Prevalence, resistance to antimicrobials, and antibiotypes of *Arcobacter* species recovered from retail meat in Wasit marketplaces in Iraq

Manal H. G. Kanaan[ID]

Department of Agriculture/Technical Institute of Suwaria, Middle Technical University, Baghdad, Iraq.
Corresponding author: Manal H. G. Kanaan, e-mail: manalhadi73@yahoo.com
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Abstract

Background and Aim: *Arcobacter* is a food-borne pathogen associated with human and animal infections. In Iraq, these infections and their adverse effects on public health have not been well investigated. For this reason, as well as to submit data on the resistance to antimicrobials and antibiotypes of the *Arcobacter* spp. and their occurrence in retail meat in the Wasit marketplaces, this study was undertaken.

Materials and Methods: A total of 83 samples of fresh raw (n=35) and chilled meat (n=48) were purchased randomly from marketplaces in various regions of the Wasit Governorate. Bacterial detection was conducted using cultural methods, biochemical analysis, and the Oxoid Biomedical Identification System Campy. Confirmation of these bacteria at the species level was performed using the multiplex polymerase chain reaction method. Susceptibility of the *Arcobacter* spp. to antimicrobials was investigated in 11 isolates comprising *Arcobacter butzleri* (n=9) and *Arcobacter cryaerophilus* (n=2) using the Kirby−Bauer disk diffusion method.

Results: A total of 32 (38.6%) of the 83 fresh raw and chilled meat samples tested positive for *Arcobacter* spp.; of those, 27 (84.4%) and 5 (15.6%) were recognized as *A. butzleri* and *A. cryaerophilus*, respectively. Maximum resistance was perceived, respectively, to tetracycline, erythromycin, and ampicillin (90.9%, 81.8%, and 81.8%, respectively). In contrast, a low resistance rate against fluoroquinolones up to 9.09% was found. Antibiograms of the *A. butzleri* and *A. cryaerophilus* isolates yielded ten antibiotypes. The vast majority of the isolates (90.91%) were resistant to no fewer than three classes of antimicrobials, and 27.3% of these showed resistance to six antibiotics. A total of 91% of the analyzed isolates had a multiple antibiotic resistance index score between 0.27 and 0.73.

Conclusion: Our outcomes demonstrated that retail meat can be a prospective vehicle for pathogenic *Arcobacter*, making these products a possible risk to human health. Our outcomes postulate that the contamination of retail meats by pathogenic *Arcobacter* is a global public health concern, particularly with the growing resistance to life-saving drugs, and emphasizes consumer understanding about the quality and safety of these products. To achieve healthy food products, good management practices, and successful control approaches must be implemented across the entire food chain, not only to protect consumers from these contaminants but also to minimize the risk of drug resistance.

Keywords: antibiotypes, *Arcobacter*, cattle meat, chicken meat, multidrug resistance.

Introduction

Arcobacter spp. has recently gained attention as an aquatic and food zoonotic pathogen [1] related to many serious conditions in animals, including enteritis and abortion, as well as to bacteremia, gastroenteritis, and diarrhea in humans [2]. Pathogenic species of the genus *Arcobacter*, which are the most important causes of medical conditions in humans and animals, include *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* [3]. Of these, *A. butzleri* in particular has been classified by the International Commission of Microbiological Specifications for Foods as a serious danger to human health [4].

Arcobacter spp. has been recovered from various types of food, in particular chicken, veal, and pork meat, raw milk, and shellfish, as well as from water and vegetables [2,5-7]. Poultry species in particular act as a vital reservoir of *Arcobacter* spp., as well as are a main source of the spread of infection [3,7]. Human infections with these pathogens have been linked to the ingesting or handling of fresh or undercooked contaminated food from animal sources or tainted water [8].

The world's population is increasing and the demand for food resources continues to grow. Therefore, the use of antimicrobials by food manufacturers is increasing worldwide, not only as a cure but also to improve growth and prophylaxis, resulting in bacterial resistance [9]. Antimicrobial resistance in bacteria is becoming a major concern in veterinary medicine, due to the fact that animals can become carriers of resistant zoonotic agents, which can then transmit resistance to the pathogens that affect humans [10]. Although the use of antibiotics in feeding animals is

still hidden from the public sphere, the application of antibiotics in meat animals has affected public health and has been recognized scientifically [11]. According to the Centers for Disease Control and Prevention, 80% of the total amount of antibiotics used in the United States was dumped in the animal production sector and not used for medical cures. At present, each year 2 million people are infected with resistant bacteria, and 23,000 die [12].

In Iraq, the demand for meat consumption is strong, and as far as we know, no documents are available on the prevalence of *Arcobacter* spp. in retail meats in the Wasit Governorate. As such, the impact of *Arcobacter* spp. remains unclear. Therefore, we conducted this study to investigate the occurrence and resistance to antimicrobials, as well as the antibiotypes of *Arcobacter* spp. recovered from retail meat, to detect the role of these products in the epidemiology of this pathogen as well as to evaluate the safety of these products in our marketplaces.

Materials and Methods

Ethical approval

Ethical approval is not needed for this study. Meat samples were collected from the reatil market.

Study period and location

The study was conducted in March 2019, and the samples were processed at the Technical Institute of Suwaria, Middle Technical University, Iraq.

Collection of samples

A total of 83 fresh raw and chilled meat samples, encompassing fresh raw chicken quarters (n=18) and cattle meat (n=17) and chilled chicken quarters (n=25) and cattle meat (n=23), were purchased randomly from native marketplaces and supermarkets in miscellaneous regions in the Wasit Governorate in Iraq and analyzed between March and August 2019. All samples were placed in a sanitized bag of ice sachets and moved instantly to the meat hygiene lab, where they were analyzed within 3 h of purchase.

