

Article

Prevalence and Antibiotic Resistance of *Salmonella* spp. and *Campylobacter* spp. Isolated from Retail Chickens in Saudi Arabia

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Abstract: Foodborne pathogens such as *Salmonella* spp. and *Campylobacter* spp. pose significant threats to the safety of broiler meat worldwide. However, data on their prevalence in retail chicken meat in Saudi Arabia are scarce. This context mainly concerns the vast poultry market in Saudi Arabia, which may double by 2030. The overall objective of this study was to determine the prevalence and antibiotic resistance of *Salmonella* spp. and *Campylobacter* spp. in retail chickens from small, medium-sized, and large production companies in Saudi Arabia. Of the 212 chicken samples tested, *Salmonella* was detected in 9.3% of samples, all identified as *Salmonella enterica* serovar Enteritidis. *Campylobacter* was more prevalent, found in 35.8% of samples, with *Campylobacter jejuni* accounting for 26.4% and *Campylobacter coli* for 9.3%. Pathogen prevalence was higher in small-scale than in medium-sized and large producers. *Salmonella* Enteritidis isolates were resistant to nalidixic acid (90%), amoxicillin-clavulanic acid, and tetracycline (70%). Most *Campylobacter coli* isolates (90%) exhibited resistance to erythromycin, clindamycin, and gentamicin, followed by tetracycline (80%). *Campylobacter jejuni* isolates showed high resistance to erythromycin, clindamycin, tetracycline, azithromycin, and nalidixic acid (75–92%). Multidrug resistance (MDR) was observed in all *Campylobacter jejuni* isolates, 90% of *Campylobacter coli* isolates, and 70% of *Salmonella* isolates. These findings underscore the urgent need for adherence to food safety guidelines, particularly in small-scale poultry farms. The pervasive presence of MDR *Salmonella* spp. and *Campylobacter* spp. in broiler meat calls for enhanced surveillance, stricter enforcement of food safety practices, and public health initiatives to mitigate the risk of foodborne diseases in Saudi Arabia.



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1. Introduction

Meat from poultry is an important source of protein; it is widely consumed across numerous cultures. However, poultry meat is highly susceptible to spoilage and is frequently implicated as a vehicle for foodborne pathogens [1]. This issue presents a significant public health hazard, not only due to the spread of foodborne diseases, such as those caused by *Salmonella*, *Shigella*, *Campylobacter*, pathogenic *Escherichia coli*, and *Listeria monocytogenes*, but also because some of these pathogens often exhibit multidrug resistance that limits treatment options in hospital settings and may complicate the management of foodborne infections [2,3]. Among these pathogens, *Salmonella* and *Campylobacter* are recognized as the

most important pathogens associated with foodborne outbreaks linked to the consumption of poultry. These two pathogens are commonly identified as major contributors to the burden of foodborne illnesses worldwide, with poultry meat serving as a major transmission vehicle [4]. Infection with these pathogens causes self-limiting symptoms, including diarrhea, vomiting, nausea, abdominal pain, and sometimes fever. However, the invasive form causes post-infection complications. In some cases, this may lead to enteric fever, focal systemic infections, bacteremia, hepatosplenomegaly, and respiratory complications, especially in immunodeficient individuals [4,5].

Salmonella is a facultative anaerobic member of the *Enterobacteriaceae*. It is Gram-negative, rod-shaped, non-lactose fermenting, non-spore-forming, and grows optimally between 35 °C and 37 °C. As the most common foodborne pathogen isolated from food-producing animals, *Salmonella* is a zoonotic bacterium capable of transmission between humans, animals, and birds. With approximately 2600 known serovars, *Salmonella* infections pose a significant public health challenge due to their widespread occurrence and potential severity [6]. On the other hand, *Campylobacter* is a microaerophilic, Gram-negative bacterium with a curved or spiral shape [7]. Most *Campylobacter* spp. are motile; they are equipped with a flagellum at one or both poles, enabling their characteristic corkscrew-like motion. *Campylobacter* spp. inhabit the gastrointestinal tracts of warm-blooded animals, particularly poultry, where the temperature of 41–42 °C creates ideal growth conditions. This adaptation makes *Campylobacter* an important zoonotic pathogen that poses significant risks to humans and animals through infectious diseases [8]. The stability of different *Campylobacter* spp. varies significantly under different environmental conditions, influencing their survival and transmission [9,10]. Human campylobacteriosis is caused primarily by *Campylobacter jejuni* and *Campylobacter coli* [11]. One severe complication associated with *Campylobacter jejuni* infections is Guillain–Barré syndrome, an autoimmune neurological disorder initiated by molecular mimicry between *Campylobacter jejuni* outer membrane lipooligosaccharides and human peripheral nerve gangliosides [12].

