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Prevalence of *Campylobacter* species in chicken meat retailed in Jazan City, Kingdom of Saudi Arabia

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Poultry meats have been considered the primary source of campylobacteriosis infections among various foods. This study was performed to detect the presence of *Campylobacter* species in retail chicken meat and organs. Eighty-six samples (thighs = 21, breasts = 16, legs = 21, wings =17, and livers = 11) were collected from supermarkets and retail shops in Jazan city. Cultivation and isolation of *Campylobacter* species were carried out according to the protocol described by ISO 10272-1: 2017. Sixty-one samples (representing six companies) showed growth on mCCDA agar plates after incubation. In contrast, no bacterial growth was observed in samples representing one company. Bacterial isolates (n = 122) were subjected to a *Campylobacter* Multiplex Polymerase Chain Reaction (Multiplex-PCR) for the identification of the genus *Campylobacter* (816 bp) and the three *Campylobacter* spp.: *Campylobacter jejuni* (323 bp), *Campylobacter coli* (126 bp), and *Campylobacter lari* (251 bp). The PCR products used to identify the *Campylobacter* spp. came only from the positive controls but not from the isolates. Because of this, all isolates were found to be non-*Campylobacter* spp (bacteria other than *Campylobacter*). Amplifying and sequencing the 16S rRNA gene allowed for identification of 19 isolates to confirm this result. Sequence analysis on the NCBI website revealed that sixteen isolates (84%) were identified as *Escherichia coli* (*E. coli*) and three isolates (16%) as *Proteus mirabilis*. Furthermore, six of the sequenced isolates were subjected to an antibiotic susceptibility test using eight antibiotics, and results showed that all isolates were multidrug resistant.

Key words: *Campylobacter* (*jejuni, coli, lari*), chicken meat, multiplex-polymerase chain reaction (PCR), *Escherichia coli, Proteus mirabilis,* antibiotic resistance.

INTRODUCTION

Campylobacter bacteria are members of the Campylobacteraceae family. The genus *Campylobacter* includes 32 different species and nine subspecies (Costa and Iraola, 2019); they are Gram-negative, microaerophilic (conditions: 5% O_2, 10% CO_2, and 85% N_2), curved or spiral-shaped rods (Gull wings), have small width (0.2-0.8 µm) and length (0.5-5.0 µm), and corkscrew-like motility using single polar unsheathed flagellum at one or both ends (Facciolà et al., 2017; Meurer et al., 2020). *Campylobacter* cells may show spherical or coccoid forms in stressed environments that are viable but non-culturable cells. The optimum growth temperature is 37 to 42°C under microaerobic conditions (Hald et al., 2000; Tholozan et al., 1999).

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Campylobacter species are found in various environments, including wild and domestic animals, unpasteurized milk, water, soil, sewage, and humans (Champion et al., 2005; Maugeri et al., 2004). The digestive system of birds and other domestic and free-living animals is the natural reservoir of *Campylobacter* as a part of normal flora (Sahin et al., 2017). *Campylobacter* spp. is the causative agent of the human foodborne disease "campylobacteriosis," one of the most frequent types of bacterial gastroenteritis globally. Campylobacteriosis is characterized by diarrhea, cramps, stomach pain, and fever (O'Brien, 2017). Handling, preparing, and consuming undercooked meats, especially poultry, are the leading causes of human campylobacteriosis (Shane, 2000). Other types of food, such as unpasteurized milk, beef, and pork, and contact with animals and infected humans have also been reported as a source of campylobacter infection (Andrzejewska et al., 2019; Schönberg-Norio et al., 2004). *Campylobacter jejuni* and *Campylobacter coli* are the leading cause of foodborne campylobacteriosis in humans (Guerry, 2007). The presence of *Campylobacter* spp. in retailed chicken meat varies significantly from country to country depending on different factors such as geographical differences in sampling, hygienic measurements at the processing plant, and different isolation techniques that use a variety of enrichment and selective media. In Japan, 64.7% of the samples were contaminated with *Campylobacter* (Sallam, 2007); in Malaysia, 70.7 and 91.4% of chicken samples from 4 wet markets and three hypermarkets were contaminated with *Campylobacter* (Tang et al., 2009); in Saudi Arabia, previous work in 2014, reported that the prevalence of *Campylobacter* in collected chicken samples was 30.3% (Yehia and AL-Dagal, 2014); in Qatar, Abu-Madi et al. (2016) examined the occurrence of *Campylobacter* spp. in 400 sold chicken meat samples and found that 36.5% showed growth in Karmali agar; in Egypt (Zagazig City, Sharkia Governorate) 25.5, 27.5, and 29.3% of the samples from neck skin, breast meat, and thigh meat were contaminated with *Campylobacter* spp. (Abd El Tawab et al., 2018); in Tunisia, 26.8% of fresh chicken samples were positive for *Campylobacter* spp. (Jribi et al., 2017); while the lowest detection rate of *Campylobacter* was in China, the analyzed data from the National Food Safety Risk Surveillance Network on *C. jejuni* between 2007 and 2010 and also from four published articles revealed that the detection rates of *C. jejuni* in raw chicken were between 0.29 and 2.28% (Jun et al., 2013). In general, the presence of *Campylobacter* in chicken meat could be attributed to the unhygienic measures during slaughtering, processing steps (stunning and bleeding, scalding, de-feathering, evisceration, washing, chilling, and post-chill) and packaging of chickens. Furthermore, the prevalence and contamination of chicken carcasses by *Campylobacter* in the processing plant increase after defeathering and evisceration but decrease after scalding and chilling (Sahin et al., 2015). This study aimed to examine the prevalence of *Campylobacter* spp. in retailed chilled chicken meat in Jazan City, Kingdom of Saudi Arabia.