Bacteria isolation and identification

We analyzed the samples using a process previously described by Molva and Atabay [5], with a slight modification. Briefly, 25 g of each sample was homogenized for 2 min in a stomacher with 225 mL of sterilized buffer peptone water (Oxoid CM0509, UK). Then, a 25-mL aliquot of the homogenate was inoculated into 25 mL of double-strength *Arcobacter* broth (Oxoid CM965, UK) encompassing a cefoperazone, amphotericin B, and teicoplanin (CAT) selective supplement (Oxoid SR0174, UK). This suspension was incubated at 30°C micro-aerobically for 2 days using Campy Gen™ atmosphere packs (Oxoid CN0025A, UK) in an anaerobic jar (Oxoid AG25, UK).

After enrichment, 200 µL of each sample was dispensed using a micropipette onto 0.45-µm-hole nitrocellulose film strainers (Sartorius) and positioned into two selective agars: Trypticase soy agar (TSA, Oxoid CM0131, UK) enhanced with 5% Laked Horse Blood (Oxoid SR0048C, UK) with CAT improvement (Oxoid SR0174, UK) and modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid CM739, UK). Then, the plates were incubated aerobically at 30°C for ~1 h before the filter was removed. The filtrate was streaked evenly across the surface of the two selective agar plates, and the plates were raised at 30°C aerobically for 2 days.

Afterward, the suspected colonies were purified by subculture onto mCCDA without supplement and raised at 30°C for 2 days. Purified isolates were extra confirmed morphologically to the species level using microscopic examination for motility (wet-mount slide for contrast microscopy) and morphology (Gram staining) accompanied by standard biochemical analysis using catalase, oxidase, indoxyl acetate hydrolysis, hippurate hydrolysis, and salt tolerance [13]. A further biochemical investigation was performed using the Oxoid Biomedical Identification System (OBIS) Campy (Oxoid ID0803M, UK) for the differentiation of *Campylobacteraceae* from other Gram-negative organisms based on detection of the L-alanyl aminopeptidase enzyme. The isolates referable at *Arcobacter* were preserved in a double-strength nutrient broth (Oxoid CM000, UK) with 20% (v/v) of pure medical glycerin at −20°C for further analysis.

Confirmation of *Arcobacter* isolates via mPCR

Stock cultures of *Arcobacter* isolates were thawed at 4°C overnight and then revived in blood agar. The extraction and purification of bacterial DNA were conducted using the Wizard® Genomic DNA Extraction and Purification Kit (Promega, USA). A multiplex polymerase chain reaction (PCR) (mPCR) method that uses primers developed by Houf *et al.* [14] has been used for the conformation of *Arcobacter* at the species level. The primers amplified (401) bp, (257) bp, and (641) bp fragments from *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, respectively (Table-1). The mPCR reactions were carried out in 50 mL of reaction combination (Promega 2× PCR master mix) comprising 2 µL of DNA model; 5 mL of 10× PCR buffer; 1.25 mM $MgCl_2$; 0.2 mM of deoxynucleoside triphosphate mixture; 50 pmol of each of the primers ARCO, BUTZ, CRY1, and CRY2; 25 pmol of the SKIR primer; and 1.5 U of Taq DNA polymerase [14].

mPCR amplification was performed in a Perkin−Elmer Thermocycler system with a preliminary denaturation at 94°C for 2 min, 32 cycles of denaturation (94°C, 45 s), annealing of the primer (61°C, 45 s), and final extension (72°C, 30 s). The amplification products were subjected to 1.5% agarose gel electrophoresis with SYBR Safe DNA gel dye at 100 V for 40 min, and the bands were visualized with a UV transilluminator (Alpha Imager HP, Alpha Innotech, CA, USA). A 100-bp DNA scale was used as a DNA molecular size marker. Reference-strain DNA was used as a positive control, and sterile DW was used as a negative control.

Determination of antimicrobial susceptibility

A total of 11 *Arcobacter* isolates comprising *A. butzleri* (n=9) and *A. cryaerophilus* (n=2) were selected to test the susceptibility of these isolates against nalidixic acid (ND; 30 μg), ampicillin (AMP; 10 μg), ciprofloxacin (CIP; 5 μg), cefotaxime (CTX; 30 μg), erythromycin (E; 15 μg), tetracycline (T; 30 μg), gentamicin (GM; 10 μg), cloxacillin (CX; 5 μg), vancomycin (VAN; 30 μg), norfloxacin (NOR; 10 μg), and amoxicillin/clavulanic acid (AMC; 10 μg) using the Kirby−Bauer disk diffusion method as cited by Quinn *et al*. [15]. In summary, the bacterial isolates that were conserved in pure medical glycerin at −20°C were thawed at 4°C and then resuscitated in blood agar (Oxoid CM0854, UK). Pure colonies of *Arcobacter* were cultured in Mueller−Hinton agar (Oxoid CM0337, UK) supplemented by 5% lysed horse blood (Oxoid SR0048C, UK) and incubated at 30°C for 48 h under aerobic conditions. Bacterial colonies from fresh pure cultures were mixed with nutrient broth (Oxoid CM000, UK); the turbidity of each inoculum was adjusted according to the 0.5 McFarland standards. Bacteria from each suspension were inoculated into Mueller−Hinton agar supplemented by 5% lysed horse blood using a sterile cotton swab. All plates were allowed to dry for 5 min at 37°C before dispensing the antimicrobial disks into the agar. Incubation of the plates took place in a micro-aerobic atmosphere at 30°C for 48 h, and the diameter of the inhibition zones was measured with calipers. The outcomes were interpreted based on the Clinical and Laboratory Standards Institute [16].

Multiple antibiotic resistance (MAR) index

The MAR index of these isolates was expressed as a result of dividing the sum of the antimicrobials to which the recovered isolates are resistant by the sum of the antimicrobials to which the isolates are exposed [17].

Statistical analysis

We used MedCalc Software Bvba version 18 (BE, USA https://www.medcalc.org/) to analyze the data. We compared the proportions using two samples. We used the Chi-squared test (χ^2) with a 5% significance level to study the significance between the proportions.

