antibiotics (ratio of resistance, 50%) was noticed for *C. jejuni* 2 A2, *C. jejuni* 1 B2, and *C. jejuni* 2 I1. The lowest resistance to antibiotics was exhibited by *C. jejuni subsp doylei* J1, with a ratio of resistance of 7.14%. The main cause of high variation in ratios of resistance among the Campylobacter strains may be due to the overuse of antibiotics, especially by poultry breeders or farmers. Transmission of *Campylobacter spp.* via chicken carcass contributes to the emergence of antimicrobial resistance in *C. jejuni*, which in turn poses public health risk.

Keywords: *Campylobacter jejuni*, Multidrug resistance, Chicken carcass.

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Introduction

Gastroenteric disease in humans is caused by various gram-negative bacteria, including *Campylobacter jejuni* [1-3]. The most common type of foodborne gastroenteritis in humans is *C. jejuni* bacterial gastroenteritis [4-5]. Campylobacteriosis, a severe illness caused by *Campylobacter sp*ecies (spp), is a self-limiting disease in healthy adults. However, in children, elderly individuals, and immunosuppressed individuals, treatment with antibiotics is required [6].

C. jejuni can be transmitted from poultry waste to humans through many ways such as exposure to birds' feces during cleaning of the coops, bird handling, petting, and kissing or through handling and consumption of contaminated eggs and meat [7,8]. Chicken carcass may also be contaminated by *C. jejuni* during the slaughtering process and handling, or from water used for cleaning the carcass. Cross-contamination between raw chicken and other ready-to-eat food via the cook's hands or kitchen utensils, knives, and cutting boards has also been reported [9].

Antimicrobial resistance (AMR) poses an additional risk because infections caused by antimicrobial resistant *Campylobacter spp*. may lead to increased morbidity and mortality due to higher chances of treatment failures with different antibiotics [10-12].

Erythromycin has been recommended in patients with severe intestinal infection and in immune compromised patients and is considered the usual drug of choice for treatment of infections by *Campylobacter spp*. [13-14]. Other antibiotics such as tetracycline, gentamycin, and fluoroquinolone are also used in the treatment of *Campylobacter spp*. infections, when antimicrobial therapy is required [15]. In a study conducted in Canada, *Campylobacter spp*. isolated from chicken samples and slaughterhouse were found to be resistant to the antibiotics fluoroquinolone and tetracycline [16-18]. High resistance of *Campylobacter spp*. to these antibiotics was also reported in studies conducted in China, Poland and Italy [19-21].

Antimicrobial agents are commonly used to control infections in poultry and act as growth promoters in poultry. Similar drugs are also used for treatment of infections in humans. Thus, there exists an increased risk of these antibiotics to become ineffective against various bacterial infections [22].

Studies have reported tetracycline, erythromycin, and gentamicin resistance of *C. jejuni* and *C. coli* isolated from poultry in Poland. These bacteria can be potentially transmitted

to humans and may compromise clinical treatment. In addition, other studies have shown that treat Campylobacter infected chickens with fluoroquinolones, develop quickly antibiotic-resistant *Campylobacter spp*., to fluoroquinolones [23-24]. The prevalence of antimicrobial-resistant *Campylobacter spp*. has been reported in animal reservoirs in different countries [25-28].

Overuse of antibiotics in animal feedings and meals is considered to be the main factor leading to antimicrobial resistance in enteric bacteria [19,20]. Until recently, the purchase and use of antibiotics without veterinary prescriptions was prevalent in many countries, including Canada, thus resulting in improper usage of antibiotics [29].

Research on multidrug resistance (MDR) of *C. jejuni* isolated from chicken carcasses in Saudi Arabia is limited. To address this knowledge gap, we evaluated MDR patterns in *C. jejuni* isolates from chickens carcasses distributed in retail markets in Riyadh city, Saudi Arabia.

Materials and Methods

C. jejuni ATCC 33291 was used as a positive control. Thirty identified strains of *C. jejuni* were supplied by the Department of Food Science and Human Nutrition, College of Food and Agriculture Sciences, King Saud University.

Media used

Bolton broth was used as the activation media for *C. jejuni* strains. Before inoculation, the broth was autoclaved at 121°C for 15-20 min, followed by cooling to 50 °C and supplemented with (Oxoid, SR0183) and laked horse blood (Oxoid SR0048). For activation, *C. jejuni* strains were inoculated in Bolton broth (Oxoid, CM0983). After inoculation, the tubes were incubated under microaerobic conditions using gas generating kits (Oxoid BR38) at 42°C for about 3-4 days. To test the antibiotic sensitivity for *C. jejuni* strains, Campylobacter Agar Base (Karmali), (Oxoid, CM0935) was used after autoclaving at 121°C, and cooling to 50°C and supplementing with (Oxoid, SR020).

A comparison of antimicrobial susceptibility tests for *C. jejuni* ATCC 33291 and thirty strains were performed. The tested bacteria were obtained from overnight cultures inoculated from single colonies into campylobacter blood-free selective agar (modified CCDA-preston), (Oxoid CM0739) supplemented with CCDA selective supplement (SR 155E), which were applied to the surface of the same medium and used for the agar disk diffusion method.