MATERIALS AND METHODS

Sample collection

Eighty-six samples of chilled chicken meat (thighs = 21, breasts = 16, legs = 21, wings = 17, and livers = 11) were collected from different supermarkets and retail shops in Jazan City. Seven commercial brands of poultry companies in Jazan City were represented in the collected samples. The collected samples were transported to the laboratory directly in an ice box for microbiological examination.

Enrichment and isolation of bacteria

The protocol described by ISO 10272-1: 2017 (Standardization, 2017) was used to detect the presence of *Campylobacter* spp. in collected samples. Ten grams from each chicken sample were aseptically transferred to a sterile bag and homogenized for 1 min in a stomacher with 90 ml of *Campylobacter* selective enrichment broth (Bolton broth, CM0983, Oxoid, UK) supplemented with Bolton broth Selective Supplement (SR0183, Oxoid, UK) and 5% lysed Horse Blood (SR0048, Oxoid, UK). Ten milliliters of the homogenate were transferred into sterile tubes, capped loosely, and then incubated in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) using gas generating kits (AnaeroPck-MicroAero, MGC, Mitsubishi Gas Chemical Co., Inc., Japan) at 37°C for 4 h followed by 41.5°C for 44±4 h.

After enrichment, a loopful (10 µl) from each tube was streaked onto modified *Campylobacter* selective agar plates (mCCDA, CM0739, Oxoid, UK) containing CCDA selective supplement (SR0155, Oxoid, UK). The plates were incubated at 41.5°C ± 0.5 for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) in anaerobic jars containing gas-generating kits. After incubation of inoculated mCCDA plates for 48 h at 42°C, the plates were examined for typical *Campylobacter* colonies, which were small, gray, drop-like, shiny, or slimy. Bacterial colonies were selected from mCCDA plates, streaked onto Columbia blood agar (Oxoid, CM331, United Kingdom) plates (containing 5% horse blood), and incubated under microaerobic conditions at 42°C for 48 h under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). Purified colonies were used for Gram staining, biochemical reactions, PCR, and preservation.

Identification of bacterial isolates

Multiplex PCR for detection and identification of Campylobacter spp.

To detect and identify *Campylobacter* spp., a polymerase chain reaction was performed using the MultiGene™ OptiMax Thermal Cycler (Labnet AG, Edison, USA). The PCR reaction was performed in a total volume of 25 µl by using 12.5 µl of 2X OnePCRTM, ready-to-use PCR reaction mixture (GeneDireX, Inc., Taiwan), 1.5 µl from forward and reverse primer, 1 µl sample for DNA template, and nuclease-free water (to a final volume of 25 µl). Negative control was included without template DNA.

All primers used in this work were manufactured by Macrogen, Seoul, South Korea, in a lyophilized form and were dissolved in nuclease-free water to give a final concentration of 100 pmol/µl and

Table 1. Primers used for the detection and identification of *Campylobacter* species.

Primer	Target bacteria	Primer sequence (5'–3')	Target gene	Amplicon (bp)	Reference
C412-F 1228-R	*Campylobacter* genus	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	*16S-rRNA*	816	Linton et al. (1996)
CJ-F CJ-R	*C. jejuni*	ACTTCTTTATTGCTTGCTGC GCCACAACAAGTAAAGAAGC	*hipO*	323	
CC-F CC-R	*C. coli*	GTAAAACCAAAGCTTATCGTG TCCAGCAATGTGTGCAATG	*glyA*	126	Wang et al. (2002)
CL-F CL-R	*C. lari*	TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC	*glyA*	251	

stored at -20°C as a stock. Then, primers were diluted to a final concentration of 10 pmol/µl by adding 10 µl of primer stock solution to 90 µl of nuclease-free water and finally stored at -20°C until used. Four pairs of primers were used to detect the genus *Campylobacter* (Linton et al., 1996) and identify *Campylobacter* spp (Wang et al., 2002). All PCR primers are listed in Table 1.

The PCR program used was one cycle of 95°C for 5 min, followed by 30 cycles of 94°C × 30 s, 54°C (Annealing temperature) × 30 s, 72°C × 50 s, and a final extension of 72°C × 7 min. The resulting PCR products were mixed with 6x RUNESAFE staining buffer (Cleaver Scientific LTD, United Kingdom), applied to a 3% agarose gel, and visualized by UV illumination.

16S rRNA gene analysis

The 16S rRNA gene of nineteen bacterial isolates was amplified using the colony-PCR method. One colony or more were picked with a sterile loop from agar plates and transferred to 1.5 ml micro-centrifuge tubes containing an adequate amount (50 µl) of 1x Colony PCR buffer to obtain a turbid cell suspension. The tubes were placed in a thermomixer or water bath, heated at 95°C for 15 min, and then cooled on ice. Finally, cell debris was sedimented by centrifugation at 11000 rpm, at 4°C temperature for 5 min. The supernatant was transferred to a new 1.5 ml micro-centrifuge tube (Eppendorf tube) and used as template DNA; 1 µl was used for PCR reaction in a 25 µl total volume, and the rest was stored at -20°C for further use. The used universal primers were: the forward primer 27F 5'- AGAGTTTGATCM*TGGCTCAG-3' (M* = C or A) and the reverse primer 1492R 5'- TACGGY* TACCTTGTTACGACTT-3' (Y* = T or G) (Song et al., 2017). The PCR conditions used were one cycle of 95°C for 5 min, followed by 30 cycles of 95°C × 30 s, 51°C (Annealing temperature) × 30 s, 72°C × 90 s, and a final extension of 72°C × 7 min. The PCR product was mixed with 6x RUNESAFE staining buffer (Cleaver Scientific LTD, United Kingdom), applied to a 2% agarose gel, and visualized by UV illumination. The nucleotide sequence of the amplified fragment was at Macrogen (Macrogen Inc., South Korea). It was analyzed by the National Centre for Biotechnology Information (NCBI)-Basic Local Alignment Search Tool (BLAST) at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antimicrobial susceptibility test