Results

In 32 (38.6%) of the 83 tested samples, morphological, microscopic, and standard biochemical inspection enabled the identification of plausible *Arcobacter* spp (Table-2) [14]. All the isolates had the same colony morphology (small, colorless, transparent, convex, and having a complete edge), motility, Gram negativity, catalase, oxidase, indoxyl acetate hydrolysis, positive salt tolerance, and negative hippurate hydrolysis. Moreover, these isolates, when passed on the OBIS system, were negative for Gram lysis state and for the acquisition of a-alanyle aminopeptidase.

The isolation percentages of *Arcobacter* spp. in fresh and chilled retail meat were 48.6% and 31.3%, respectively (Table-2). A total of 32 presumptive isolates were confirmed as *Arcobacter* spp. using mPCR, with *A. butzleri* and *A. cryaerophilus* accounting for 84.4% and 15.6%, respectively (Table-2). Furthermore, the peak occurrence for *A. butzleri* was in fresh and chilled chicken meat (90% and 100%, respectively), while the highest occurrence for *A. cryaerophilus* was in fresh and chilled cattle meat (28.6% and 33.3%, respectively).

Statistically, we found no significant effects (p>0.05) for the occurrence of *Arcobacter* (χ^2=2.525; p=0.1120). However, the effect of the sample category on the occurrence of the two species is highly significant (χ^2=29.821; p<0.0001).

Table-1: Primers sequences used in the multiplex PCR assay [14].

Arcobacter species	Primer	Sequence of primers (5′−3′)	Size in bp
Arcobacter butzleri	BUTZ	CCTGGACTTGACATAGTAAGAATGA	401 bp
	ARCO	CGTATTCACCGTAGCATAGC	
Arcobacter skirrowii	SKIR	GGCGATTTACTGGAACACA	641 bp
	ARCO	CGTATTCACCGTAGCATAGC	
Arcobacter cryaerophilus	CRY1	TGCTGGAGCGGATAGAAGTA	257 bp
	CRY2	AACAACCTACGTCCTTCGAC	

Table-2: Prevalence of *Arcobacter* spp. in retail meat traded in Wasit marketplaces.

Samples type		Samples inspected	*Arcobacter* positive samples (%)	*Arcobacter butzleri* (%)	*Arcobacter cryaerophilus* (%)
Fresh meat	Chicken quarters	18	10 (55.6)	9 (90)	1 (10)
	Cattle meat	17	7 (41.2)	5 (71.4)	2 (28.6)
	Total	35	17 (48.6)	14 (82.4)	3 (17.6)
Chilled meat	Chicken quarters	25	9 (36)	9 (100)	0 (0)
	Cattle meat	23	6 (26.1)	4 (66.7)	2 (33.3)
	Total	48	15 (31.3)	13 (86.7)	2 (33.3)
Total		83	32 (38.6)	27 (84.4)	5 (15.6)
p-value		χ^2=2.525	p=0.1120	χ^2=29.821	p<0.0001

Antibiotic resistance

Most of the tested isolates exhibited resistance to T, E, AMP, CX, and VAN at 90.9%, 81.8%, 81.8%, 72.7%, and 72.7%, respectively (Table-3). In contrast, we detected a low resistance rate against fluoroquinolones, GM, CTX, and AMC, ranging from 0% to 27.3%. Furthermore, *A. butzleri* had the highest resistance rate to AMP, E, GM, AMC, CX, CIP, and NOR, ranging from 11.1% to 88.9%, while *A. cryaerophilus* exhibited high resistance to VAN, NA,T, and CTX, ranging from 22.2% to 88.9% (Figure-1). Chicken *Arcobacter* isolates showed the highest resistance rates to AMP, NA, GM, CIP, CTX, AMC, and NOR, while cattle *Arcobacter* isolates showed the highest resistance rates to VAN, E, T, and CX (Figure-2).

Depending on the species of the bacteria, we found a significant effect ($p < 0.05$) in the level of resistance perceived to T ($\chi^2 = 6.290$; $p = 0.012$) and CX ($\chi^2 = 6.740$; $p = 0.01$). Furthermore, this effect is highly significant toward AMP and E ($\chi^2 = 8.785$; $p = 0.003$). However, there is no significant effect ($p > 0.05$) for sample category on the occurrence of resistance to these antimicrobials ($p = 0.673$, 0.902, 0.902, 0.259, 0.449, 0.902, 0.449, 0.902, 0.902, 0.902, and 0.449) for AMP, VAN, NA, E, T, GM, CIP, CTX, AMC, CX, and NOR, respectively.

The antibiogram and MAR index of the *A. butzleri* and *A. cryaerophilus* isolates are shown in Table-4; these isolates yielded ten antibiotypes in six antibiogroups based on the number of antimicrobials to which every isolate exhibited resistance. The most important remark in this study is that the vast majority of the *Arcobacter* isolates (10/11, 90.91%) established resistance to no less than three antimicrobial classes, and 27.3% displayed resistance to six antibiotics. In addition, the occurrence of the MDR phenomenon in the *Arcobacter* isolates recovered from chicken and cattle meat was 85.7% and 100%, respectively. On the basis of sample category, we found no significant effects ($p > 0.05$) on the existence of this phenomenon in the *Arcobacter* isolates ($\chi^2 = 1.617$; $p = 0.204$). Furthermore, percentages of the *Arcobacter* isolates reporting MAR index scores of 0.27, 0.36, 0.45, 0.55, 0.64, and 0.73 were 9.1%, 9.1%, 18.2%, 27.3%, 9.1%, and 18.2%, respectively (Table-4).