Antimicrobial susceptibility test

The antimicrobial susceptibility testing was performed by the disk diffusion method according to the protocol of the Clinical and Laboratory Standards Institute-CLSI for the following antimicrobials. A total of 25 different antibiotic discs (Oxoid, UK) containing the following components were prepared and tested against *C. jejuni* strains: chloramphenicol (C 30 µg CT0013B), kanamycin (K 30 µg, CT0025B), ciprofloxacin (Cip 5 µg, CT0425B), linezolid (LZD 30 µg CT1650B), nalidixic acid (NA 10 CT0424B), Neomycin (N 30 µg, CT0033B), ampicillin (AMP 10 µg, CT0003B), Amoxycillin/clavulanic acid (AMC 30 µg, CT0223B), Colistin (CT 25 µg CT0065B), Ticarcillin (TIC 75 µg CT0167B), Vancomycin (VA 5 µg CT0188B), Doxycycline (DO 30 µg CT0018B), Nitrofurantoin (F 100 µg CT0034B) and Erythromycin (E 15 µg CT0020B). The diameters of the zones of inhibition (mm) were measured using the criteria recommended for *C. jejuni* by CLSI [30].

Results and Discussion

The results of antimicrobial susceptibility testing are explained in Table 1. Resistance to antibiotics in *C. jejuni* strains was categorized in three groups. The first group exhibited the high rates of resistance to antibiotics and included *C. jejuni subsp doylei* H1 (92.85%), *C. jejuni* 2 C2 and *C. jejuni* 2 A5 (78.57%), and *C. jejuni* 2 E1, E2, E3, *C. jejuni* 2 A1, and *C. jejuni* subsp. doylei B1 (71.42%). Moderate resistance to antibiotics was observed in *C. jejuni* 2 A6, *C. jejuni* 2 C4, *C. jejuni* subsp. Doylei D3, *C. jejuni* 2 F2, and *C. jejuni* 2 H1 (64.28%), *C. jejuni* 2 A3, A4, and *C. jejuni* 2 G1, G3 (57.14%). Also, moderate antibiotic resistance of 50% was observed in *C. jejuni* 2 A2, *C. jejuni* 1 B2, and *C. jejuni* 2 I1. The third group with the lowest resistance to antibiotics included *C. jejuni* ATCC 33291, *C. jejuni subsp doylei* F1, and *C. jejuni* 2 B3, 57.15%, 42.85%, 35.71%, respectively, *C. jejuni* 2 C3, *C. jejuni* 2 D2, *C. jejuni* 2 E4 and *C. jejuni* 2 G2 (28.57% each), and *C. jejuni* 2 C1 (21.42%), *C. jejuni* 1 D1 (14.28%) and *C. jejuni subsp doylei* J1 (7.14%).

Table 1. *Antimicrobial resistance of 24 h cultures of Campylobacter jejuni based upon development of inhibitory zone diameters after application of discs containing specific antimicrobial agents.*

Antibiotics Microorganisms	C 30	K 30	CIP 5	LZD 30	NA 30	N 30	AMP 25	AMC 30	CT 25	TIC 75	VA 5	DO 30	F300	E 15	No. of strains	Resistance %	Sensitivity %
C. jejuni ATCC 33291	20	25	20	20	20	20	10	R	R	15	R	R	R	R	6	42.85	57.15
C. jejuni 2 A1	R	12	12	R	R	13	R	R	R	R	R	R	20	R	10	71.42	28.58
C. jejuni 2 A2	12	13	13	R	15	10	R	R	R	R	R	20	R	15	7	50	50
C. jejuni 2 A3	R	R	15	R	17	10	12	R	R	R	R	8	18	R	8	57.14	42.86

C. jejuni 2 A4	R	8	16	R	18	8	R	R	10	R	R	R	20	R	8	57.14	42.86
C. jejuni 2 A5	20	15	R	R	R	R	R	R	R	R	R	R	20	R	11	78.57	21.43
C. jejuni 2 A6	R	10	12	R	R	12	R	R	15	R	R	R	22	R	9	64.28	35.72
C. jejuni subsp. Doylei B1	R	8	12	R	10	R	R	R	R	R	R	20	R	R	10	71.42	28.58
C. jejuni 1 B2	R	13	R	R	15	12	R	14	R	15	R	18	R	15	7	50	50
C. jejuni 2 B3	R	15	14	R	15	14	15	14	1 4	15	R	18	R	12	4	28.57	71.43
C. jejuni 2 C1	20	15	15	R	15	14	14	15	R	15	R	20	10	12	3	21.42	78.58
C. jejuni 2 C2	R	R	16	R	10	R	R	R	R	R	R	R	18	R	11	78.57	21.43
C. jejuni 2 C3	12	12	14	R	22	12	12	R	14	14	R	R	15	12	3	28.57	71.43
C. jejuni 2 C4	R	R	18	R	20	R	R	R	25	R	R	12	20	R	9	64.28	35.72
C. jejuni 1 D1	20	20	12	20	R	16	20	15	R	75	20	20	18	18	2	14.28	85.72
C. jejuni 2 D2	R	R	18	R	10	15	8	15	18	15	R	20	13	13	4	28.57	71.43
C. jejuni subsp. Doylei D3	R	10	R	R	R	13	R	R	15	R	R	8	21	R	9	64.28	35.72
C. jejuni 2 E1	R	14	20	R	R	R	R	R	12	R	R	R	20	R	10	71.42	28.58
C. jejuni 2 E2	R	R	R	R	20	R	R	R	10	R	R	10	20	R	10	71.42	28.58
C. jejuni 2 E3	R	R	12	R	R	R	R	R	8	R	R	R	20	8	10	71.42	28.58
C. jejuni 2 E4	18	R	15	R	R	12	15	15	R	15	20	12	20	20	4	28.57	71.43
C. jejuni subsp doylei F1	13	15	16	22	R	R	16	20	R	15	R	15	20	R	5	35.71	64.29
C. jejuni 2 F2	R	12	20	R	20	20	R	R	15	R	R	R	R	R	9	64.28	35.72
C. jejuni 2 G1	R	13	10	R	R	12	R	R	13	R	R	8	20	R	8	57.14	42.86
C. jejuni 2 G2	1 6	14	12	16	R	10	R	16	15	R	14	15	18	R	4	28.57	71.43
C. jejuni 2 G3	R	R	14	22	R	R	15	15	R	22	R	10	R	R	8	57.14	42.86
C. jejuni subsp doylei H1	R	R	R	R	R	R	R	R	R	R	R	R	20	R	13	92.85	7.15