The increasing prevalence of antibiotic-resistant bacteria, facilitated by horizontal gene transfer, presents a significant challenge to modern medicine and public health [13]. Resistance in *Salmonella* spp. and *Campylobacter* spp. has escalated sharply in recent years, driven largely by the overuse of antimicrobial agents in agriculture and healthcare settings [14]. Of particular concern are MDR *Salmonella* serovars, which have acquired resistance to three or more classes of antibiotics, complicating treatment options and undermining efforts to control infections [15].

Recent epidemiological data indicate that the consumption of poultry meat could be implicated in over 30% of global foodborne salmonellosis cases and over 50% of campylobacteriosis cases [16]. According to the Ministry of Health (MOH) in Saudi Arabia, *Salmonella* spp. is the leading cause of foodborne-related outbreaks, accounting for over 70% of reported foodborne outbreaks [14]. *Campylobacter* spp. is also among the leading causes of bacterial gastroenteritis; it is often associated with the consumption of contaminated poultry products [17]. The antibiotic resistance of *Salmonella* spp. and *Campylobacter* spp. has recently increased due to the overuse of antibiotics, which is considered a problem for public health [18].

In the last few decades, poultry meat consumption has increased worldwide and is projected to double by 2050 [19]. In Saudi Arabia, the chicken meat industry is dominated by ten companies, including three large ones and seven medium- to small-sized farms. Together, these companies control up to 95 percent of the country's chicken production. In 2021, poultry production in Saudi Arabia was estimated to be over 900,000 metric tons (MT), reaching a 60% self-sufficiency level. That year, Saudi Arabia also imported about 500,000 MT of chicken meat, with over 70% sourced from Brazil. The Saudi Ministry of

Environment, Water, and Agriculture (MEWA) has revealed plans to increase the self-sufficiency level from 60% (as of 2020) to over 85% by 2030. Therefore, enhancing the safety of broiler chicken and other poultry products is warranted. The overall objective of this paper was to determine the prevalence and antibiotic resistance of *Salmonella* spp. and *Campylobacter* spp. in retail chickens from small, medium-sized, and large production companies in Saudi Arabia [20].

2. Materials and Methods

2.1. Sample Collection

Between January and March 2023, a total of 212 chilled retail chickens were purchased from supermarkets in Qassim and Riyadh, Saudi Arabia. The supermarkets were selected randomly, representing a mix of large retail chains with multiple branches and smaller independent stores. The purchased chickens, representing 106 sampling points, were collected in pairs from the same producer, with the same production date, and purchased from the same store. The samples, representing nine local poultry brands in Saudi Arabia, were categorized by production capacity into large-sized (3 brands), medium-sized (3 brands), and small-sized (3 brands) farms according to the classifications provided by the MEWA, the official governmental body overseeing poultry production in Saudi Arabia [20]. Qassim and Riyadh were chosen for sampling based on their centralized facilities that distribute poultry products nationwide. Therefore, collecting samples from these locations provided a representative overview of retail chicken products available throughout Saudi Arabia. All collected samples were immediately transported under refrigerated conditions to the laboratory for analysis.

