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Original Article

Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of *Campylobacter* Species Isolated from Poultry

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Abstract

Campylobacter is a major public health problem, leading to foodborne diarrhea in the world. The current study aimed to isolate *Campylobacter* in different sources of poultry and determine antimicrobial susceptibility. A total of 150 fecal and 29 cloacal swabs were obtained from poultry farms (84 cloacal swabs) and live bird markets (LBMs), respectively, and 37 cecal swabs were also acquired from a local slaughterhouse located in the middle Euphrates region. *Campylobacter* Species (spp.) was first isolated and characterized by conventional bacteriological methods. Secondly, the antimicrobial susceptibility of isolates was investigated by disc diffusion method. The overall prevalence of *Campylobacter* spp. isolated from fecal cloacal and cecal poultry samples was 24% (36 out of 150). All strains were resistant to Nalidixic acid and Ciprofloxacin (100%), with high resistance to Tetracycline (88.8%), Ampicillin (83.3%), Sulpha/Trimethoprim (80.5%), Erythromycin (50%), and Ceftriaxone (50%), but less resistant to Gentamicin (30.5%), Amoxi-Clav (27.7%), and Chloramphenicol (22.2%). The majority of isolates (97.2%) scored a multiple antibiotic resistance (MAR) index of 0.3 or more, and 35 (97.2%) isolates were resistant to three or more antibiotic classes. Particularly, 61.1% of the isolates were multidrug resistance (MDR), 36.1% of the isolates were extensively drug resistant, and 2.8% of the isolates were Pan drug resistant. Moreover, the current study detected 24 multiple resistance patterns from 36 isolates of *Campylobacter* spp., and most of the isolates (27 out of 36) displayed an important route of resistance to Nalidixic acid, Ciprofloxacin, and Tetracycline. Based on the results, increased resistance rates to commonly used antibiotics in *Campylobacter* were recovered from poultry farms, LBMs, and local slaughterhouses. The majority of strains were MDR to commonly used antimicrobials with elevated MAR indices, requiring implementation of a national strategy to improve husbandry practice and the effective use of antibacterial agents, alternatives, and vaccines.

Keywords: Antimicrobial resistance, *Campylobacter*, LBMs, MAR index, Poultry farms, Slaughterhouse

1. Introduction

Campylobacter is a major public health problem, leading to food-borne diarrhea and the most prevalent bacterial cause of gastroenteritis in humans and Campylobacteriosis in the world. However, the course of this disease is usually self-limited, characterized by fever, abdominal pain, and diarrhea. The infections can be deadly in young children, immunocompromised people, and old age. Individuals immunocompromised with underlying chronic conditions and the elderly may develop a systemic spread of infection and septicemia (1).

Campylobacter is a zoonotic and thermophilic organism with an optimum growth temperature of about 42°C that is similar to avian body temperature, and poultry serves as a reservoir for these bacteria (2). Poultry and poultry products are considered the main source of pathogenic *Campylobacter*, resulting in transmitting these bacteria to humans. The treatment of the domestic animals with antimicrobials to improve the overall health and produce higher-quality products could result in developing resistance bacteria to these antibiotics and suppressing sensitive bacteria. Therefore, human consumption of such contaminated food from animal origin may lead to adverse and unwanted health consequences (3).

More recently, literature has reported an increased frequency of antimicrobial-resistant and multidrug resistance (MDR) *Campylobacter* strains, particularly macrolides, fluoroquinolones, and tetracyclines (4). The valuable surveillance strategies to control campylobacteriosis should focus not only on tracing bacteria in humans, but also on the significant sources of *Campylobacter* species (spp.), mainly poultry production chain, endorsed under the "One World-One Health" approach that represents collective efforts of ministries of health and agriculture, workers in medical and veterinary sectors, researchers, and politicians (5).

In Iraq, few studies have been conducted on the prevalence and antimicrobial resistance in *Campylobacter* isolated from poultry farms, live bird markets (LBMs), and slaughterhouses.

Therefore, the current study was conducted to isolate *Campylobacter* from poultry farms, LBMs, and a local slaughterhouse in the middle Euphrates region and determine the susceptibility of the isolates to commonly used antimicrobial agents.