Discussion

Arcobacter spp. has been reported to be a significant public health hazard [5]. Contaminated foods may transmit these microorganisms to humans [18]. Contamination of chicken and cattle carcasses possibly caused by feces or other propagation routes during handling or initial processing, in addition to unhealthy food-handling practices, can lead to plausible persistence or cross-contamination of retail meat in the marketplace [5,19,20]. These microorganisms' ability to form biofilms on various pipe surfaces may be related to their persistence, resulting in colonization of water delivery systems and contamination of slaughterhouse water and equipment [21].In the current study, 38.6% of the retail meat tested positive for *Arcobacter* spp.; 84.4% and 15.6% were identified as *A. butzleri* and *A. cryaerophilus*, respectively (Table-2). Similarly, study conducted in Spain [22], and in Malaysia [23] have detected *Arcobacter* spp. in 32% and 39% of their food samples, respectively. In addition, they have established *A. butzleri* as the prevailing species, with occurrence rates ranging from 63% to 100%, followed by *A. cryaerophilus*, with an occurrence rate of 26.6%.

Lower isolation frequencies than those found in the current research were formerly attained by Di Noto *et al*. [2] in Italy; they recovered *Arcobacter* spp. from 14.3% of the food samples retailed in Sicily, finding *A. butzleri* and *A. cryaerophilus* at rates of 92.3% and 7.7%, respectively. Other studies conducted in Belgium [24], Malaysia [25], and Turkey [26] have found the occurrence of *Arcobacter* spp. in retail minced meat, in various other sources, and in animal fecal samples 9%, 15%, and 13%, respectively.

Table-3: Prevalence of antibiotic resistance in *Arcobacter* spp. recovered from retail meat in Wasit marketplaces.

Antibiotics	Prevalence of antibiotic resistance in *Arcobacter*				
	Sample's type				
	Chicken meat (7)		Cattle meat (4)		Total 11 (%)
	Arcobacter butzleri (%)	*Arcobacter cryaerophilus* (%)	*Arcobacter butzleri* (%)	*Arcobacter cryaerophilus* (%)	
Ampicillin	5 (71.4)	1 (14.3)	3 (75)	0 (0)	9 (81.8)
Vancomycin	4 (57.1)	1(14.3)	2 (50)	1 (25)	8 (72.7)
Nalidixic acid	1 (14.3)	1(14.3)	1 (25)	0 (0)	3 (27.3)
Erythromycin	5 (71.4)	0 (0)	3 (75)	1 (25)	9 (81.8)
Tetracycline	5 (71.4)	1 (14.3)	3 (75)	1(25)	10 (90.9)
Gentamycin	2 (28.6)	0 (0)	1 (25)	0 (0)	3 (27.3)
Ciprofloxacin	1 (14.3)	0 (0)	0 (0)	0 (0)	1 (9.09)
Cefotaxime	1 (14.3)	1 (14.3)	1 (25)	0 (0)	3 (27.3)
Amoxicillin/clavulanic acid	2 (28.6)	0 (0)	1 (25)	0 (0)	3 (27.3)
Cloxacillin	4 (57.1)	1(14.3)	3 (75)	0 (0)	8 (72.7)
Norfloxacin	1 (14.3)	0 (0)	0 (0)	0 (0)	1 (9.09)
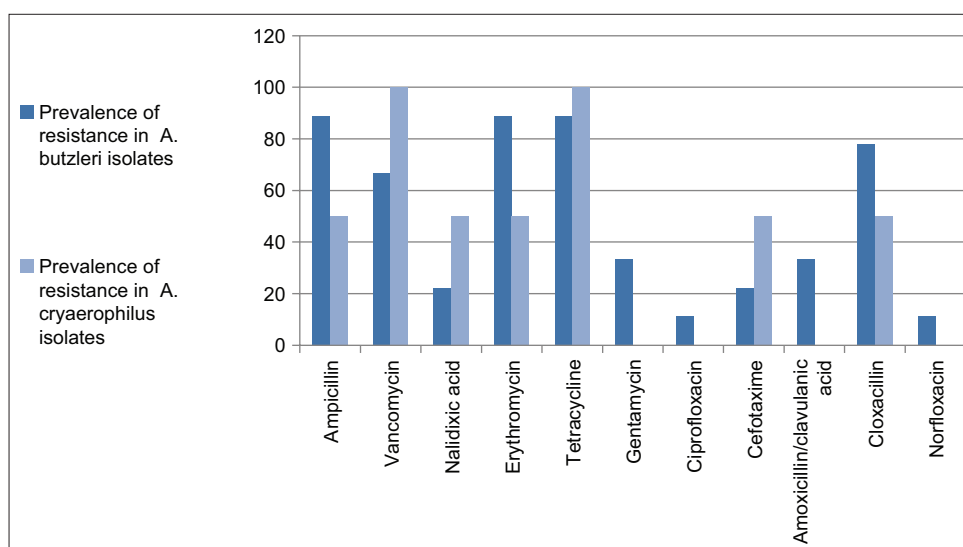
Figure-1: Prevalence of resistance in *Arcobacter* isolates based on species.

Table-4: Antibiogram and MAR index of *Arcobacter* spp. recovered from retail meat in Wasit marketplaces.

| Antibiotypes	Chicken *Arcobacter* isolates (7)	Cattle *Arcobacter* isolates (4)	Antibiogroups	Total 11 (%)	MAR index
CTX AMP CX AMC E VAN NA T	1 (14.3)	0 (0)	1A	2 (18.2)	0.73
AMP CX AMC E VAN NA GM T	0 (0)	1 (25)	1B		
AMP CX E VAN CIP GM T	1 (14.3)	0 (0)	2A	1 (9.1)	0.64
AMP CX E VAN GM T	1 (14.3)	0 (0)	3A	3 (27.3)	0.55
AMP CX AMC E NOR T	1 (14.3)	0 (0)	3B		
CTX AMP CX VAN NA T	1 (14.3)	0 (0)	3C		
AMP CX E VAN T	0 (0)	1 (25)	4A	2 (18.2)	0.45
CTX AMP CX E T	0 (0)	1 (25)	4B		
AMP E VAN T	1 (14.3)	0 (0)	5A	1 (9.1)	0.36
E VAN T	0 (0)	1 (25)	6A	1 (9.1)	0.27
Sensitive	1 (14.3)	0 (0)	-	1 (9.1)	
Total	6/7 (85.7)	4/4 (100)	6	10 (90.91)	