2.2. Bacterial Isolation and Identification

2.2.1. *Salmonella* Isolation and Serotyping

The skins and internal organs from two chickens at the same sampling point were placed in a sterile plastic bag, rinsed with 225 mL of buffered peptone water for 2 min, and then homogenized for 1 min. The resulting mixture was transferred into a sterile jar and incubated overnight at 36 °C to allow for the recovery of injured cells. After incubation, 1 mL of the suspension was added to 10 mL of Rappaport Vassiliadis soy broth (RVS) (Neogen, Lansing, MI, USA), and it was incubated at 42 °C for 24 h. Following selective enrichment, the suspensions were streaked onto xylose lysine deoxycholate (XLD) agar (Neogen) and incubated overnight at 37 °C. Candidate colonies were then streaked onto Hektoen agar (Biolife, Layton, Utah, USA) for another overnight incubation at the same temperature. Single colonies exhibiting typical *Salmonella* phenotypes were biochemically confirmed by streaking onto triple sugar iron (TSI) agar, as well as urease and lysine decarboxylase tests as previously described [21,22]. After incubation at 37 °C for 24 h, colonies identified as *Salmonella* were preserved at −80 °C for further analyses. Positive isolates were serotyped according to the White Kauffmann Le Minor scheme via slide agglutination using specific O and H antisera [23].

2.2.2. *Campylobacter* Isolation

Campylobacter spp. were isolated following the protocols outlined in the FDA bacteriological analytical manual [24] and ISO 10272-1 [25]. Each 25 g broiler sample was homogenized for 5 min with 100 mL of peptone broth. The homogenates were then enriched by mixing 2 mL of the sample with 10 mL of modified Preston media supplemented with acumedia (Neogen), and the solution was incubated at 37 °C for 48 h under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂) using the CampyGen gas-generating kit (Oxoid, Thermo Fisher Scientific, Basingstoke, UK). Post-enrichment, 200 µL of each suspension was

plated onto modified charcoal cefoperazone deoxycholate agar, and five suspect colonies with characteristic *Campylobacter* spp. morphology were purified through sub-culturing. Identification at the genus level involved assessing bacterial growth at different temperatures, cell morphology, and motility using dark-field microscopy, as well as oxidase tests (Biolife) [26,27].

2.2.3. Molecular Identification

Isolates stored at $-80\text{ }^{\circ}\text{C}$ were inoculated into BHI broth and incubated at $37\text{ }^{\circ}\text{C}$ for *Salmonella* spp. and at $42\text{ }^{\circ}\text{C}$ under microaerophilic conditions for *Campylobacter* spp. for 24 h. Genomic DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Venlo, The Netherlands) according to the standard protocol, with minor modifications. Briefly, 200 μL of the sample was mixed with 20 μL of proteinase K and 200 μL of lysis buffer, and the samples were incubated at $56\text{ }^{\circ}\text{C}$ for 10 min. After incubation, 200 μL of 100% ethanol was added to the lysate. The sample was then washed and centrifuged according to the manufacturer's recommendations, with nucleic acids eluted in 100 μL of the provided elution buffer. PCR amplification was performed using primers for virulent genes sourced from the literature: the *mapA* gene for *Campylobacter jejuni* [28], the *ceuE* gene for *Campylobacter coli* [29], and the *invA* gene for *Salmonella* [30]. The 25 μL PCR reaction contained 12.5 μL of EmeraldAmp Max PCR Master Mix (Takara, Shiga, Japan), 1 μL of each primer at a concentration of 20 pmol, 5.5 μL of water, and 5 μL of DNA template. The amplification was carried out in an Applied Biosystems 2720 thermal cycler. PCR products were separated via electrophoresis on a 1.5% agarose gel in $1\times$ TBE buffer at room temperature, using a voltage gradient of 5 V/cm. For gel analysis, 20 μL of the PCR products were loaded into each slot, and fragment sizes were determined using a 100 bp ladder (Fermentas, Burlington, ON, USA). Gels were photographed by gel documentation system (Biometra BDA digital 2.64.11.20, Göttingen, Germany) which allowed the camera to capture images of gels separated via electrophoresis. The data were analyzed using gel documentation system (Alpha Innotech, Biometra, 3.0 Germany) and the accompanying automatic image capture software (Protein Simple, Formerly Cell Bioscience, 6.3.0, Santa Clara, CA, USA).