2. Materials and Methods

2.1. Sample Collection

In this cross-sectional study, 150 fecal and 29 cloacal swabs were obtained from poultry farms (84 cloacal swabs) and LBMs, respectively; moreover, 37 cecal swabs were acquired from a local slaughterhouse located in Al-Najaf and Al-Qadisiyah Governorates in the middle Euphrates region from December 2020 to the end of March 2021. Sterile cotton swabs were used for fecal sample collection, immediately inoculated on C&S Modified Cary Blair Transport media (Hardy diagnostics/USA), and delivered by cold box to Public Health Laboratory of milk and meat hygiene, Department of Public Health, Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq.

2.2. Bacteriological Isolation of *Campylobacter*

According to the guidelines of OIE (2017) for the isolation of *Campylobacter* spp., each swab from the transport medium was streaked onto Campylobacter Agar Base (CRITERION, Hardy Diagnostic), enriched with 5%-10% (vol/vol) human blood and *Campylobacter* selective supplement (Karmali, Oxoid) with Sodium pyruvate 50 mg, Vancomycin 10 mg, Cefoperazone 16 mg, and Cycloheximide 50 mg. Subsequently, the streaked plates were incubated at 42°C under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2), generated by CampyGenTM (Oxoid CN0035) for 48 h in the anaerobic jar (Oxoid, UK).

2.3. Identification of *Campylobacter* Isolates

Colonies with a small, not hemolytic, mucoid, grayish, and flat appearance, as well as irregular edges after 48 h from the incubation or those that were convex, round, entire, and glistening with 1-2 mm in diameter were presumptively identified as *Campylobacter* spp. (6) and subjected for further identification with Gram stain, oxidase, catalase, and indoxyl hydrolysis tests (7). All suspected isolates were subjected for agglutination with Microgen® Bioproducts Campylobacter Latex kit (Hardy diagnostics/USA) according to the manufacturer's instructions.

2.4. Disk Diffusion Antibacterial Susceptibility Testing

The antimicrobial susceptibility test for all confirmed isolates was conducted using the disc diffusion method on Mueller-Hinton/blood agar plates in accordance with Gblossi Bernadette and Eric Essoh (8). Moreover, the susceptibility of strains was evaluated against 10 antimicrobial agents, including Erythromycin (15 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Sulph/Trimethoprim (25 µg), Amoxi/Clav (30 µg), Ampicillin (10 µg), Ceftriaxone (30 µg), and Nalidixic acid (30 µg). The recorded results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines for fastidious bacteria (CLSI, 2018) and CLSI criteria for *Enterobacteriaceae* (CLSI, 2019).

The multiple antibiotic resistance (MAR) index was estimated as the total number of medicines for each isolate and obtained as follows:

MAR=R/E (9)

where R and E are resistant and the number of antibiotics examined for each isolate, respectively.

This study utilized the concepts of resistance patterns, defined by Magiorakos and Srinivasan (10) who defined MDR strains that are resistant to at least one drug in three antimicrobial medication classes. Extensively drug-resistant (XDR) refers to strains that are resistant to all drugs, except for two classes of medications, and pan drug resistance (PDR) also refers to an ability of strain to withstand all antimicrobial agents in all classes examined.

2.5. Statistics

The Chi-square and Fisher's Exact tests were used to compare significance between two percentages, and a *P*-value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Prevalence of *Campylobacter* Species in Poultry

The overall prevalence of *Campylobacter* spp. isolated from fecal cloacal and cecal poultry samples confirmed by standard bacteriological methods was 36 (24%) isolates out of 150 samples. In reviewing the literature, the prevalence of *Campylobacter* spp. was very dynamic and varied according to geographic area, season, sampling, and culturing methods, as well as other confounding factors. The findings of the current study are consistent with the results of studies conducted by Karikari and Obiri-Danso (11) who found a prevalence rate of 22.5% after examining the broiler and layer fecal samples. However, a systemic review and meta-analysis study reported a high mean prevalence (40.2%) in cecal/fecal poultry samples among 14 African countries (12), and the prevalence varied from 2% to 100% among 28 European.

countries (13). Furthermore, the sampling season substantially affected the prevalence of *Campylobacter* due to the high temperature of summer (14). Therefore, all samples were collected in the winter, leading to a low prevalence rate of colonization in the current study.

3.2. Antibacterial Susceptibility Testing

All *Campylobacter* spp. isolates (100%) were resistant to Ciprofloxacin and Nalidixic acid emphasized in table 1. Moreover, high resistance frequency was recorded to Tetracycline (88.8%), Ampicillin (83.3%), Sulpha/Trimethoprim (80.5%), Erythromycin (50%), and Ceftriaxone (50%) with less resistant to Gentamicin (30.5%), Amoxi-Clav (27.7%), and Chloramphenicol (22.2%).