Antibiotic susceptibility test was carried out using the disk diffusion method on Mueller-Hinton agar (CM0337, Oxoid, UK) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) (Humphries et al., 2018).

RESULTS AND DISCUSSION

Isolation of *Campylobacter* spp.

After enrichment of the collected samples in Bolton Selective Enrichment Broth and streaking onto modified *Campylobacter* selective agar (mCCDA) containing CCDA antibiotic supplement, out of eighty-six examined collected samples (representing seven companies named A, B, C, D, E, F & G) of chicken meat and organs (Meat: thighs = 21, breasts = 16, legs = 21, wings =17, and Organs: liver = 11), 61 samples (71%) showed bacterial growth on mCCDA agar plates (Figure 1A). Interestingly, company "B" representatives showed no bacterial growth on mCCDA agar plates, and this might be due to the post-slaughtering treatments during chicken processing like freezing, hot water treatment, irradiation, and chemical decontamination (sodium hypochlorite, calcium hypochlorite, sodium bisulfate, lactic acid, and trisodium phosphate, ozone, chlorine gas, chlorine dioxide). Although chemical decontamination is commonly used in the USA, it is not allowed in EU countries (Sahin et al., 2015). In most cases, the lack of proper hygiene throughout the slaughtering, processing, staining, bleeding, de-feathering, evisceration, washing, chilling, and post-chill phases of birds is to blame for the presence of *Campylobacter* in chicken flesh. In addition, defeathering and evisceration raise the incidence and contamination of *Campylobacter* in chicken carcasses in the processing plant, but scalding and chilling lower it (Sahin et al., 2015). One hundred and twenty-two bacterial isolates were selected, purified on Columbia blood agar plates containing 5% horse blood (Figure 1B), and used for further identification.