CTX=Cefotaxime, AMP=Ampicillin, CX=Cloxacillin, AMC=Amoxicillin/clavulanic acid, E=Erythromycin, VAN=Vancomycin, ND=Nalidixic acid, T=Tetracycline, GM=Gentamicin, CIP=Ciprofloxacin, NOR=Norfloxacin, MAR index=Multiple-drug resistance index

The increased presence of these bacteria could reflect unhealthy practices in slaughterhouses and processing plants, with the consequent contamination of livestock and poultry. Because the products have been stored at 4°C and/or room temperature, these temperatures can promote bacterial colonization [27].Even higher occurrences of *Arcobacter* spp. in meat previously have been found in Spain [8] and Chile [27], with rates up to 92%.

Our results reveal a larger occurrence of *Arcobacter* in chicken than in cattle (Table-2), in accordance with the previous findings [22,24,26,28]. Our results also proved greater contamination levels of *Arcobacter* spp. in fresh rather than in chilled meat, which is in accordance with findings published earlier [23,24].These results can be attributed to the death of bacteria after chemical changes in the lipid bilayer, leading to permanent physical damage to the cells, as well as when the temperature drops to a frost, which can lead to the formation of ice minerals that penetrate the cell membrane, with the consequent release of cellular components [24]. Furthermore,

this effect is greater in cattle carcasses, which can be explained by the longer cooling process and the absence of skin versus that in chickens, where feather follicles provide an excellent atmosphere to protect bacterial cells from dehydration and lower temperatures [29].We found that *A. butzleri* was more common than *A. cryaerophilus* in 84.4% of the positive samples. This result corresponds with that of many previous findings [2,5,22,25,27]. However, other investigators have stated that *A. cryaerophilus* was more common than *A. butzleri* [24,26].The dissimilarities in the occurrence of *Arcobacter* spp. maybe attributed to the different processing plant conditions and procedures, seasonal variances, geographical locations, experimental designs and analyses, and water sources [30,31].

Antimicrobials are used in humans and animals to treat diseases. Therefore, the resistance of the *Arcobacter* spp. to antibiotics creates a large concern with respect to curing these diseases [32]. However, the lack of cautious use and the excessive use of antimicrobials can facilitate the spread of resistant genes.
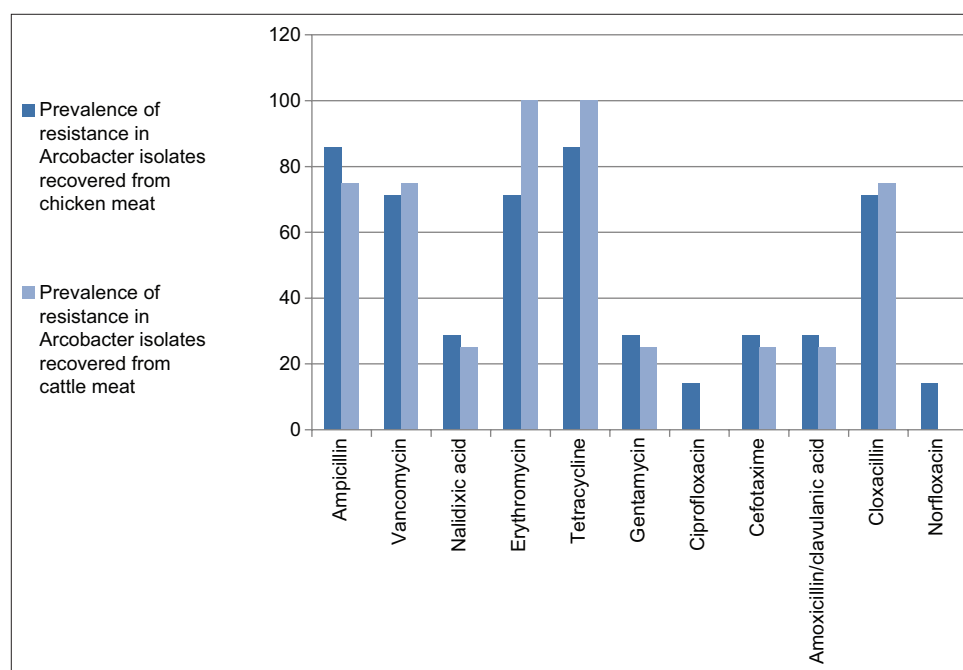
Figure-2: Prevalence of resistance in *Arcobacter* isolates based on samples origin.

Finding *Arcobacter* spp. in dissimilar sources such as humans, bird carcasses, meat, and the environment has publicized the issue of drug resistance [26], and our results support this finding.

In the current study, *Arcobacter* spp. from retail meat expressed high resistance to T, E, AMP, CX, and VAN. Antimicrobial resistance can arise through various intrinsic or acquired mechanisms that can vary by organism and class of antimicrobial agents involved. Intrinsic resistance is caused by natural genes present in the host animal's DNA, while acquired resistance involves the acquisition of the genes that encoded the resistance [33].

The increased resistance to β-lactam can be linked in large part to the application of penicillin as a food additive or a growth stimulant [34]. The resistance to macrolides can be concomitant to their unlimited to cure common infections in food-producing animals and to the recurrent use of spiramycin as a growth stimulant in poultry production [35], favoring the emergence of strains resistant to E [34]. The generous use of T in human and veterinary care and as a supplement to poultry and livestock feed can be concomitant to the increase in organisms with high resistance [36]. VAN resistance may be tied to the use of avoparcin, a related glycopeptide antibiotic used in agriculture [37]. Furthermore, environmental bacteria such as *Enterococci* spp. have been found in a variety of foods; these bacteria transfer resistance to various antibiotics by delivering several resistance genes, which can be transmitted to food-borne pathogens [38,39]. Therefore, meat can be vulnerable to such resistant bacteria, in particular to VAN-resistant *Enterococci* (VRE). Tremendous contamination of poultry and minced meat by VRE previously has been

detected in Turkey, with frequencies of 57.1% and 36.5%, respectively [40].