Mean zones of inhibition for common antibiotics tested as; chloramphenicol (C 30 µg), kanamycin (K 30 µg), ciprofloxacin (Cip 5 µg), linezolid (LZD 30 µg), nalidixic acid (NA 10), neomycin (N 30 µg), ampicillin (AMP 10 µg), amoxycillin/clavulanic acid (AMC 30 µg), colistin (CT 25 µg), ticarcillin (TIC 75 µg), vancomycin (VA 5 µg), doxycycline (DO 30 µg), nitrofurantoin (F 100 µg), and erythromycin (E 15 µg).

Company A=1, Company B=2, Company C=3, Company D=4, Company E=5, Company F=6, Company G=7, Company H=8, Company I=9 and Company J=10

Figure 1 shows the percentage of resistance to different antibiotics tested against all *C. jejuni* strains. Fourteen commonly antimicrobial agents used against *Campylobacter sp*. were used. Vancomycin (VA 5 µg) exhibiftated resistance with 26 Campylobacter strains (86.66%), followed by linezolid (LZD 30 µg) with 24 strains (80%). Resistance to Erythromycin (E 15 µg), amoxycillin/clavulanic acid (AMC) was observed in 20 strains of Campylobacter (66.66%). Resistance to ticarcillin (TIC 75 µg), ampicillin (AMP 10 µg), and chloramphenicol (C 30 µg) were observed equally in 19 different strains (63.33%). Fifteen (50%) were resistant to colistin (CT 25 µg), and 13 (43.33%) resistant to nalidixic acid (NA 10), 12 (40.00%) doxycycline (DO 30 µg), 11 (36.66%) kanamycin (K 30 µg), 10 (33.33%) resistant to neomycin (N 30 µg,), 7 (23.33%) were resistant nitrofurantoin (F 100 µg), 5 (16.66%) resistant to ciprofloxacin (Cip 5 µg).

Results in Table 2. depict the antimicrobial class and the ratio of resistance to every antibiotic by *C. jejuni*. All classes of antibiotics showed to be resistant by *C. jejuni*; for example, vancomycin of glycopeptide class is the first antibiotic has shown resistant by *C. jejuni* strains than other antibiotic tested, followed by linezolid of oxazolidone class. Macrolides class included erythromycin and nitrofurantoin also considered from high resistant by *C. jejuni*. Doxycycline from tetracycline group was resistant by *C. jejuni* strains. Also, Penicillin class comprised Ticarcillin and Ampicillin were resistant by *C. jejuni*. Quinolones class contained ciprofloxacin and nalidixic acid appeared resistant by *C. jejuni*. Aminoglycosides class of antibiotics (neomycin and kanamycin) moderate efficacy against *C. jejuni*.

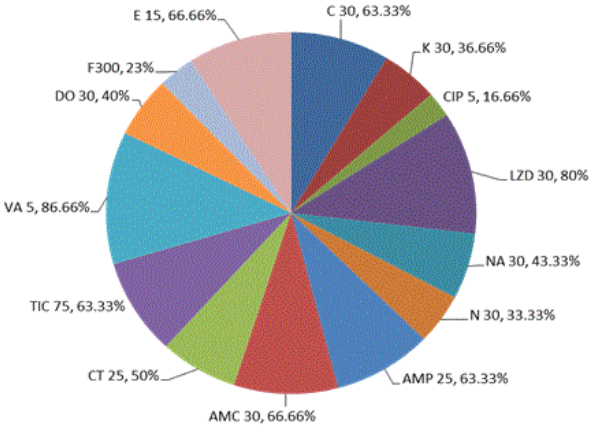

Figure 1. *Ratio of antibiotic resistant against C. jejuni strains.*

Table 2. *Antibiotic resistance profile of C. jejuni strains against 10 antibacterial classes and 14 agents.*