2.3. Antimicrobial Susceptibility Testing

2.3.1. *Salmonella* Isolates

Antibiotic susceptibility of *Salmonella* spp. isolates was studied using the Kirby-Bauer disc diffusion method. *Salmonella* spp. isolates were cultured in BHI broth, and 20 μL of broth was streaked onto Mueller-Hinton agar using a sterile loop. The selected antibiotics were then applied, and the plates were incubated at $37\text{ }^{\circ}\text{C}$. The antibiotics tested were ampicillin (10 $\mu\text{g}/\text{Disc}$), cefotaxime (30 $\mu\text{g}/\text{Disc}$), ceftazidime (30 $\mu\text{g}/\text{Disc}$), amoxicillin-clavulanic acid (30 $\mu\text{g}/\text{Disc}$), gentamicin (10 $\mu\text{g}/\text{Disc}$), ciprofloxacin (5 $\mu\text{g}/\text{Disc}$), streptomycin (10 $\mu\text{g}/\text{Disc}$), chloramphenicol (30 $\mu\text{g}/\text{Disc}$), tetracycline (300 $\mu\text{g}/\text{Disc}$), ofloxacin (5 $\mu\text{g}/\text{Disc}$) and nalidixic acid (30 $\mu\text{g}/\text{Disc}$). Interpretations were made according to the instructions of the Clinical and Laboratory Standards Institute [31,32]. Isolates exhibiting inhibition zones of ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime were considered positive extended-spectrum beta-lactamase (ESBL). On the other hand, isolates exhibiting inhibition zones of ≤ 18 mm were considered β -lactamase producers [33–35]. The parameters of MDR [36,37] were defined as non-susceptibility to three or more antibiotics of different groups.

2.3.2. *Campylobacter* Isolates

Antibiotic sensitivity of *Campylobacter* spp. isolates was conducted according to protocols from The European Committee on Antimicrobial Susceptibility Testing (EUCAST) [38].

Tests were administered on Muller–Hinton agar supplemented with 3% blood and incubated for 24 h at 42 °C under microaerophilic conditions. The following antibiotics were used: doxycycline (30 µg/Disc), erythromycin (15 µg/Disc), azithromycin (15 µg/disc), ciprofloxacin (5 µg/Disc), gentamicin (10 µg/disc), clindamycin (2 µg/Disc), nalidixic acid (30 µg/Disc), and tetracycline (300 µg/Disc).

2.4. Statistical Analysis

The association between farm size and pathogen prevalence was examined with Fisher’s exact test using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The level of significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Prevalence of *Salmonella* spp. and *Campylobacter* spp. in Retail Chickens

The prevalence of *Salmonella* spp. and *Campylobacter* spp. isolated from retail chicken samples in Saudi Arabia is summarized in Table 1. Of the 212 chicken samples tested, *Salmonella* was detected in 9.3% of samples, with all identified as *Salmonella enterica* subsp. *enterica* serovar Enteritidis (serotype 1,9,12, m:-). *Campylobacter* was more prevalent, appearing in 35.8% of samples, with 26.4% attributed to *Campylobacter jejuni* and 9.3% to *Campylobacter coli*. Details of culture characteristics of *Salmonella* and *Campylobacter* isolates along with agar gel electrophoresis images are provided in the Supplementary Figures S1–S8.

Table 1. Prevalence of *Campylobacter* spp. and *Salmonella* Enteritidis in retail chicken from large, medium-sized, and small producers.

Size of Poultry Farm	Brand	No. *	No. of Positive Samples		
			<i>Salmonella</i> Enteritidis	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>
Large	A	14	0	8	0
	B	12	1	1	0
	C	13	0	0	3
Medium	D	13	0	4	1
	E	12	0	5	0
	F	13	0	5	0
Small	G	6	4	0	3
	H	10	1	4	0
	I	13	4	1	3
Total		106 (100%)	10 (9.3%)	28 (26.4%)	10 (9.3%)

* Each sample represents a composite of two chicken carcasses that were collected from the same producer, with the same production date, and purchased from the same retail location.

The prevalence of *Salmonella* in retail chicken exhibits significant variability across the literature. In a study conducted in Al-Ahsa Province, Saudi Arabia [39], Al-Dughaym and Altabari reported a 1% prevalence of *Salmonella*, identifying the serovar as Arizona. Another study in the same province detected *Salmonella enterica* subsp. *enterica* serovars Enteritidis and Typhimurium in chicken shawarma sold at fast food restaurants, with prevalences of 19% and 8%, respectively [40]. However, these studies did not specify whether the chicken was locally produced or imported. In Jeddah, Saudi Arabia, a study reported that retail chilled chicken from local companies had a *Salmonella* prevalence of 35.25% [41]. Studies across the Middle East have reported a relatively higher prevalence of *Salmonella*, with serovars Kentucky, Typhimurium, and Newport being more common than Enteritidis [42–44].