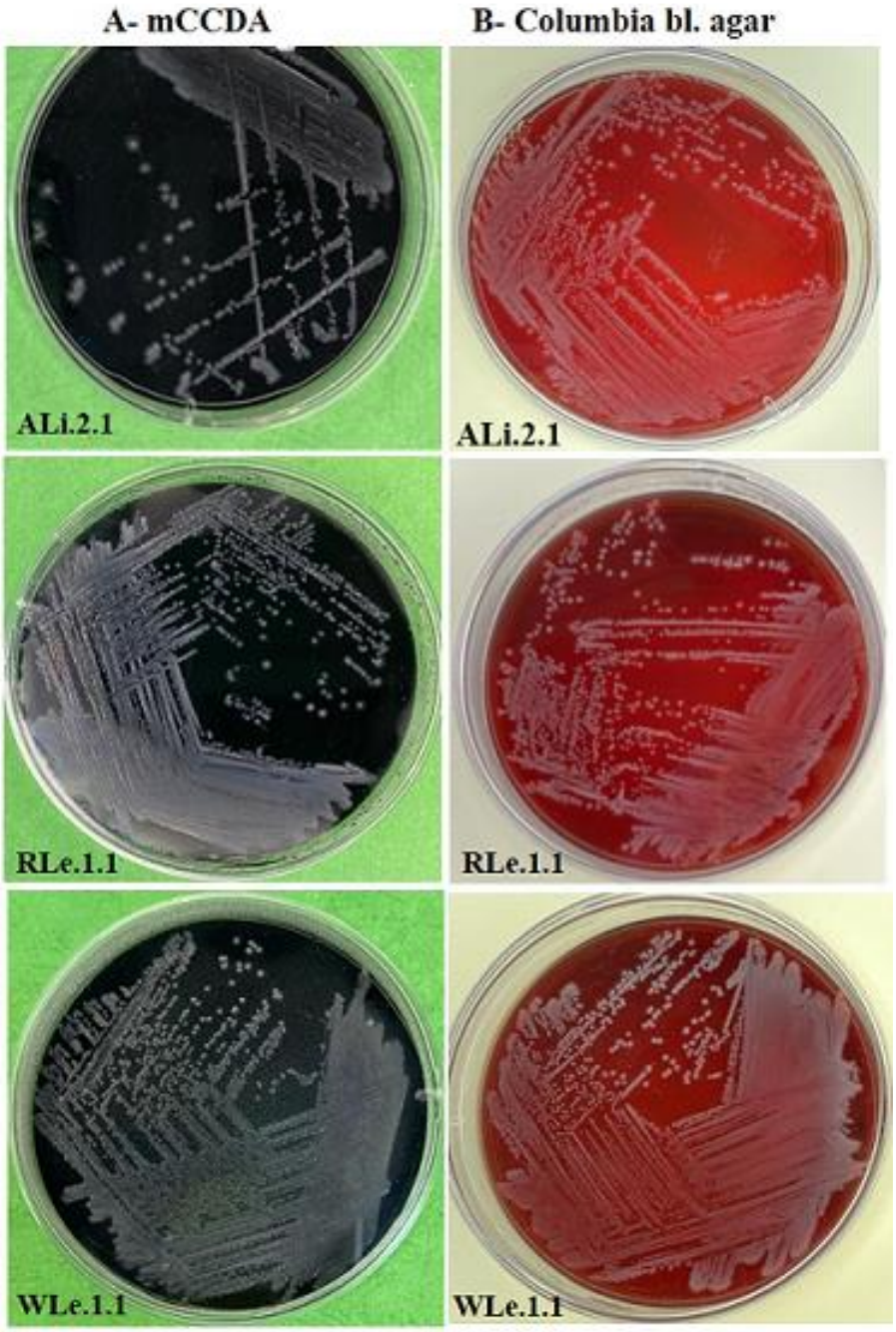

Figure 1. (A) mCCDA agar plates showing bacterial growth from chicken meat samples after enrichment in Bolton broth. (B) Streaking of bacterial isolates onto Columbia blood agar from mCCDA agar plates.

Multiplex PCR for detection and identification of isolated bacteria

To confirm whether the isolated bacteria belonged to *Campylobacter* or non-*Campylobacter* spp., 122 bacterial isolates were subjected to the *Campylobacter* multiplex PCR. After visualization of the PCR products on the agarose gel, PCR obtained products for *Campylobacter* bacteria only were from the three reference strains (*C. jejuni* = 323 bp, *C. coli* = 126 bp and *Campylobacter lari* = 251 bp). In contrast, no PCR fragments were obtained from the isolated bacteria (Figure 2). This finding disagrees with previously reported studies (Abd El Tawab et al., 2018; Abu-Madi et al., 2016; Jribi et al., 2017; Jun et al., 2013; Yehia and AL-Dagal, 2014).

To reduce the number of non-*Campylobacter* bacteria

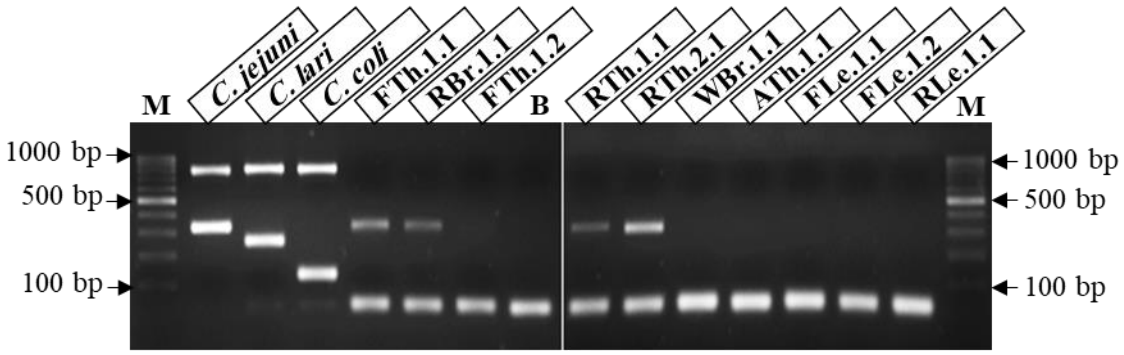

Figure 2. *Campylobacter* Multiplex PCR for identification of the isolated bacteria. Agarose gel (3%) electrophoresis of PCR products obtained from the isolated bacteria. Reference Strains: *C. jejuni* ATCC 33291 = 323 bp, *C. coli* ATCC 33559 = 126 bp, *C. lari* ATCC 35221 = 251 bp, while no PCR fragments were obtained from bacterial isolates. Lane B = Negative control. Lane M: 100 bp DNA Marker (Cleaver Scientific LTD, United Kingdom).

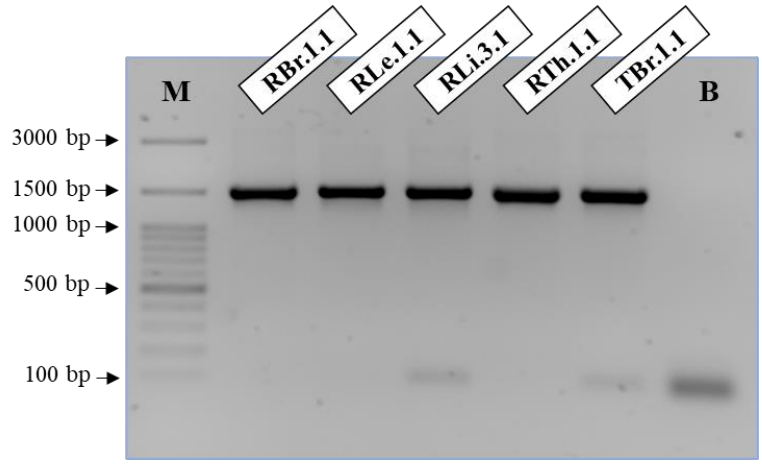

Figure 3. RCR of the 16S-rRNA gene from the isolated bacteria. Agarose gel (2%) electrophoresis showed PCR products of 1500 bp length. Lane B: Negative control (Blank). Lane M: 100 bp DNA Marker (GeneDireX, inc., Taiwan).