Antimicrobial		**Chicken**		
Class	**Agent**	*C. jejuni* **(n=30)**	**Prevalence of resistance %**	**Prevalence of sensitivity %**
β- lactam	Amoxycillin/Clavulanic acid	20	66.66	33.34
Aminoglycosides	Neomycin	10	33.33	66.67
	Kanamycin	11	36.66	63.34
Macrolides	Erythromycin	20	66.66	33.34
	Nitrofurantoin	7	23.33	
Quinolones	Ciprofloxacin	5	16.66	83.34
	Nalidixic acid	13	43.33	56.67
Tetracyclines	doxycycline	12	40	60
Polymyxin	Colistin	15	50	50
	Ticarcillin	19	63.33	36.67
Penicillin	Ampicillin	19	63.33	36.67
Glycopeptide	Vancomycin	26	86.66	13.34
Oxazolidone	Linezolid	24	80	20
Other	Chloramphenicol	19	63.33	36.67
10	14	-	-	-

All Campylobacter strains found in chicken obtained from different companies were identified as MDR. They were resistant to the tested antibiotics and either more or less resistant than *C. jejuni* ATCC 33291. This observation may be attributed to the overuse of antibiotics in animal feed by poultry breeders or farmers. These results are consistent with those of several similar studies that reported that the antibiotic resistance of *C. jejuni* isolated from chicken was due to improper and/or overuse of antibiotics in the human population and in animal husbandry, thus leading to increase in the incidence of antibiotic-resistant infections and emergence of new and more resistant strains. The understanding of the mechanism of AMR and its effect on *C. jejuni* is important to identify new strategies to reduce AMR and develop novel therapies for both human and veterinary populations [31]. The problem of antibiotic overuse and the resistance developed by *C. jejuni* is a major concern for public health. Several studies have been carried out in the last decade to investigate the antibiotic-resistance in *C. jejuni* strains and have concluded that the resistance is influenced by drug usage in chicken feedings or in animal production and human medicine. Contamination of carcasses in slaughterhouse, and through

equipment also exhibited antibiotic resistant strains of *C. jejuni* and *C. coli* [32-35].

In a study on antibiotic resistance of Campylobacter strains, it was reported that resistance to tetracycline, amoxicillin-clavulanic acid, erythromycin, and gentamicin were 77.8, 55.6, 25.3, and 9.1%, respectively [36]. Similar prevalences (up to 70%) for macrolide, tetracycline, and amoxicillin-clavulanic acid resistance have been previously described [37-38].

Varga et al., 2019 found that 40% of *Campylobacter spp*. isolates exhibited high prevalence of resistance to tetracycline and low prevalence of resistance (5-14% of isolates) to ciprofloxacin and nalidixic acid and a very low prevalence of resistance (<5% of isolates) to gentamicin, telithromycin, clindamycin, azithromycin, erythromycin, and florfenicol [38]. *C. jejuni* isolates from chicken showed a high frequency of resistance to tetracycline, while *C. coli* demonstrated a moderate frequency of resistance (15-39% of isolates). *C. jejuni* isolates from turkey exhibited a high frequency of resistance to tetracycline. *C. coli* isolates from turkey showed a high frequency of resistance to tetracycline and a moderate frequency of resistance to ciprofloxacin and nalidixic acid. In the *C. jejuni* isolates from other poultry species, a high frequency of resistance to ciprofloxacin was observed. In the *C. coli* isolates from other poultry species, a high frequency of resistance to tetracycline and a moderate frequency of resistance to nalidixic acid were seen.

Campylobacter isolates were resistant to nalidixic acid (91.4%) ciprofloxacin (87.9%), tetracycline (87.2%), kanamycin (30.6%), erythromycin (19.4%), and chloramphenicol (1.3%). Campylobacter isolates from human also showed similar resistance to the six antibiotics tested. Emergence of MDR in Campylobacter isolates to four or more antimicrobials with resistance ratio ranging from 28 to 43.5% is a serious health-threatening concern [39]. Resistance to fluoroquinolones and ciprofloxacin is closely correlated to resistance to nalidixic acid [1]. In Korea, ciprofloxacin is currently used in the poultry industry instead of nalidixic acid [40]. In European Union, reduced use of antibiotics has led to decrease in antibiotic resistant microorganisms without compromising animal health and significantly increased the cost of production [41].

Our results corroborate with those by Ge et al. [42], who isolated 378 Campylobacter strains from raw meat sold in retail stores and studied the antibiotic resistance. The isolates showed the highest resistance to tetracycline (82%), followed by erythromycin (54%), nalidixic acid (44%), and ciprofloxacin (35%). In the United States, Campylobacter strains were found to be resistant to the following three major antibiotics: tetracycline, ciprofloxacin and nalidixic acid. Reports indicate that antibiotics resistance of *Campylobacter sp*. isolated from poultry meat and human samples are increasing each year [42-45]. Increased use of fluoroquinolone antibiotics in animal food in different has led to the its enhanced resistance in Campylobacter and is considered to be a big problem all over the world [41,45-46].

Nalidixic acid (a first-generation drug of quinolone)-susceptible strains of Campylobacter were also found to be susceptible to ciprofloxacin and can be considered as markers of ciprofloxacin susceptibility [45]. In Korea, data obtained from 2000-2002 showed that Campylobacter isolates demonstrated MDR to four or more antibiotics ranging from 28 to 43.5%, thus indicating that it could be a health-threatening factor.