Yehia and AL-Dagal [45] found that almost half of the retail chicken samples tested in Saudi Arabia were positive for the presence of *Campylobacter jejuni* in Saudi Arabia. Abu-Madi et al. [46] found that *Campylobacter jejuni* was present in 45.9% of retail chicken produced by Saudi companies and sold in Qatar. Unlike *Campylobacter jejuni*, the incidence of *Campylobacter coli* in retail chicken from Saudi producers has rarely been documented in the literature. However, one study reported a single positive sample of *Campylobacter coli*. By contrast, a study conducted in the United Arab Emirates by Habib et al. [47] found that 28.5% of chicken samples were positive for the presence of *Campylobacter*, with the majority of these (83%) identified as *Campylobacter coli*. In Iraq, a study by Kanaan and Mohammed [48] reported that *Campylobacter jejuni* was detected in 25% of retail frozen chicken meat, while *Campylobacter coli* was found in 50% of the samples. In Jordan, the prevalence of *Campylobacter jejuni* in broiler chicken carcasses was found to be 17% [49].

Similarly to our findings, Gritli et al. reported a 100% prevalence of *Salmonella enteritidis* in poultry in Tunisia [50]. In Egypt, Nicodème et al. [51] reported a prevalence of 91.6% for *Campylobacter* and 44.4% for *Salmonella* across various poultry sources. By contrast, Gharbi et al. [52] in Tunisia found no detection of either *Campylobacter jejuni* or *Campylobacter coli* in broiler chickens. Similarly, in Morocco, Asmai et al. [53] observed a prevalence of 0% for *Campylobacter jejuni* and 40% for *Campylobacter coli* in broiler chickens. Our findings align with those of Azizian et al. [54] in Iran, who reported a prevalence of 14.9% for *Campylobacter coli* and 85.1% for *Campylobacter jejuni*. In West Africa, Kouglénou et al. [55] similarly observed a prevalence of 23.4% for *Campylobacter jejuni* and 7.8% for *Campylobacter coli*.

3.2. Producer Size and Pathogen Prevalence

Overall, pathogen prevalence was higher in small-scale producers compared to medium- and large-scale producers. The prevalence of *Salmonella* Enteritidis was significantly higher in small-scale poultry farms (31%) compared to large-scale (2.6%, $p < 0.001$) and medium-scale farms (0%, $p < 0.0002$). This could be attributed to greater experience, well-established systems, and advanced laboratories for food safety monitoring typically found in large- and medium-scale poultry companies. In contrast, the prevalence of *Campylobacter* spp. was 30%, 39%, and 37% in large-, medium-, and small-scale poultry farms, respectively, with no significant differences among the producer sizes. Most *Campylobacter* isolates from large- and medium-scale poultry farms were identified as *Campylobacter jejuni* (75% and 93%, respectively), whereas over half of the isolates from small farms were *Campylobacter coli* (55%).

3.3. Antibiotic Susceptibility of *Salmonella* spp. and *Campylobacter* spp. Isolates

The antibiotic susceptibilities of *Salmonella* Enteritidis and *Campylobacter* spp. isolates are shown in Tables 2 and 3, respectively. A higher proportion of *Salmonella* isolates were resistant to nalidixic acid (nine isolates, 90%), followed by amoxicillin–clavulanic acid and tetracycline (seven isolates, 70%). Among the 10 isolates, 70% exhibited MDR, while 30% were identified as positive for extended-spectrum beta-lactamase production (Table 4). All *Salmonella* isolates were susceptible to ciprofloxacin, gentamicin, as well as chloramphenicol; nine isolates (90%) were susceptible to ceftazidime and cefotaxime, and eight isolates (80%) were susceptible to ofloxacin.

Table 2. Number and percentage (in parentheses) of antibiotic-resistant *Salmonella* Enteritidis isolates from retail chicken meat.