High resistance rates to T, AMP, CX, and E previously have been observed in *Arcobacter* spp. isolated from domestic geese and animal feces in Turkey, with rates ranging from 61.4% to 100% [26,32], as well as high rates of resistance tot T, AMP, and E in *A. butzleri* from pets in Malaysia, up to 80% [41]. In contrast with the results of our study, susceptibility to T, E, and AMP has been detected previously in Turkey, Belgium, and Japan, ranging from 78.7% to 100% [32,42,43].

Low levels of resistance to VAN in the *Arcobacter* spp. recovered from animal feces of up to 31.8% has been reported earlier [26]. This finding contradicts ours, which reveals a resistance frequency of up to 72.7%. Over the breeding period, poultry habitually interact with antimicrobials such as enrofloxacin and sarafloxacin, which possibly explains the appearance of quinolone resistance [44]. Apramycin has been used expansively in veterinary therapy, which probably correlates to the rise of GM resistance in *Arcobacter* [44].

Our results reveal a low resistance rate to fluoroquinolones, GM, CTX, and AMC, ranging from 9.09% to 27.3%, which is consistent with previous results from Turkey, showing rates ranging from 6.8% to 31.8% [26]. These outcomes also conflict with those from Malaysia, which have expressed *Arcobacter* spp. in pets having resistance to enrofloxacin, GM, CTX, and AMC, in rates of more than 70% [41]. These researchers attributed the extraordinary resistance to these antibiotics to their high usage in cures for humans and pet animals. On the other hand, their susceptibility to CIP was 100% that rate in our study was 90%. This variability could be due to the lack of

standardized protocols and resistance cutoff points for the isolates [45].

A. butzleri had the highest resistance rate to AMP, E, GM, AMC, CX, CIP, and NOR, while *A. cryaerophilus* exhibited a high resistance rate to VAN, NA, T, and CTX. Furthermore, the chicken *Arcobacter* isolates had high resistance rates to AMP, NA, GM, CIP, CTX, AMC, and NOR, while the cattle *Arcobacter* isolates exhibited more resistance toward VAN, E, T, and CX (Figures-1 and 2). These results contradicted the Turkey outcomes [26]. Variations in resistance and susceptibility rates have been linked to differences in antimicrobial agents, antibiotics used, organism types, and isolate origins [26].

The over application of antimicrobial agents has interfered with the balance of the ecosystem, thus enriching MDR bacteria [46,47]. The rise of this phenomenon could conceivably reveal the acquisition of lonely or miscellaneous resistance traits on DNA particles, such as during the use of multiple drug pumps [48]. Genetic resistance occurs either as a result of chromosomal or plasmid bearing and is expressed as a combination of endogenic and captured genes [44]. In this study, we found that 90.91 of the *Arcobacter* isolates established MDR to no less than three antimicrobial classes (Table-4), which mirrors earlier results [26,30]. MDR is considered a great threat to humanity that can affect the choice of antimicrobials for the cure of infections [49].

This study supports the findings of discrepancies in farming practices used for the raising of animals [50]. This is illustrated in the discrepancies in the MAR index among the *Arcobacter* strains recovered from retail meat. When we realize that most supplemental drugs in feed or water are not wholly absorbed in the gastrointestinal tract of these animals, and nearly 90% of the absorbed antimicrobials can be expelled in the feces, fresh waste can be a vigorous exporter of drug residues [51]. Accordingly, a high MAR index can imply that these strains were recovered from meat exposed to contamination with animal manure. That these animals were raised on farms using dissimilar agriculture practices may explain the discrepancies in the MAR index in this study (from 0.27 to 0.73).

Conclusion

Our data show that retail meat can be a prospective vehicle for pathogenic *Arcobacter* spp. The occurrence of *Arcobacter* spp. in meat represents a possible risk for human health because it may cause serious diseases. In addition, these results can add newly available data for the significant zoonotic pathogens.

Furthermore, the two pathogenic species of *Arcobacter* recovered in this study were highly resistant to CX, T, AMP, and E, with cumulative resistance to GM and CIP. This finding should be considered when therapy decisions are assessed. These results highlight the necessity to execute further studies on the existence, distribution, and MDR rates of the *Arcobacter* spp. in diverse foods for human ingestion in other areas in Iraq to provide additional data about this food-borne pathogen. Moreover, it is crucial to apply good hygiene practices and effective control plans throughout the entire food chain to achieve safe food product and protect consumers against these pathogens.

Author's Contributions

All parts of the current study (study proposal, collection of samples, laboratory work, manuscript preparation, analysis of data, and revisions) were implemented by MHGK. She read, confirmed, and approved the final manuscript.

Competing Interests

The author declares that she has no competing interests.