Our data regarding antimicrobial class appeared that group under each class different in the effect on Campylobacter strains; these may be due to concentration differences or effectiveness of active substances between agents. Resistance to β-lactam antibiotics appears to be mediated by β-lactamase production, intrinsic resistance, and limited efficacy against *Campylobacter spp*. Our results indicated that *C. jejuni* was resistant to amoxycillin/clavulanic acid containing β-lactam structure [46].

Aminoglycoside class of antibiotics have efficacy against *C. jejuni* and little resistance appeared in comparison to other classes of antibiotics. The resistance of *C. jejuni* to aminoglycoside class of antibiotics was conferred by drug modification proteins. *Campylobacter spp*. has been reported to have different modifying enzymes against aminoglycoside class of antibiotics, including 3-aminoglycoside phosphotransferase types I, III, IV and VII, 3,9-aminoglycoside adenyl transferase and 6-aminoglycoside adenyl transferase [46].

Macrolides class of antibiotics also exhibited ineffectiveness against *C. jejuni* strains and our results concurred with other authors who reported that macrolide resistance in Campylobacter is mainly associated with target modification and active efflux [27,47-50]. Resistance in Campylobacter to macrolides is mediated by modification of the ribosomal target and can occur either by enzyme-mediated methylation or by point mutation in the 23S rRNA and/or ribosomal proteins L4 and L22 [27,51]. It has been reported that rRNA methylation leads to macrolide resistance in *C. rectus* [52]. The most common mechanism for macrolide resistance in *C. jejuni* and *C. coli* is point mutations in domain V of the 23S rRNA [27,51,53].

C. jejuni also showed resistance to quinolones class of antibiotics (ciprofloxacin and nalidixic acid). It has been reported that *Campylobacter spp*. when exposed to fluoroquinolone (FQ), ciprofloxacin-resistant mutants will inevitably emerge [54]. Many studies about rapid development of FQ resistant mutants in chickens originally infected with FQ-susceptible *C. jejuni*, but this happened when treated with enrofloxacin [24,55-58].

Resistance of Campylobacter isolates, recovered from various animal species, to tetracycline has been reported to be conferred by tet(O) [5]. There are no other test resistance genes that have been found in Campylobacter until now, tet(O) encodes a ribosomal protection protein [59]. It binds on an open A site on the bacterial ribosome of *Campylobacter spp*. and binds it in such a manner that it induces a conformational

change that results in the release of the bound tetracycline molecule [60]. Resistance of *C. jejuni* to tetracycline is also associated with the CmeABC multidrug efflux pump [71,27]. Other studies have explained that resistance of *Campylobacter spp*. to tetracycline can be attributed to a plasmid-encoded tet(O) gene [72], transferred between *C. jejuni* and *C. coli* in this genes through plasmids take place horizontally in the intestinal tract of human and animals [72-74].

In recent years, the resistance of *C. jejuni* and *C. coli* against different antibiotics has increased throughout the world. A high rate of resistance to macrolides, fluoroquinolones, and aminoglycosides is emerging and has been investigated in both human and animal isolates.

Many antimicrobial classes of antibiotics known by their broad activity spectrum such as tetracyclines and macrolides, are often used in the treatment of poultry diseases. They have been used in poultry production as growth promoter for over 30 years. These antibiotics inhibit the pathogenic microflora and create unfavourable metabolites such as cadaverine, putrescine, and increasing the poultry production effects. Unfortunately, humans and animals are also treated with the antibiotics of the same chemical group such as tetracycline, used in human to treat respiratory disease, erythromycin used to treat of campylobacteriosis, while aminoglycosides used in general therapy because its oto and nephrotoxic activities.

Conclusion

Resistance gene of *Campylobacter spp* can be transferred horizontally between *C. jejuni* strains in the intestinal tract of food animals and humans. To reduce the development and spread of MDR in Campylobacter strains, antibiotic or addition in poultry meals or water as growth promotion or to kill the pathogenic bacteria without any veterinary prescription must be stopped.