	AMC	AMP	NAL	CIP	OFX	GEN	CAZ	CTX	TET	CHL	STR
Resistance	7 (70)	6 (60)	9 (90)	0 (0)	2 (20)	0 (0)	1 (10)	1 (10)	7 (70)	0 (0)	3 (30)
Intermediate	1 (10)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (30)	0 (0)	3 (30)
Susceptible	2 (20)	4 (40)	0 (0)	10 (100)	8 (80)	10 (100)	9 (90)	9 (90)	0 (0)	10 (100)	4 (40)

AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; AMC, amoxicillin–clavulanic acid; GEN, gentamicin; CIP, ciprofloxacin; STR, streptomycin; CHL, chloramphenicol; TET, tetracycline; OFX, ofloxacin; NAL, nalidixic acid.

Table 3. Number and percentage (in parentheses) of antibiotic-resistant *Campylobacter* spp. isolates from retail chicken meat.

Species		CIP	TET	NAL	ERT	CLI	GEN	AZM	DOX
<i>Campylobacter coli</i>	Resistance	0 (0)	8 (80)	5 (50)	9 (90)	9 (90)	9 (90)	3 (30)	0 (0)
	Intermediate	2 (20)	1 (10)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	1 (10)
	Susceptible	8 (80)	1 (10)	5 (50)	1 (10)	0 (0)	1 (10)	7 (70)	9 (90)
<i>Campylobacter jejuni</i>	Resistance	11 (42.8)	24 (86)	21 (75)	26 (92.8)	26 (92.8)	18 (64.3)	22 (78.6)	7 (25)
	Intermediate	10 (35.7)	2 (7.1)	4 (14.3)	2 (7.1)	1 (3.6)	4 (14.3)	0 (0)	7 (25)
	Susceptible	8 (28.6)	2 (7.1)	3 (10.7)	0 (0)	1 (3.6)	6 (21.4)	6 (21.4)	14 (50)

DOX, doxycycline; ERT, erythromycin; AZM, azithromycin; CIP, ciprofloxacin; GEN, gentamicin; CLI, clindamycin; NAL, nalidixic acid; TET, tetracycline.

Table 4. Patterns of antibiotic resistance in isolates of *Salmonella* Enteritidis, *Campylobacter coli*, and *Campylobacter jejuni*.

Antimicrobial Resistance Pattern	Number of Isolates		
	<i>Salmonella</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>
AMC-AMP-NAL-CAZ-TET	1		
AMC-AMP-NAL-CTX-STR	1		
AMC-AMP-NAL-OFX-TET	1		
AMC-AMP-NAL-TET	1		
AMC-AMP-NAL-OFX-CTX	1		
AMC-AMP-NAL-TET-STR-CHL	1		
NAL-CTX-TET-STR	1		
TET-ERT-CLI-GEN		5	1
NAL-ERT-CLI-GEN-AZM		1	
TET-NAL-ERT-CLI-GEN-AZM		2	6
TET-NAL-ERT-CLI-GEN		1	
TET-NAL-ERT-CLI-GEN-AZM-DOX			2
AMC-NAL	1		
AMC	1		
NAL	1	1	
CIP-TET-NAL-ERT-CLI-GEN-AZM-DOX			1
CIP-ERT-CLI-AZM-DOX			1
CIP-TET-NAL-ERT-CLI-GEN-AZM			2
TET-ERT-CLI			3
CIP-NAL-CLI			1
TET-NAL-ERT-CLI-AZM			2
CIP-TET-CLI-GEN-AZM			1
CIP-NAL-ERT-CLI-GEN			1
TET-NAL-ERT-CLI-AZM-DOX			1
TET-NAL-E-CLI-GEN-AZM			1
CIP-TET-NAL-ERT-AZM-GEN-DOX			1
TET-ERT-CLI-GEN-AZM			1
CIP-NAL-AZM			1
TET-ERT-CLI-GEN-AZM-DOX			1

AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; AMC, amoxicillin–clavulanic acid; GEN, gentamicin; CIP, ciprofloxacin; STR, streptomycin; CHL, chloramphenicol; TET, tetracycline; OFX, ofloxacin; NAL, nalidixic acid; DOX, doxycycline; ERT, erythromycin; AZM, azithromycin; CLI, clindamycin.

Similar to our findings, Alzahrani et al. [56] reported that ~77% of *Salmonella* Enteritidis isolates isolated from retail chicken in Riyadh were resistant to ampicillin and 88% were resistant to nalidixic acid and tetracycline. However, our study observed higher resistance levels to amoxicillin–clavulanic acid (70%) compared to the study of Alzahrani et al. [56], who reported lower levels (11%).