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References

1. Shrestha, R.G., Tandukar, S., Bhandari, D., Sherchan, S.P., Tanaka, Y., Sherchand, J.B. and Haramoto, E. (2019) Prevalence of *Arcobacter* and other pathogenic bacteria in river water in Nepal. *Water*, 11(7): 1416.
2. Di Noto, A.M., Sciortino, S., Cardamone, C., Ciravolo, C., Napoli, C., Alio, V., Arculeo, P., Oliveri, G. and Costa, A. (2018). Detection of *Arcobacter* spp. in food products collected from Sicilia region: A preliminary study. *Ital. J. Food Saf.*, 7(2): 7171.
3. Ramees, T.P., Dhama, K., Karthik, K., Rathore, R.S., Kumar, A., Saminathan, M., Tiwari, R., Malik, Y.S. and Singh, R.K. (2017) *Arcobacter*: An emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control a comprehensive review. *Vet. Q.*, 37(1): 136-161.
4. ICMSF. (2002) Microorganisms in food. Microbiological testing in food safety management. In: International Commission on Microbiological Specifications for Foods. Kluwer Academic Publication, New York, USA.
5. Molva, C. and Atabay, H.I. (2016) Prevalence and diversity of *Arcobacter* spp. in retail chicken meat in Turkey. *Microbiol. Res.*, 7(1): 29-31.
6. Tanaka, R., Cleenwerck, I., Mizutani, Y., Iehata, S., Bossier, P. and Vandamme, P. (2017) *Arcobacter haliotis* sp. nov., isolated from abalone species *Haliotis gigantea*. *Int. J. Syst. Evol. Microbiol.*, 67(8): 3050-3056.
7. Hassan, A.K. (2017) Detection and identification of *Arcobacter* species in poultry in Assiut Governorate, Upper Egypt. *J. Adv. Vet. Res.*, 7(2): 53-58.
8. Collado, L. and Figueras, M.J. (2011) Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clin. Microbiol. Rev.*, 24(1): 174-192.

9. Ballard, D.P., Peterson, E.A., Nadler, J.L. and Khardori, N.M. (2015) Antibiotic use in animal feed and its impact on antibiotic resistance in human pathogens. *Food Microbiol.,* 61: 137-155.

10. Weese, J.S. (2008) Antimicrobial resistance in companion animals. *Anim. Health Res. Rev.*, 9(2): 169-176.

11. Kanaan, M.H.G. and Al-Isawi, A.J.O. (2019) Prevalence of methicillin or multiple drug-resistant *Staphylococcus aureus* in cattle meat marketed in Wasit Province. *Biochem. Cell Arch.*, 19(1): 495-502.

12. Osman, K., Badr, J., Al-Maary, K.S., Moussa, I.M., Hessain, A.M., Girah, Z., Abo-Shama, U.H., Orabi, A. and Saad, A. (2016) Prevalence of the antibiotic resistance genes in coagulase-positive-and negative-*Staphylococcus* in chicken meat retailed to consumers. *Front. Microbiol.*, 7(222): 1-12.

13. Collado, L., Cleenwerck, I., Van Trappen, S., De Vos, P. and Figueras, M.J. (2009) *Arcobacter mytili* sp. nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels. *Int. J. Syst. Evol. Microbiol.*, 59(6): 1391-1396.

14. Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J. and Vandamme, P. (2000) Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Microbiol. Lett.*, 193(1): 89-94.

15. Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (2004) Clinical Veterinary Microbiology. 2nd ed., Mosby Int., USA.

16. Clinical and Laboratory Standards Institute. (2015) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

17. Kanaan, M.H.G. and Abdulwahid, M.T. (2019) Prevalence rate, antibiotic resistance and biotyping of thermotolerant *Campylobacter* isolated from poultry products vended in Wasit markets. *Curr. Res. Nutr. Food Sci. J.*, 7(3): 905-917.

18. Ferreira, S., Queiroz, J.A., Oleastro, M. and Domingues, F.C. (2014) Genotypic and phenotypic features of *Arcobacter butzleri* pathogenicity. *Microbiol. Pathog.*, 76(1): 19-25.

19. Shah, A.H., Saleha, A.A., Zunita, Z. and Murugaiyah, M. (2011) *Arcobacter*-an emerging threat to animals and animal origin food products? *Trends Food Sci. Technol.*, 22(5): 225-236.

20. Ho, H.T., Lipman, L.J. and Gaastra, W. (2008) The introduction of *Arcobacter* spp. in poultry slaughterhouses. *Int. J. Food Microbiol.*, 125(3): 223-229.

21. Khoshbakht, R., Tabatabaei, M., Shirzad Aski, H. and Seifi, S. (2014) Occurrence of *Arcobacter* in Iranian poultry and slaughterhouse samples implicates contamination by processing equipment and procedures. *Br. Poult. Sci.*, 55(6): 732-736.

22. Collado, L., Guarro, J. and Figueras, M.J. (2009) Prevalence of *Arcobacter* in meat and shellfish. *J. Food Prot.*, 72(5): 1102-1106.

23. Amare, L.B., Saleha, A.A., Zunita, Z., Jalila, A. and Hassan, L. (2011) Prevalence of *Arcobacter* spp. on chicken meat at retail markets and in farm chickens in Selangor, Malaysia. *Food Control*, 22(5): 732-736.

24. De Smet, S., De Zutter, L., Van Hende, J. and Houf, K. (2010) *Arcobacter* contamination on pre-and post-chilled bovine carcasses and in minced beef at retail. *J. Appl. Microbiol.*, 108(1): 299-305.

25. Shah, A.H., Saleha, A.A., Zunita, Z., Cheah, Y.K., Murugaiyah, M. and Korejo, N.A. (2012) Genetic characterization of *Arcobacter* isolates from various sources. *Vet. Microbiol.*, 160(3-4): 355-361.

26. Yesilmen, S., Vural, A., Erkan, M.E. and Yildirim, I.H. (2017) Isolation and determination of antimicrobial resistance of *Arcobacter* species isolated from animal faeces in the Diyarbakir region of Turkey using the 16S rDNA-RFLP method. *Vet. Med.*, 62(6): 301-307.

27. Fernandez, H., Villanueva, M.P., Mansilla, I., Gonzalez, M. and Latif, F. (2015) *Arcobacter butzleri* and *A. cryaerophilus* in human, animals and food sources, in southern Chile. *Braz. J. Microbiol.*, 46(1): 145-147.

28. Lehmann, D., Alter, T., Lehmann, L., Uherkova, S., Seidler, T. and Gölz, G. (2015) Prevalence, virulence gene distribution and genetic diversity of *Arcobacter* in food samples in Germany. *Berl. Munch. Tierarztl.*, 128(3-4): 163-168.