Author Contributions

Conceptualization, H.M.Y. and K.M.A. M.F.E. data curation, formal analysis; investigation; methodology and visualization, H.M.Y. writing-original draft; funding acquisition; validation; project administration, resources, M.F.E. writing-review and editing.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Thomas M, MurrayR. Flockhart L, Pintar K, Fazil A, Nesbitt A, Marshall B, Tataryn J, Pollari F. Estimates of foodborne illness-related hospitalizations and deaths in Canada for 30 specified pathogens and unspecified agents. Foodborne Pathog Dis. 2015;12: 820-827.
2. Scallan E, Hoekstra RM, Mahon BE, Jones TF, Griffin PM. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. Epidemiol Infect 2015;143:2795-804.
3. Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleesschauwer B, Dopfer D, Fazil A, Fischer-Walker CL, Hald T, Hall AJ. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS medicine 2015;12:12.
4. The community summary report on trends and sources of zoonoses and zoonotic. Agents in the European union in 2007. EFSA J 2009;223:223-440.
5. Blaser MJ, Berkowitz ID, Laforce FM, Cravens J, Reller LB, Wang WL. Campylobacter enteritis: clinical and epidemiologic features. Annals of internal medicine. 1979;91:179-85.
6. Cox LA. Re-examining the causes of campylobacteriosis. Int J Infect Dis 2002;6:26-36.
7. Blaser MJ, Berkowitz ID, Laforce FM, Cravens J, Reller LB, Wang WL. Outbreaks of human Salmonella infections associated with live poultry. Emerg Infect Dis 2016;22:1705-1711.
8. Sahin O, Kassem II, Shen Z, Lin J, Rajashekara G, Zhang Q. Campylobacter in poultry: Ecology and potential interventions. Avian Dis 2015;59:185-200.
9. Lorenz RJ. Grundbegriffe der Biometrie, 4th ed. G. Fischer Verlag, Germany, 1996.
10. Moore JE, Barton MD, Blair IS, Corcoran D, Dooley JS, Fanning S, Kempf I, Lastovica AJ, Lowery CJ, Matsuda M, McDowell DA. The epidemiology of antibiotic resistance in Campylobacter. Microbes Infect 2006;8:1955-1966.
11. Thakur S, Zhao S, McDermott PF, Harbottle H, Abbott J, English L, Gebreyes WA, White DG. Antimicrobial resistance, virulence, and genotypic profile comparison of Campylobacter jejuni and Campylobacter coli isolated from humans and retail meats. Foodborne Pathog Dis 2010;7:835–844.
12. Doyle ME. Multidrug-resistant pathogens in the food supply. Foodborne Pathog Dis 2015;12:261–279.
13. Nachamkin I, Engberg J, Aarestrup FM. Diagnosis and antimicrobial susceptibility of Campylobacter spp. In: Nachamkin I. Blaser MJ, eds. Campylobacter, 2nd edn. Washington: American Society for Microbiology, 2000.
14. Petruccelli BP, Murphy GS, Sanchez JL, Walz S, DeFraites R, Gelnett J, Haberberger RL, Echeverria P, Taylor DN.

Treatment of traveler's diarrhea with ciprofloxacin and loperamide. J Infect Dis 1992;165:557-560.