during *Campylobacter* isolation in the laboratory, the traditional microbiological methods have been improved over time by using selective media, antibiotics, and better-growing conditions (Ricke et al., 2019). A higher incubation temperature (42°C) and a microaerophilic environment are required to promote the growth of thermophilic *Campylobacter* bacteria. Moreover, some antibiotics are used to enhance the growth of *Campylobacter* isolates that are naturally resistant to antibiotics while suppressing the growth of non-*Campylobacter* spp (Eberle and Kiess, 2012). Unlike other foodborne bacteria, *Campylobacter* shows an adaptable physiological and metabolic biological feature that can hinder the efficiency of culture techniques (On et al., 1996; Ricke et al., 2019). Also, it was said that *Campylobacter* can become stressed when exposed to psychrotrophic conditions like refrigeration and freezing. This makes it viable but non-culturable (VBNC), which means that many common microbiological methods cannot find it (Castro et al., 2018; Ziprin et al., 2003). This case (VBNC) might be a reason for the failure of *Campylobacter* detection in the present study.

Amplification and sequencing of 16S rRNA gene

The 16S-rRNA gene from 19 bacterial isolates was amplified using 27F and 1492R universal primers (De Lillo et al., 2006). A PCR product of about 1.5-kb fragment (Figure 3) was amplified as previously mentioned.

The amplified 16S rRNA gene from the 19 bacterial

Table 2. Results of the 16S ribosomal RNA gene sequence alignments for the nineteen bacterial isolates in the NCBI GenBank database.

No.	Code	Name	Organism	E value	Identity (%)	Accession No.
1	*ST-1*	*ATh.1.1*	*Escherichia coli* strain ST95-32	0.0	99.33	CP043950.1
2	*ST-2*	*ALi.2.1*	*Escherichia coli* strain RRL-36	0.0	96.31	JQ398845.1
3	*ST-3*	*FBr.1.1*	*Escherichia coli* strain DSM 103246	0.0	95.85	CP019944.1
4	*ST-4*	*FLe.1.1*	*Escherichia coli* strain UT1 & UT22	0.0	97.15	KP276714.1 KP276735.1
5	*ST-5*	*FTh.1.2*	*Escherichia coli* strain SJC148	0.0	97.11	MN367952.1
6	*ST-6*	*RBr.1.1*	*Proteus mirabilis* strain UFV 128	0.0	96.57	JX293281.1
7	*ST-7*	*RLe.1.1*	*Escherichia coli* strain SJC148	0.0	96.66	MN367952.1
8	*ST-8*	*RLi.3.1*	*Escherichia coli* strain CFSAN061761	0.0	97.17	CP042903.1
9	*ST-9*	*RTh.1.1*	*Proteus mirabilis* strain MPE4069	0.0	99.11	JF775423.1
10	*ST-10*	*TBr.1.1*	*Escherichia coli* strain SJC148	0.0	98.14	MN367952.1
11	*ST-11*	*TLe.2.1*	*Escherichia coli* strain SJC148	0.0	98.52	MN367952.1
12	*ST-12*	*TLi.3.1*	*Escherichia coli* strain SJC148	0.0	98.59	MN367952.1
13	*ST-13*	*TTh.2.1*	*Escherichia coli* strain W3	0.0	99.04	MN086363.1
14	*ST-14*	*TaLe.3.1*	*Escherichia* spp. strain Es_1	0.0	98.36	MK095771.1
15	*ST-15*	*TaLi.1.1*	*Escherichia coli* strain CFSAN027350	0.0	98.31	CP037941.1
16	*ST-16*	*TaTh.2.1*	*Escherichia coli* strain UFV 209	0.0	95.98	MN557804.1
17	*ST-17*	*TaWi.3.1*	*Escherichia coli* strain UT1	0.0	95.43	KP276714.1
18	*ST-18*	*WBr.3.1*	Proteus mirabilis strain OA18	0.0	99.20	OQ026396.1
19	*ST-19*	*WLe.1.1*	*Escherichia coli* strain UT1	0.0	96.70	KP276714.1

isolates was sequenced at Macrogen (Macrogen Inc., South Korea) to confirm the PCR results. Sequence analysis on the NCBI website (Table 2 and Figure 4) showed that 16 isolates (84%) were *E. coli* strains with 95 to 99% similarity. Three isolates (16%) were identified as *Proteus mirabilis* (*RBr.1.1*, *RTh.1.1* and *WBr.3.1*) with the identity of 96, 99, and 99%, respectively. These results of the present study were in close agreement with a previous study from Kim et al. (2019), in which different combinations of culture media were tested using 40 retail chicken samples, such as Bolton broth-Bolton agar (BB-BA), Preston broth-Preston agar (PB-PA), and Preston broth-Bolton agar PB-BA, to see effect of combining Bolton and Preston selective media on the frequencies of *Campylobacter* isolation. Lower isolation frequencies were seen in the combinations that included BA as the second selective culture medium. Kim et al. (2019) found that, at the genus level, Bolton broth (BB) significantly increased the proportion of *Escherichia coli* (70.6%) and decreased the number of *Campylobacter* bacteria. In addition, the growing *E. coli* in BB was resistant to the three antibiotics of BB (Cefoperazone, Vancomycin, and Trimethoprim). Recently, bacteria resistant to cefoperazone, named extended-spectrum beta-lactamases-producing (ESBL-producing), have become more common (Chon et al., 2020). In many countries, ESBL-producing *E. coli* strains resistant to cefoperazone (a plasmid-mediated β-lactamase) have also been frequently isolated from raw chicken. Recent studies have reported that ESBL-producing *E. coli* may overgrow on mCCDA supplemented with Cefoperazone, making it difficult to cultivate and isolate suspected *Campylobacter* colonies (Kim et al., 2019; Moran et al., 2011). In addition, Gram-positive bacteria are sensitive to vancomycin, while Gram-negative bacteria typically exhibit intrinsic resistance to vancomycin. Consistently, *Escherichia* was predominant in the selective enrichment media (Zhou et al., 2015).