Dishan et al. identified MDR *Salmonella* Enteritidis with similar antigenic profiles (9:g, m:-) in poultry meat products in Turkey, with the highest resistance observed to doxycycline (96.42%) and trimethoprim–sulfamethoxazole (71.42%) [57]. In our study, *Salmonella* isolates showed a 60% resistance to ampicillin, differing from Thai et al., who reported low resistance to this antibiotic [58]. Our findings were consistent with those of Yu et al. [59], who recorded ampicillin resistance at 95.3% and tetracycline resistance at 72.7% in *Salmonella* isolates from chicken broilers in Shandong Province, China, where approximately 75% of *Salmonella enteritidis* isolates were MDR. Similarly, Waghamare et al. reported a 100% resistance to doxycycline in *Salmonella* Enteritidis isolates, while Elkenany et al. observed a resistance rate of 40% in chicken carcasses [60,61].

Most *Campylobacter coli* isolates (nine isolates, 90%) exhibited resistance to erythromycin, clindamycin, and gentamicin, followed by tetracycline (eight isolates, 80%). Lower resistance was observed for doxycycline (10%), ciprofloxacin (20%), and azithromycin (30%). In contrast, a higher proportion of *Campylobacter jejuni* isolates was resistant to erythromycin and clindamycin (26 isolates, 92.8%), tetracycline (24 isolates, 86%), azithromycin (22 isolates, 78.1%), nalidixic acid (21 isolates, 75%), and gentamicin (18 isolates, 64.3%). The highest susceptibility of *Campylobacter jejuni* was observed to doxycycline (fourteen isolates, 50%) and to ciprofloxacin (eight isolates, 28.6%). MDR was observed in all *Campylobacter jejuni* isolates (100%) and the majority of *Campylobacter coli* isolates (90%) (Table 4). These findings align with those of Yeh et al., who reported resistance to quinolone antibiotics in *Campylobacter* isolates from chicken liver [45].

A systematic review highlighted resistance levels across the Middle East that were similar to our findings [62]. Habib et al. [47] reported that approximately 40% of *Campylobacter coli* isolates from retail chicken in the United Arab Emirates were resistant to tetracycline, aligning with other reports from Canada [63], Italy [64], and China [65], where tetracycline resistance ranged from 45 to 95%. The resistance to ciprofloxacin in *Campylobacter jejuni* (28.6%) and *coli* (10%) was notably lower than that reported from Jordan, where Alaboudi et al. [66] observed complete resistance in all isolates. However, our study found a significantly higher level of resistance to gentamicin compared to the lower rates reported by Alaboudi et al. Our results revealed a low correlation between resistance to ciprofloxacin and nalidixic acid, contrasting with the findings by Varga et al. [63] Wei et al. [67] reported significantly lower erythromycin resistance levels in *Campylobacter jejuni* (16.7%) and *coli* (0%) compared to our study (~90%).

Azithromycin resistance is uncommon in *Campylobacter* species. Varga et al. [63] reported a lower level of resistance (6%) in *Campylobacter* isolates from retail chicken in Canada. Similarly, Wei et al. [67] observed azithromycin resistance levels of 19% in *Campylobacter jejuni* and 0% in *Campylobacter coli* from retail chicken in South Korea. In contrast, our findings indicate a higher level of resistance to azithromycin, particularly in *Campylobacter jejuni* (78.1%), posing a public health concern. The extensive and often inappropriate use of antibiotics in poultry is recognized as a significant factor in the development of MDR among commensal and zoonotic enteric bacteria [68].