Fluoroquinolones, Macrolides, and Tetracyclines are used to treat clinical cases of campylobacteriosis (15). However, antibiotic resistance, such as fluoroquinolones resistant *Campylobacter* among bacteria recovered from foods of animal origin is known as the main concern during the past two decades (16, 17).

Table 1. Antibiotic susceptibility testing of 36 *Campylobacter* spp. recovered from different poultry sources

Antibiotic class	Agent	Interpretation	
		Susceptible (%)	Resistant (%)
Macrolides	Erythromycin	18 (50)	18 (50)
Tetracyclines	Tetracycline	4 (11.1)	32 (88.8)
Fluoroquinolones	Ciprofloxacin	0 (0)	36 (100)
Phenicols	Chloramphenicol	26 (72.2)	10 (27.7)
Aminoglycosides	Gentamicin	25 (69.4)	11 (30.5)
Folate pathway inhibitor	Sulpha / Trimeth	7 (19.4)	29 (80.5)
Penicillin	Amoxi-Clav	26 (72.2)	10 (27.7)
Penicillin	Ampicillin	7 (19.4)	29 (80.5)
Cephalosporin	Ceftriaxone	18 (50)	18 (50)
Quinolones	Nalidixic acid	0 (0)	36 (100)

In the present study, ultimate resistance to Nalidixic acid and Ciprofloxacin and high resistance to Tetracycline and Erythromycin was observed, reflecting the great risk of current isolates on public health. A previous study conducted on *Campylobacter* from chicken meat in Baghdad reported a high level of resistance to Tetracycline (86.7%), Erythromycin (86.7%), and Norfloxacin (56.7%), but not to Nalidixic acid and Ciprofloxacin (23.3% and 13.3%, respectively) (18). In Algeria, *Campylobacter* recovered from broilers at farms and slaughterhouses was resistant to Nalidixic acid with a high frequency of resistance to Tetracycline, Ciprofloxacin, and Ampicillin (19). Some studies have documented increased levels of resistance to many classes of antimicrobials, including Fluoroquinolones and Tetracyclines in *Campylobacter* isolated from fecal samples in poultry (20-22). The misuse and heavy use of quinolones in poultry production may result in emerging and spreading quinolone and fluoroquinolone resistance in the bacterial population.

The data on the prevalence of resistance is an important milestone to assess the risk connected with antimicrobial resistance in *Campylobacter* surveillance programs (23). The use of Tetracycline in the food of poultry to prevent infection in breeding fields can lead to higher rates of resistance to it (88.8%), resulting in emerging and spreading antimicrobial resistance. These results are consistent with those of a study conducted in Canada in which resistance to Tetracycline was due to its overuse in the food of small poultry flocks (21).

In the current study, Ampicillin resistance was 83.3% that is in agreement with that in some previous studies (24, 25). Out of 36 isolates, only 7 (19.4%) isloates showed susceptibility to Sulpha/Trimethoprim, showing that the less susceptible *Campylobacter* isolates to Sulpha/Trimethoprim seem increasing which is in line with the results of other studies (11, 26). Furthermore, the antimicrobial susceptibility testing of the current study documented significant Erythromycin resistance (50%) among *Campylobacter* isolates, and a similar resistance rate (54.5%) was observed in *Campylobacter* isolated from chicken cloacal swabs in Egypt (27). However, a study in Tunisia determined Erythromycin resistance in all *Campylobacter* isolated from broilers (28). The increased Erythromycin resistance causes its less use as a first-line treatment option for human campylobacteriosis.

The resistance ratio of the current poultry isolates to Ceftriaxone was 50%. Moreover, high Ceftriaxone resistance was recorded in *Campylobacter* strains reported in broiler chicken products in Lithuania (20). However, the results of other studies are not in line with the findings of a study conducted by Rashid and

Azeem (29) in Pakistan. They found very low Ceftriaxone resistance. Ceftriaxone is frequently recommended as an empirical treatment for individuals admitted to the hospital with stomach pain and fever.

On the contrary, very low resistance frequencies were reported in *Campylobacter* spp. recovered from poultry in countries that implemented Fluoroquinolone ban and/or restricted antibiotics usage by poultry farms, such as Australia and Scandinavian countries (30). Therefore, revising the Iraqi guidelines seems necessary to regulate the distribution and the use of these antimicrobial agents as growth promotors and prophylactic for therapeutic purposes in poultry production.