The isolate *ST-1* (*ATh.1.1*) showed 99% to *E. coli* strain ST95-32 chromosome; it is a member of Extra-intestinal pathogenic *E. coli* (ExPEC) which is responsible for multi-system diseases in humans, other mammals, and birds, including typical urinary tract infections (UTI), neonatal meningitis, and bloodstream infections. Strain ST95-32 is a multidrug-resistant strain resistant to more than 12 antibiotics, including ampicillin, ceftazidime, levofloxacin, and chloramphenicol (Ewers et al., 2007; Jiang et al., 2022). Depending on their different pathogenic subtypes, ExPECs are subdivided into uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC), and avian pathogenic *E. coli* (APEC) (Köhler and Dobrindt, 2011). ExPEC strains contain nine STs (ST10, ST12, ST69, ST73, ST95, ST117, ST127, ST131, and ST405), which are the major avian pathogenic *E. coli* (APEC) associated with avian colibacillosis (Riley, 2020). Recent studies have shown

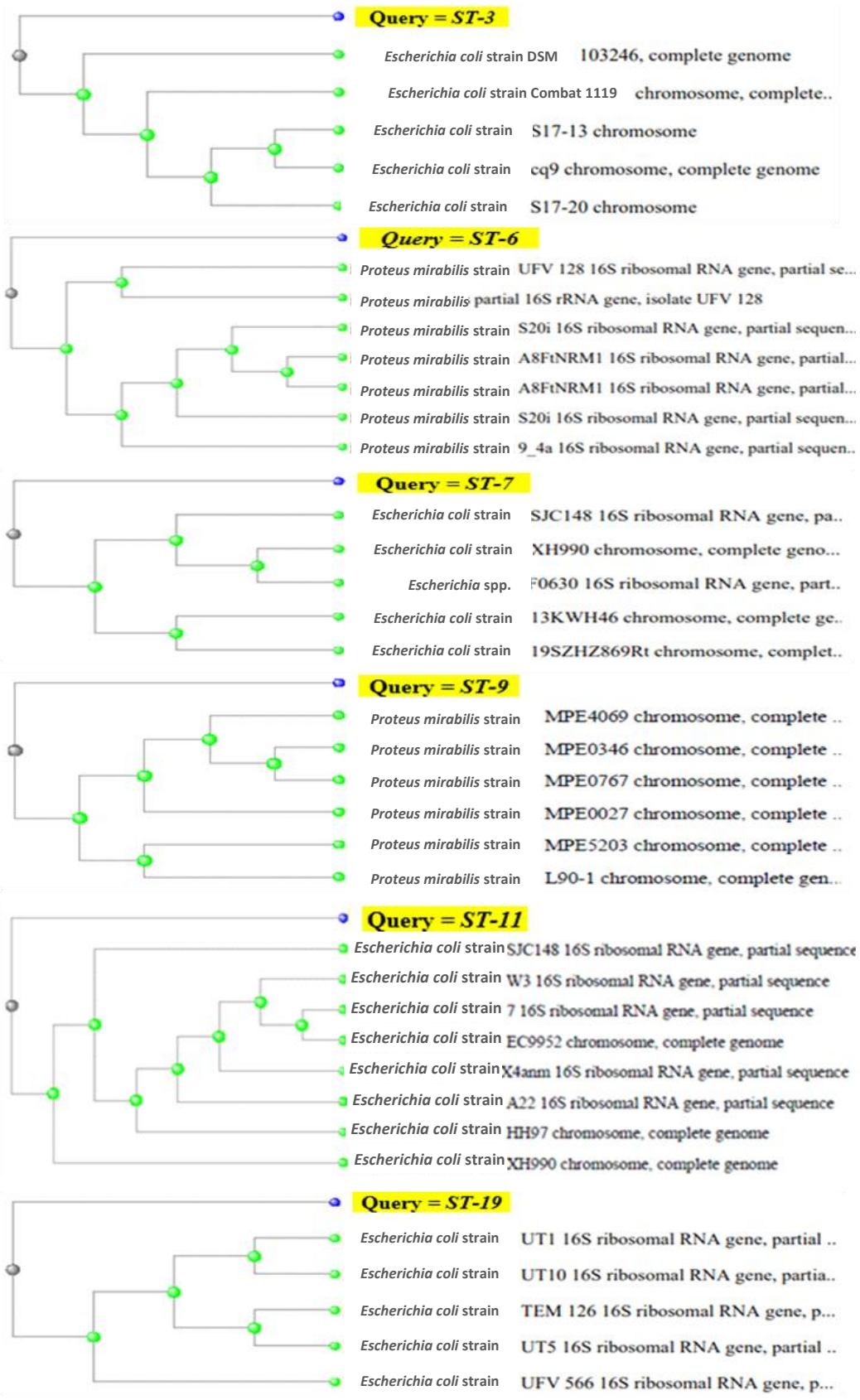

Figure 4. Phylogenetic tree displaying relatedness between the isolate *ST-3, ST7, ST7, ST9, ST11, ST19,* and other strains based on the nucleotide sequence of the 16S rRNA gene.