Our results align with Castro-Vargas [69], who noted that nalidixic acid and ampicillin are typically the first antibiotics to cause resistance in poultry pathogens. Additionally, our study confirmed *Campylobacter* resistance after screening samples from chicken skin and liver, consistent with findings by Taniguchi et al. [70]. We observed 90% resistance

to clindamycin and erythromycin in both *Campylobacter jejuni* and *Campylobacter coli*, as well as resistance rates of 50% in *Campylobacter coli* and 72% in *Campylobacter jejuni* to nalidixic acid. These findings are similar to those of Yeh et al. [45], who reported quinolone resistance in *Campylobacter* isolates from chicken liver. Among *Campylobacter* species, Azizian et al. [54] identified tetracycline resistance as the most common (70.6%), followed by ciprofloxacin (63.7%) and amoxicillin (27.5%), with gentamicin being the most effective antibiotic. Similarly, Kouglenou et al. [55] reported that 55.8% of *Campylobacter* strains were MDR, with resistance rates of 72.7% for ciprofloxacin, 71.4% for ampicillin, and 71.4% for tetracycline.

According to data from Saudi MOH, half of the foodborne outbreaks occur in private homes [14]. In addition, an integrative review highlighted significant gaps in food safety knowledge and practices among consumers in Saudi Arabia [71]. Therefore, retail chicken may act as a vehicle and introduce MDR *Salmonella* and *Campylobacter* species into consumers' homes, potentially leading to foodborne infections. This underscores the necessity for developing routine monitoring systems to assess contamination levels in retail chicken meat. The pervasive presence of MDR *Salmonella* and *Campylobacter* in broiler meat calls for enhanced surveillance, stricter enforcement of food safety practices, and public health initiatives to mitigate the risk of foodborne diseases in Saudi Arabia. Future research should focus on sequencing the isolates to elucidate their genotypic profiles and their correlations to phenotypic profiles. It is also imperative to screen for other foodborne pathogens in retail chicken produced in Saudi Arabia to identify any other risks of pathogenic contamination.

4. Conclusions

This study underscores the importance of monitoring the prevalence of *Salmonella* spp. and *Campylobacter* spp., as well as antibiotic resistance in these pathogens, in Saudi Arabia's poultry industry. The findings highlight the need for targeted interventions in small-scale poultry farms, stricter implementation of food safety regulations, and enhanced surveillance programmes. Future research should investigate the prevalence of other foodborne pathogens in poultry and identify the sources contributing to the development and spread of antimicrobial resistance. Genotypic analyses of resistant isolates should also be prioritized to better understand the genetic mechanisms driving resistance, paving the way for effective control strategies and improved public health outcomes.

Supplementary Materials: The following supporting information can be downloaded at the following website: <https://www.mdpi.com/article/10.3390/microbiolres16010027/s1>, Figure S1. Culture characteristics of *Salmonella* Enteritidis serovar 1, 9, 12:g, m:- isolated from chicken retails. A. Black-centered colonies with blue background on Hektoen agar. B. Colonies were red with or without H2S on XLD-agar; Figure S2. Culture characteristics with different colonial forms with the metallic sheen of *Campylobacter jejuni* (A) and *Campylobacter coli* (B) on modified Charcoal Cefoperazone Deoxycholate Agar (MCCD) which incubated microaerophilic at 41–42 °C; Figure S3. Agar gel electrophoresis showed results of conventional PCR virulent *invA* genes in *Salmonella* Enteritidis serovar 1, 9, 12:g, m:-. Isolates number 3 to 14 were positive *invA* 284 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder); Figure S4. Agar gel electrophoresis showed results of conventional PCR virulent *mapA* gene in *Campylobacter jejuni*. Isolates number 1, 2, 3, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, and 22 were positive *mapA* 589 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder); Figure S5. Agar gel electrophoresis showed results of conventional PCR virulent *mapA* gene in *Campylobacter jejuni*. Isolates number 23, 29, 30, 31, 34, 35, 38, 39, and 40 were positive *mapA* 589 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder). Figure S6. Agar gel electrophoresis showed results of conventional PCR virulent *mapA* gene in *Campylobacter jejuni*. Isolates number 43, 44, 45, 46, 47, 48, and 56 were

positive *mapA* 589 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder); Figure S7. Agar gel electrophoresis showed results of conventional PCR virulent *ceuE* gene in *Campylobacter coli*. Isolates number 20, 25, 26, 28, 37, and 49 were positive *ceuE* gene 462 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder); Figure S8. Agar gel electrophoresis showed results of conventional PCR virulent *ceuE* gene in *Campylobacter coli*. Isolates number 51, 52, 53, and 55 were positive *ceuE* gene 462 bp. P was the positive control, and N was the negative control. L: represented the molecular size marker (100 pb ladder).

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