Although the current results showed the low resistance of *Campylobacter* to Gentamicin (30.5%), Amoxi-Clav (27.7%), and Chloramphenicol (22.2%), they are close to those recorded in *Campylobacter* isolated from different sources of poultry from different parts of the world (31, 32). On the other hand, in eastern Algeria, a study was conducted on ultimate resistance to Amoxi-Clave and a moderate level of Chloramphenicol resistance (52.6%), all *Campylobacter* isolates were sensitive to Gentamycin (7), utilized rarely in Iraq for both preventive and therapeutic reasons, leading to the low prevalence of resistance.

3.3. Multiple Antibiotic Resistance Index and Multidrug Resistance

As presented in table 2, the MAR indices of the current isolates ranged between 0.2 and 0.7, and 35 out of 36 (97.2%) isolates were scored a MAR index of 0.3 or more.

Table 2. Multiple antibiotic resistance indices of *Campylobacter* spp. isolated from the poultry sample

MAR index	No. of antimicrobials to which the isolates were resistant (10)	No of Isolates (%)
0.2	3	1 (2.77)
0.3	4	2 (5.55)
0.3	6	1 (2.77)
0.4	5	12 (33.33)
0.4	6	5 (13.88)
0.4	7	1 (2.77)
0.5	6	2 (5.55)
0.5	8	4 (11.11)
0.6	8	4 (11.11)
0.6	9	1 (2.77)
0.7	7	1 (2.77)
0.7	9	1 (2.77)
0.7	10	1 (2.77)

MAR=multiple drug resistance

MAR index is used to measure how bacteria are affected by antibiotic usage, used as a tool to differentiate between animal isolates with high risk (MAR index over than 0.2) and low risk (MAR index less than 0.2). Furthermore, a MAR index of greater than 0.3 indicates that bacteria had already developed in an area in which antibiotics were often administered (33).

In the present study, however, the MAR index of 0.3 and more were recorded for the majority (97.2%) of *Campylobacter* spp. isolates were considered the most interesting finding. One isolate showed a 0.2 MAR index, indicating certainly the link to heavy and uncontrolled use of antibiotics which might create a high antibiotic selective pressure in the poultry production chain. Therefore, the current isolates represent a high public health risk.

Additionally, the findings of the current study are consistent with those of previous studies conducted on *Campylobacter* spp. isolated from frozen chicken meat in Iraq (9). Moreover, similar results were reported in many countries overusing antimicrobial agents in the poultry field (34, 35). In India, a MAR index between 0.11 and 0.78 in *Campylobacter* isolated from broilers and slaughterhouse background was reported (36).

Furthermore, 35 (97.2%) isolates were resistant to three or more antibiotic classes, and 22 (61.1%) isolates were particularly MDR, showing resistance to 3-4 different antimicrobial classes. In addition, our findings revealed the presence of 11 (30.55%) XDR isolates (resistant to 5-7 different antimicrobial classes) with 1 (2.77%) PDR (resistant to all examined antimicrobials) as shown in table 3.

Table 3. Prevalence of MDR, XDR, and PDR *Campylobacter* spp. isolated from poultry samples

Character of resistant strains	No. (%) of *Campylobacter* spp. isolates	No. of antibiotics to which the isolates were resistant	No. of antibiotic classes (n=7)
MDR (22=61.1%)	2	4	3
	1	6	3
	12	5	4
	5	6	4
	1	7	4
XDR (11=30.55%)	4	8	5
	2	6	5
	1	9	6
	4	8	6
	1	7	7
	1	9	7
PDR (1=2.77%)	1	10	7

MDR= Multidrug resistance
XDR= Extensively drug-resistant
PDR= Pan drug resistance

Emerging and spreading MDR *Campylobacter* spp. is a vital and challenging issue that may compromise the effective treatment options for human campylobacteriosis. Therefore, the present data revealed that all isolates, except for one, were resistant to at least three of the most widely used antibiotics. However, similar studies in Iraq, South Korea, and China have reported that the majority of *Campylobacter* isolates from poultry were MDR (9, 35, 37), and lower prevalence (54.4%) was demonstrated in *Campylobacter* isolates from broilers and slaughterhouse background swabs in India (36); moreover, a very lower prevalence of MDR was recorded among poultry isolates in Brazil (34).

Additionally, XDR and PDR were characterized in the present isolates, requiring more efforts toward controlling *Campylobacter* in the food chain in Iraq. The current results are apparently consistent with those of the other research conducted on an increased prevalence of MDR, XDR, and PDR *Campylobacter* strains of poultry in Egypt (38). Such categories of resistant bacteria may lead to antibiotic treatment failure both in veterinary and medical practices.