Table 3. Susceptibility of six isolated bacterial to eight antibiotics.

Antibiotics	***ST-3* (*FBr.1.1*) *E. coli***	***ST-6* (*RBr.1.1*) *Proteus* spp.**	***ST-7* (*RLe.1.1*) *E. coli***	***ST-9* (*RTh.1.1*) *Proteus* spp.**	***ST-11* (*TLe.2.1*) *E. coli***	***ST-19* (*WLe.1.1*) *E. coli***
AMX (10 µg/disc)	R	R	R	R	R	R
AZM (15 µg/disc)	S (24 mm)	R	R	R	S (26 mm)	S (18 mm)
C (30 µg/disc)	S (26 mm)	S (20 mm)	R	R	R (11 mm)	R
E (15 µg/disc)	I (20 mm)	R	R	R	I (20 mm)	R (7 mm)
NA (30 µg/disc)	I (14 mm)	R	I (14 mm)	R	R	R
NX (10 µg/disc)	R (12 mm)	R (8 mm)	S (18 mm)	R	R (10 mm)	R (7 mm)
S (10 µg/disc)	R (8 mm)	R	R	R (7 mm)	I (13 mm)	R
TE (30 µg/disc)	R	R	R	R (8 mm)	R	R

R = Resistant; I = Intermediate; S = Susceptible.

that ST73, ST95, and ST117 isolates are highly virulent and zoonotic, causing several diseases (sepsis, meningitis, and UTI) in poultry and humans (Manges et al., 2015; Zhuge et al., 2019).

The isolate *ST-3* (*FBr.1.1*) showed 96% to *E. coli* strain DSM 103246 (strain E28), which is considered multidrug-resistant. *E. coli* strain DSM 103246 was isolated from a chicken carcass in Germany in 2012 and pre-examined with *E. coli* PanType (Alere Technologies GmbH) and the phenotypic assay Gen III MicroPlate (Biolog), where the virulence markers *astA* (heat-stable enterotoxin one gene), *prfB* (P-related fimbriae regulatory gene), *ireA* (iron-regulated gene), *hemL* (glutamate 1-semialdehyde aminotransferase gene), and the resistance to potassium tellurite and kanamycin potassium tellurite and kanamycin were identified. It was identified as an extended-spectrum β-lactamase-producing strain (blaCTX-M-9) and characterized as serotype H34 (Schmidt et al., 2017).

The isolates *RBr.1.1*, *RTh.1.1*, and *WBr. 3.1 were* identified as *P. mirabilis* with the identity of 96, 99, and 99%, respectively (Table 2). Bacteria of the genus *Proteus* are commonly found in nature, and frequently, they are considered opportunistic pathogens and cause a variety of infections in humans. They belong to the Enterobacteriaceae family. The genus *Proteus* has four species: *P. mirabilis*, *Proteus vulgaris*, *Proteus penneri*, and *Proteus myxofaciens*. Some members of *Proteus* spp. are pathogenic for humans, such as *P. mirabilis,* responsible for 70 to 90% of human infections. *P. mirabilis* is frequently associated with urinary tract infections in young boys and the elderly. The presence of *Proteus* spp. in food indicates improper storage or contamination with fecal material. *Proteus* spp. are sensitive to heat (killed by moist heating at 55°C for 1 h), by common disinfectants such as halogens, ozone, and formaldehyde, and by ultraviolet and γ irradiation (Robinson, 2014).

Three isolates (*FLe.1.1, TaWi.3.1* and *WLe.1.1*) with 95 to 97% identity to the 16S ribosomal RNA gene of *E. coli* strain UT1 and UT22 that were quinolone-resistant and were isolated from human urinary tract infection (Accession No. KP276714.1 and KP276735.1). The isolate *ALi.2.1* showed 96% identity to the 16S ribosomal RNA gene of *E. coli* strain RRL-36, identified as uropathogenic *E. coli* strains responsible for urinary tract infections. While, the isolate *RLi.3.1* showed 97% identity to the genome of *E. coli* strain CFSAN061761, which is a Colistin and Cefotaxime-resistant *E. coli* strain from Egypt (Accession No. CP042903.1), and *TaLi.1.1* revealed 98% identity to the complete sequence of *E. coli* strain CFSAN027350, that was identified as Shiga toxin-producing *E. coli* (Accession No. CP037941.1).

Finally, the isolates *TTh.2.1, Tale.3.1,* and *TaTh.2.1* revealed 99, 98, and 96% identity to the 16S ribosomal RNA gene of *E. coli* strain W3, strain Es_1, and UFV 209, respectively (Accession No. MN086363.1, MK095771.1 and MN557804.1).