29. Jang, K., Kim, M., Ha, S., Kim, K., Lee, K., Chung, D., Kim, C.H. and Kim, K. (2007) Morphology and adhesion of *Campylobacter jejuni* to chicken skin under varying conditions. *J. Microbiol. Biotechnol.*, 17(2): 202.

30. Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J. and Harrison, M.A. (2007) Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int. J. Food Microbiol.*, 113(1): 16-22.

31. González, I., García, T., Fernández, S. and Martín, R. (2012) Current status on *Arcobacter* research: An update on DNA-based identification and typing methodologies. *Food Anal. Method.*, 5(5): 956-968.

32. Ünver, A., Atabay, H.I., Şahin, M. and Çelebi, Ö. (2013) Antimicrobial susceptibilities of various *Arcobacter* species. *Turk. J. Med. Sci.*, 43(4): 548-552.

33. Umber, J.K. and Bender, J.B. (2009) Pets and antimicrobial resistance. *Vet. Clin. North Am. Small*, 39(2): 279-292.

34. Chantziaras, I., Boyen, F., Callens, B. and Dewulf, J. (2014) Correlation between veterinary antimicrobial use and anti-microbial resistance in food-producing animals: A report on seven countries. *J. Antimicrob. Chemother.*, 69(3): 827-834.

35. Padungtod, P., Kaneene, J.B., Hanson, R., Morita, Y. and Boonmar, S. (2006) Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in Northern Thailand. *FEMS Immunol. Med. Microbiol.*, 47(2): 217-225.

36. Nguyen, T.N.M., Hotzel, H., Njeru, J., Mwituria, J., El-Adawy, H., Tomaso, H., Neubauer H. and Hafez, H.M. (2016) Antimicrobial resistance of *Campylobacter* isolates from small scale and backyard chicken in Kenya. *Gut Pathog.*, 8(1): 39.

37. Lauderdale, T.L., Shiau, Y.R., Wang, H.Y., Lai, J.F., Huang, I.W., Chen, P.C., Chen, H.Y., Lai, S.S. and Ho, M. (2007) Effect of banning vancomycin analogue avoparcin on vancomycin-resistant enterococci in chicken farms in Taiwan. *Environ. Microbiol.*, 9(3): 819-823.

38. Diarra, M.S. and Malouin, F. (2014) Antibiotics in Canadian poultry productions and anticipated alternatives. *Front. Microbiol.*, 5(1): 282.

39. Santestevan, N.A., de Angelis Zvoboda, D., Prichula, J., Pereira, R.I., Wachholz, G.R., Cardoso, L.A., de Moura, T.M., Medeiros, A.W., de Amorin, D.B., Tavares, M., d'Azevedo, P.A., Franco, A.C., Frazzon, J. and Frazzon, A.P. (2015) Antimicrobial resistance and virulence factor gene profiles of *Enterococcus* spp. isolates from wild *Arctocephalus australis* (South American fur seal) and *Arctocephalus tropicalis* (Subantarctic fur seal). *World J. Microbiol. Biotechnol.*, 31(12): 1935-1946.

40. Elmal, M. and Can, H.Y. (2018) The prevalence, vancomycin resistance and virulence gene profiles of *Enterococcus* species recovered from different foods of animal origin. *Vet. Arhiv.*, 88(1): 111-124.

41. Goni, M.D., Osman, A.Y., Abdul Aziz, S., Zunita, Z., Dhaliwal, G.K., Jalo, M.I., Bitrus, A.A., Jajere, S.M. and Abbas, M.A. (2018) Antimicrobial resistance of *Campylobacter* spp. and *Arcobacter butzleri* from Pets in Malaysia. *Am. J. Anim. Vet. Sci.*, 13(4): 152-161.

42. Vandenberg, O., Houf, K., Douat, N., Vlaes, L., Retore, P., Butzler, J.P. and Dediste, A. (2006) Antimicrobial suscepti-bility of clinical isolates of non-jejuni/coli campylobacters and arcobacters from Belgium. *J. Antimicrob. Chemother.*, 57(5): 908-913.

43. Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y. and Mikami, T. (2004) Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int. J. Food Microbiol.*, 90(3): 303-308.

44. Kanaan, M.H. (2018) Antibacterial effect of ozonated water against methicillin-resistant *Staphylococcus aureus* contaminating chicken meat in Wasit Province, Iraq. *Vet. World*, 11(10): 1445.

45. Houf, K., Devriese, L.A., Haesebrouck, F., Vandenberg, O., Butzler, J.P., Hoof, J.V. and Vandamme, P. (2004) Antimicrobial susceptibility patterns of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from humans and broilers. *Microbiol. Drug Resist.*, 10(3): 243-247.

46. Levy, S.B. (1997) Antibiotic resistance: An ecological imbalance. *Ciba Found Symp.*, 207: 1-9; discussion 9-14.

47. Kanaan, M.H.G. and Abdullah, S.S. (2019) Methicillin-resistant *Staphylococcus aureus* as a Superbug Foodborne Pathogen. LAP Academic Publisher, Germany.

48. Thakur, S., Zhao, S., McDermott, P.F., Harbottle, H., Abbott, J., English, L., Gebreyes, W.A. and White, D.G. (2010) Antimicrobial resistance, virulence, and genotypic profile comparison of *Campylobacter jejuni* and *Campylobacter coli* isolated from humans and retail meats. *Foodborne Pathog. Dis*., 7(7): 835-844.

49. Kurinčič, M., Berce, I., Zorman, T. and Možina, S.S. (2005) The prevalence of multiple antibiotic resistance in *Campylobacter* spp. from retail poultry meat. *Food Technol. Biotechnol*., 43(2): 157-163.

50. Kanaan, M.H.G. and Mohammed, F.A. (2020) Antimicrobial resistance of *Campylobacter jejuni* from poultry meat in local markets of Iraq. *Plant Arch*., 20(Suppl 1): 410-415.

51. Furtula, V., Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard, J. and Diarra, M.S. (2010) Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. *Poult. Sci.*, 89(1): 180-188.

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