Table 4 tabulates 24 different resistant patterns in that the most frequent patterns include:

- CIP + NA / SXT / Te / AM (9 isolates)
- CIP + NA / SXT / Te / AM + CRO + AmC / E (3 isolates)
- CIP + NA / SXT / Te / AM + CRO (2 isolates)
- CIP + NA / Te / AM + CRO / E (2 isolates)

Other patterns were in each isolate that most of isolates (27 out of 36) displayed an important route of resistance to Nalidixic acid, Ciprofloxacin, and Tetracycline.

Table 4. Diverse resistance patterns among poultry-derived *Campylobacter* isolates

Resistance category	Resistance patterns	Code of isolates	No. of isolates
2 class	CIP, NA, SXT	B56	1
3 class	CIP, NA, SXT, Te	L73	1
3 class	CIP, NA, SXT, GM	B55	1
3 class	CIP, NA, SXT, AM, CRO, AmC	F123	1
4 class	CIP, NA, SXT, Te, AM	L81, L84, L85 F109, F110, F111, F113, F114, F115	9
4 class	CIP, NA, SXT, Te, AM, CRO	F106, F107	2
4 class	CIP, NA, Te, AM, CRO, E	B7, C1	2
4 class	CIP, NA, Te, GM, E	B62	1
4 class	CIP, NA, AM, E, C	B66	1
4 class	CIP, NA, Te, AM, CRO, E, AMC	C20	1
4 class	CIP, NA, Te, AM, CRO, GM	F117	1
4 class	CIP, NA, SXT, CRO, E	F126	1
5 class	CIP, NA, SXT, Te, AM, E	F108	1
5 class	CIP, NA, SXT, Te, AM, CRO, E, AmC	F119, B64, C2	3
5 class	CIP, NA, SXT, Te, E, C	B65	1
5 class	CIP, NA, SXT, Te, AM, CRO, GM, AMC	F124	1
6 class	CIP, NA, SXT, Te, AM, CRO, E, C	B11	1
6 class	CIP, NA, SXT, Te, AM, E, C, CRO	B44	1
6 class	CIP, NA, SXT, Te, AM, CRO, GM, E, AmC	B67	1
6 class	CIP, NA, SXT, Te, AM, GM, E, AmC	C3	1
6 class	CIP, NA, Te, AM, CRO, GM, E, C	C4	1
7 class	CIP, NA, SXT, Te, AM, CRO, GM, E, C	F93	1
7 class	CIP, NA, SXT, Te, AM, CRO, GM, E, C, AmC	F112	1
7 class	CIP, NA, SXT, Te, GM, E, C, AmC	H22	1

*Resistant to antimicrobials at normal dosages (μg): Erythromycin (E,15μg), Tetracycline (Te, 30μg), Ciprofloxacin (CIP, 5μg), Chloramphenicol (C, 30μg), Gentamycin (GM, 10μg), Sulpha-trieth (SXT, 25μg), Amoxicillin/Clav.Acid (AmC, 30μg), Ampicillin (AM, 10μg), Ceftriaxone (CRO 30μg), and Nalidixic acid (NA, 30μg).

In this study, the high prevalence of MDR strains among *Campylobacter* spp. isolates from poultry was an important finding in resistance pattern including NA/CIP/Te, detected previously in 90% of *Salmonella* Ohio isolated from poultry farms in Al-Najaf and Al-Muthana provinces (39) (i.e., the resistance traits could be circulating between pathogenic commensal bacteria colonizing the gut of birds may be via transmissible genetic elements).

To the best of our knowledge, this was the first survey study on isolation and antimicrobial susceptibility of *Campylobacter* in the middle Euphrates region that also found a significant prevalence of *Campylobacter* in poultry, regardless of its type and source with a very high prevalence of MDR strains. Therefore, the high levels of MAR index were in different poultry samples, showing a worrisome food safety situation in the middle Euphrates region.

Authors' Contribution

Study concept and design: Z. M. S.
Acquisition of data: A. O. A.
Analysis and interpretation of data: T. I. K.
Drafting of the manuscript: H. M. A.
Critical revision of the manuscript for important intellectual content: Z. M. S.
Statistical analysis: Z. M. S.
Administrative, technical, and material support: Z. M. S.

Ethics

Since this study used fecal samples from cloacal and cecal from slaughtered chicken, no ethical approval was needed.

Conflict of Interest

The authors declare that they have no conflict of interest.

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