Antimicrobial susceptibility test

To evaluate the antibiotic susceptibility of the sequenced isolated bacteria, six sequenced isolates were selected and tested for their antibiotic resistance using Amoxycillin (AMX), Azithromycin (AZM), Chloramphenicol (C), Erythromycin (E), Tetracycline (TE), Nalidixic acid (NA), Norfloxacin (NX), and Streptomycin (S). Results showed that one isolate (*RTh.1.1*) was resistant to all antibiotics, four strains were sensitive to one antibiotic (*RBr.1.1*, *RLe.1.1*, *TLe.2.2* and *WLe.1.1*), while only one isolate (*FBr.1.1*) was susceptible to 2 antibiotics (Table 3 and Figure 5). Nowadays, antimicrobials are extensively used in animal production, mainly in poultry, especially in developing countries where antimicrobials are not only used to treat infections but also prophylactically and as growth promoters (Van Boeckel et al., 2015). Thus, poultry production is a favorable zone for studying antimicrobial resistance (AMR) because of the common usage of antibiotics in this industry. Consequently,

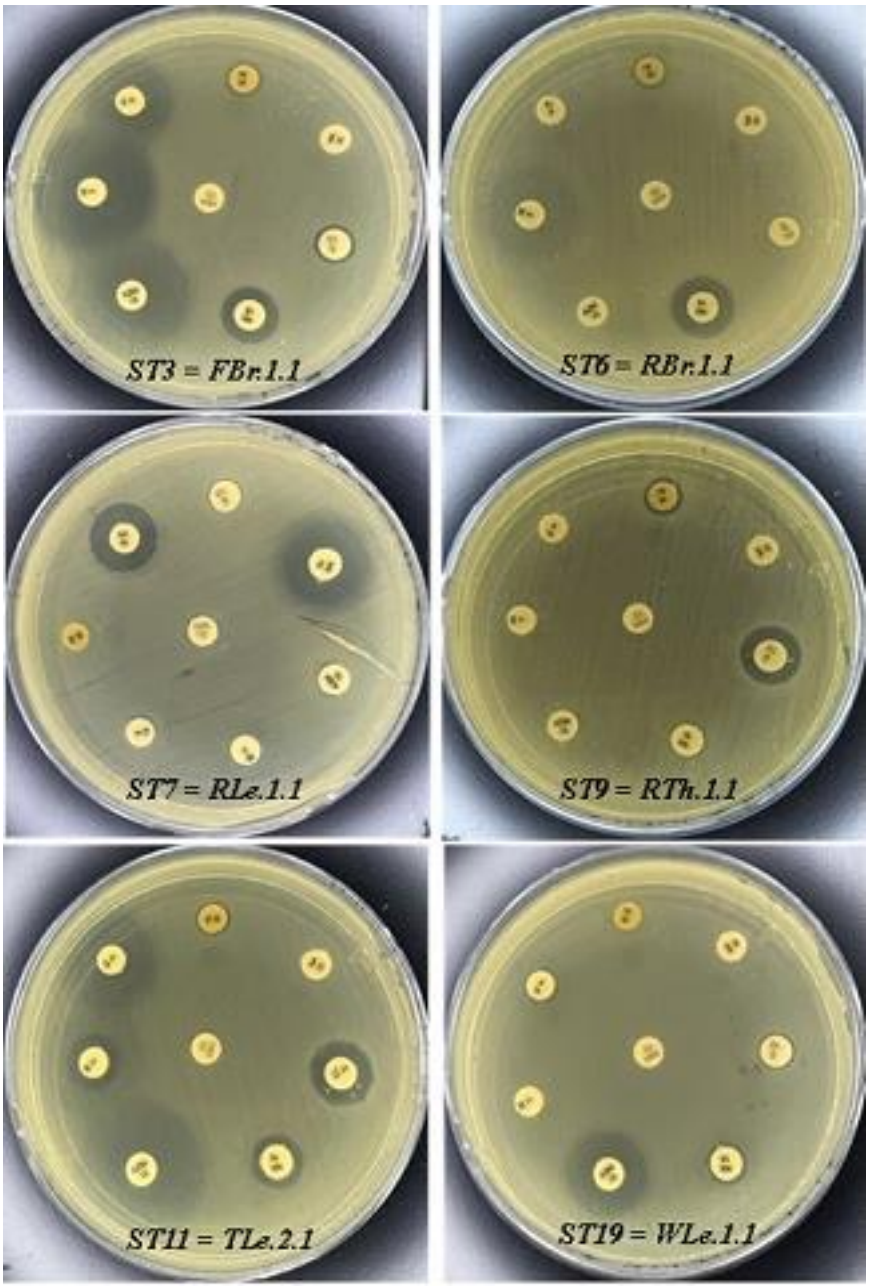

Figure 5. Antibiotic disk diffusion test for six isolates against eight antibiotics. The diameter of inhibition zones was measured, and values were translated to categories of susceptible, intermediate, or resistant using instructions from the manufacturer.

several studies reported a correlation between antimicrobial use and increased antibiotic-resistant bacteria (Vinueza-Burgos et al., 2017). In the present study, six isolates showed multidrug resistance (that is, bacterial isolates were resistant to 3 or more classes of antibiotics) against eight antibiotics used in the antibiotic susceptibility test. The present study showed a high rate of multidrug resistance which might be attributed to the massive use of antimicrobial agents in the poultry industry as a therapy, prophylaxis and growth promoter (Van Boeckel et al., 2015; Vinueza-Burgos et al., 2017).

Conclusion

The present study showed no *Campylobacter* bacteria in the examined retailed chicken meats. In contrast, other non-*Campylobacter* bacteria, such as *E. coli* and *P.*

mirabilis, were detected, which could be hazardous to human health. In addition, most of the isolated bacteria showed multidrug resistance. Thus, cross-contamination during the handling and preparation of chicken meat and meat products and the consumption of undercooked chicken meat products must be avoided. Also, strict hygienic and control measures must be applied during chicken slaughtering, processing, and packaging. Last but not least, traditional enrichment detection methods, are prone to the growth of non-*Campylobacter* bacteria, which are antibiotic-resistant. To circumvent this, faster and more direct *Campylobacter* detection methods (without enrichment) are required.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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