15. Blaser MJ, Engberg J. Clinical aspects of Campylobacter jejuni and Campylobacter coli infections. In: Nachamkin I, Szymanski CM, Blaser MJ, editors. Campylobacter. 3. ASM Press; Washington DC, USA, 2008.
16. Agunos A, Léger D, Avery BP, Parmley EJ, Deckert A, Carson CA, Dutil L. Ciprofloxacin-resistant Campylobacter spp. in retail chicken, western Canada. Emerg Infect Dis 2013;19:1121-1124.
17. Agunos A, Arsenault RK, Avery BP, Deckert AE, Gow SP, Janecko N, Léger DF, Parmley EJ, Reid-Smith RJ, McEwen SA. Changes in antimicrobial resistance levels among Escherichia coli, Salmonella, and Campylobacter in Ontario broiler chickens between 2003 and 2015. Can J Vet Res 2018;82:163-177.
18. Government of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2016 Annual Report. 2018.
19. Woźniak-Biel A, Bugla-Płoskońska G, Kielsznia A, Korzekwa K, Tobiasz A, Korzeniowska-Kowal A, Wieliczko A. High prevalence of resistance to fluoroquinolones and tetracycline Campylobacter spp. isolated from poultry in Poland. Microb Drug Resist 2018;24:314-322.
20. Li B, Ma L, Li Y, Jia H, Wei J, Shao D, Liu K, Shi Y, Qiu Y, Ma Z. Antimicrobial resistance of Campylobacter species isolated from broilers in live bird markets in Shanghai, China. Foodborne Pathog Dis 2017;14:96-102.
21. Giacomelli M, Salata C, Martini M, Montesissa C, Piccirillo A. Antimicrobial resistance of Campylobacter jejuni and Campylobacter coli from poultry in Italy. Microb Drug Resist 2014;20:181-188.
22. WoŹNiak AN, Wieliczko A. Tetracycline, erythromycin, and gentamicin resistance of Campylobacter jejuni and Campylobacter coli isolated from poultry in Poland. Bull Vet Inst Pulawy 2011;55:51-54.
23. Luo N, Sahin O, Lin J, Michel LO, Zhang Q. In vivo selection of Campylobacter isolates with high levels of fluoroquinolone resistance associated with gyrA mutations and the function of the CmeABC efflux pump. Antimicrob Agents Chemother 2003;47:390-394.
24. McDermott PF, Bodeis SM, English LL, White DG, Walker RD, Zhao S, Simjee S, Wagner DD. Ciprofloxacin resistance in Campylobacter jejuni evolves rapidly in chickens treated with fluoroquinolones. J Infect Dis 2002;185:837-840.
25. Alfredson DA, Korolik V. Antibiotic resistance and resistance mechanisms in Campylobacter jejuni and Campylobacter coli. FEMS Microbiol Lett 2007;277:123-132.
26. Bachoual R, Ouabdesselam S, Mory F, Lascols C, Soussy CJ, Tankovic J. Single or double mutational alterations of gyrA associated with fluoroquinolone resistance in Campylobacter jejuni and Campylobacter coli. Microb Drug Resist 2001;7:257-261.
27. Gibreel A, Taylor DE. Macrolide resistance in Campylobacter jejuni and Campylobacter coli. J Antimicrob Chemother 2006;58:243-255.
28. Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q. Antibiotic resistance in Campylobacter: emergence, transmission and persistence. Future Microbiol 2009;4:189-200.
29. Government of Canada. Food and Drugs Act. Regulations Amending the Food and Drug Regulations (Veterinary Drugs-Antimicrobial Resistance), 2017.
30. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Wayne, Pennsylvania, USA: CLSI; 2012.
31. Marotta F, Garofolo G, di Marcantonio L, Di Serafino G, Neri D, Romantini R, Sacchini L, Alessiani A, Di Donato G, Nuvoloni R, Janowicz A. Antimicrobial resistance genotypes and phenotypes of Campylobacter jejuni isolated in Italy from humans, birds from wild and urban habitats, and poultry. PLoS One 2019;14:e0223804.
32. Tang Y, Sahin O, Pavlovic N, LeJeune J, Carlson J, Wu Z, Dai L, Zhang Q. Rising fluoroquinolone resistance in Campylobacteria isolated from feedlot cattle in the United States Sci Rep 2017;7:494.
33. Szczepanska B, Andrzejewska M, Spica D, Klawe JJ. Prevalence and anti-microbial resistance of Campylobacter jejuni and Campylobacter coli isolated from children and environmental sources in urban and suburban areas. BMC Microbiol 2017;17:80.
34. Pollett S, Rocha C, Zerpa R, Patiño L, Valencia A, Camiña M, Guevara J, Lopez M, Chuquiray N, Salazar-Lindo E, Calampa C. Campylobacteria antimicrobial resistance in Peru: a ten-year observational study. BMC Infect Dis 2012;16:193.
35. Torralbo A, Borge C, García-Bocanegra I, Méric G, Perea A, Carbonero A. Higher resistance of Campylobacter coli compared to Campylobacter jejuni at chicken slaughterhouse. Comp Immunol Microbiol Infect Dis 2015;39:47-52.
36. Melo RT, Grazziotin AL, Júnior EC, Prado RR, Mendonça EP, Monteiro GP, Peres PA, Rossi DA. Evolution of Campylobacter jejuni of poultry origin in Brazil. Food Microbiol 2019;82:489-496.
37. EFSA (European Food Safety Authority) EFSA J, 2015.
38. Nguyen TN, Hotzel H, El-Adawy H, Tran HT, Le MT, Tomaso H, Neubauer H, Hafez HM. Genotyping and antibiotic resistance of thermophilic Campylobacter isolated from chicken and pig meat in Vietnam. Gut Pathog 2016;8:19.
39. Varga C, Guerin MT, Brash ML, Slavic D, Boerlin P, Susta L. Antimicrobial resistance in Campylobacter jejuni and Campylobacter coli isolated from small poultry flocks in Ontario, Canada: A two year surveillance study. PLoS ONE 2019;14:e0221429.
40. Kang YS, Cho YS, Yoon SK, Yu MA, Kim CM, Lee JO, Pyun YR.. International Association for Food Protection Prevalence and Antimicrobial Resistance of Campylobacter

jejuni and Campylobacter coli Isolated from Raw Chicken Meat and Human Stools in Korea. J Food Protect 2006;69:2915-2923.

41. Korea Food and Drug Administration, National Antimicrobial Resistance Management Program. Establishment of control system of antibiotics for livestocks in 2003, 2003.
42. Angulo FJ, Baker NL, Olsen SJ, Anderson A, Barrett TJ. Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. Pediatr Infect Dis J 2004;15:78-85.
43. Ge B, White DG, McDermott PF, Girard W, Zhao S, Hubert S, Meng J. Antimicrobial-resistant Campylobacter species from retail raw meat. Appl Environ Microbiol 2003;69: 3005-3007.
44. Kassenborg HD, Smith KE, Vugia DJ, Rabasky-Ehr T, Bates MR, Carter MA, Dumas, NB, Cassidy MP, Marano N, Tauxe RV, Angulo FJ. Fluoroquinolone-resistant Campylobacter infection: Eating poultry outside of home and foreign travel are risk factors. Clin Infect Dis 2004;38:S279–S284.
45. Ledergerber U, Regula G, Stephan R, Danuser J, Bissig B, Stärk KD. Stark. Risk factors for antibiotic resistance in Campylobacter spp. isolated from raw poultry meat in Switzerland. BMC Public Health 2003;3:1-9.
46. Gaudreau C, Gilbert H. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of Campylobacter jejuni subsp. jejuni and Campylobacter coli. J. Antimicrob Chemother 1997;39:707-712.
47. Zhang Q, Plummer P. Mechanisms of antibiotic resistance in Campylobacter. In: Nachamkin I, Szymanski CM, Blaser MJ, editors. USA: 2008.
48. Harrow SA, Gilpin BJ, Klena JD. Characterization of erythromycin resistance in Campylobacter coli and Campylobacter jejuni isolated from pig offal in New Zealand. J Appl Microbiol 2004;97:141-148.
49. Mamelli L, Prouzet-Mauléon V, Pagès JM, Mégraud F, Bolla JM. Molecular basis of macrolide resistance in Campylobacter: role of efflux pumps and target mutations. J Antimicrob Chemother 2005;56:491-497.
50. Lin J, Yan M, Sahin O, Pereira S, Chang YJ, Zhang Q. Effect of macrolide usage on emergence of erythromycin-resistant Campylobacter isolates in chickens. Antimicrob Agents Chemother 2007:51:1678-1686.
51. Cagliero C, Mouline C, Cloeckaert A, Payot S. Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in Campylobacter jejuni and Campylobacter coli. Antimicrob Agents Chemother 2006; 50:3893–3896.
52. Payot S, Bolla JM, Corcoran D, Fanning S, Mégraud F, Zhang Q. Mechanisms of fluoroquinolone and macrolide resistance in Campylobacter spp. Microbes Infect 2006; 8:1967-1971.
53. Roe DE, Weinberg A, Roberts MC. Mobile rRNA methylase genes in Campylobacter (Wolinella) rectus. J Antimicrob Chemother 1995;36:738-740.
54. Corcoran D, Quinn T, Cotter L, Fanning S. An investigation of the molecular mechanisms contributing to high-level erythromycin resistance in Campylobacter. Int J Antimicrob Agents 2006;27:40–45.
55. Han J, Sahin O, Barton YW, Zhang Q. Key role of Mfd in the development of fluoroquinolone resistance in Campylobacter jejuni. PLoS Pathog 2008;4:e1000083.
56. Luo N, Sahin O, Lin J, Michel LO, Zhang Q. In vivo selection of Campylobacter isolates with high levels of fluoroquinolone resistance associated with gyrA mutations and the function of the CmeABC efflux pump. Antimicrob Agents Chemother 2003;47:390-394.
57. van Boven M, Veldman KT, de Jong MC, Mevius DJ. Rapid selection of quinolone resistance in Campylobacter jejuni but not in Escherichia coli in individually housed broilers. J Antimicrob Chemother 2003;52:719-723.
58. Griggs DJ, Johnson MM, Frost JA, Humphrey T, Jørgensen F, Piddock LJ. Incidence and mechanism of ciprofloxacin resistance in Campylobacter spp isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. Antimicrob Agents Chemother 2005;49:699-707.
59. Farnell MB, Donoghue AM, Cole K, Reyes‐Herrera I, Blore PJ, Donoghue DJ. Campylobacter susceptibility to ciprofloxacin and corresponding fluoroquinolone concentrations within the gastrointestinal tracts of chickens. J Appl Microbiol 2005;99: 1043–1050.
60. Taylor DE, Hiratsuka K, Ray H, Manavathu EK. Characterization and expression of a cloned tetracycline resistance determinant from Campylobacter jejuni plasmid pUA466. J Bacteriol 1987:169:2984-2989.
61. Connell SR, Tracz DM, Nierhaus KH, Taylor DE. Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrob Agents Chemother 2003;47: 3675–3681.
62. Lin J, Michel LO, Zhang Q. CmeABC functions as a multidrug efflux system in Campylobacter jejuni. Antimicrob Agents Chemother 2002;46:2124-2131.
63. Andersen SR, Saadbye P, Shukri NM, Rosenquist H, Nielsen NL, Boel J. Antimicrobial resistance among Campylobacter jejuni isolated from raw poultry meat at retail level in Denmark. Int J Food Microbiol 2006;107:250-255.
64. Hakkinen M, Heiska H, Hänninen ML. Prevalence of Campylobacter spp in cattle in Finland and antimicrobial susceptibilities of bovine Campylobacter jejuni strains. Appl Environ Microbiol 2007;73:3232-3238.
65. Samie A, Ramalivhana J, Igumbor EO, Obi CL. Prevalence, haemolytic and haemagglutination activities and antibiotic susceptibility profiles of Campylobacter spp isolated from human diarrhoeal stools in Vhembe District, South Africa. J Health Popul Nutr 2007;25:406-413.
66. Gaudreau C, Gilbert H. Antimicrobial resistance of Campylobacter jejuni subsp jejuni strains isolated from humans in 1998 to 2001 in Montreal, Canada. Antimicrob Agents Chemother 2003;47:2027-2029.

67. Papavasileiou E, Voyatzi A, Papavasileiou K, Makri A, Andrianopoulou I, Chatzipanagiotou S. Antimicrobial susceptibilities of Campylobacter jejuni isolates from hospitalized children in Athens, Greece, collected during 2004–2005. Eur J Epidemiol 2007;22:77-78.
68. Nachamkin I, Ung H, Li M. Increasing fluoroquinolone resistance in Campylobacter jejuni, Pennsylvania, USA, 1982-2001. Emerg Infect Dis 2002;8:1501-1503.
69. Hakanen AJ, Lehtopolku M, Siitonen A, Huovinen P, Kotilainen P. Multidrug resistance in Campylobacter jejuni strains collected from Finnish patients during 1995-2000. J Antimicrob Chemother 2003;52:1035-1039.
70. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic Campylobacter. Veterinary Research 2001;32:311-321.
71. Altekruse SF, Stern NJ, Fields PI, Swerdlow DL. Campylobacter jejuni an emerging food borne pathogen. Emerg Infect Dis 1999;5:28-35.

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