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ORIGINAL ARTICLE

Prevalence, antimicrobial susceptibility, and virulence of *Campylobacter jejuni* isolated from chicken meat

Abdul Samad[1] | Ferhat Abbas[1] | Zafar Ahmed[1] | Ali Akbar[2] | Muhammad Naeem[1] | Muhammad Bilal Sadiq[3] | Imran Ali[4] | Saima[1] | Roomeela[1] | Farah Sabeen Bugti[1] | Safiullah Khan Achakzai[1]

[1]Centre for Advanced studies in Vaccinology and Biotechnology, Faculty of life science, University of Balochistan, Quetta, Pakistan

[2]Department of Microbiology, Faculty of life science, University of Balochistan, Quetta, Pakistan

[3]Department of Biological Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan

[4]Institute of Biochemistry, University of Balochistan, Quetta, Pakistan

Correspondence
Ali Akbar, Department of Microbiology, Faculty of life science, University of Balochistan Quetta 87300, Pakistan.
Email: aliakbar.uob@gmail.com; ali.akbar@um.uob.edu.pk

Abstract

The aim of this study was to determine the prevalence and antimicrobial resistance of *Campylobacter jejuni* isolated from retail chicken meat. The identification of *Campylobacter* isolates and the presence of virulence factor were evaluated by polymerase chain reaction (PCR). Furthermore, clove oil, cinnamon, and turmeric extracts were evaluated for the antimicrobial potential against *Campylobacter* isolates. Out of 200 chicken meat samples, 80 (40%) samples were found contaminated with *Campylobacter jejuni*. Antibacterial susceptibility testing indicated that out of 80 isolates 60 (75%) were resistant to tetracycline followed by 31 (38.75%) to ciprofloxacin, 12 (15%) to ampicillin, 8 (10%) to erythromycin, and 2 (2.5%) were resistant to chloramphenicol. Clove oil and cinnamon extract showed antibacterial potential against *Campylobacter* isolates. Furthermore, all the 80 isolates (100%) were found positive for virulence genes (*cad*F, *fla*A, and *dnaJ*). The presence of antibacterial resistance and virulence factors in *C. jejuni* highlighted the risk associated with retail poultry meat.

Practical applications

Campylobacter jejuni is associated with foodborne illnesses such as gastrointestinal intestinal complications. This study demonstrated that raw chicken meat should be subjected to pretreatment to avoid the foodborne illnesses associated with multidrug-resistant (MDR) *Campylobacter jejuni*. Moreover, the use of antibiotics should be strictly monitored in developing countries to avoid the emergence of multidrug-resistant pathogens.

1 | INTRODUCTION

Foodborne diseases or food poisoning is the gastrointestinal complications which occur due to recent consumption of food items or drinks contaminated with foodborne pathogens or toxins or both. One-third of the population in developing countries is affected by foodborne pathogens annually (Chanseyha, Sadiq, Cho, & Anal, 2018). Even in developed countries, billions are spent on foodborne illnesses caused by major pathogens (Akbar & Anal, 2013; CDC, 2013).

During processing food and personal hygienic conditions can prevent several foodborne diseases (Chanseyha et al., 2018). Several hygiene practices such as poor environmental and personal hygiene, improper cooking and preparation, insufficient storage of drinks and food are known to compromise the safety of food (Sriwattanachai, Sadiq & Anal, 2018).

Campylobacter jejuni is a gram-negative, flagellated thermophile, that can grow at the temperature of 37–42°C (Vandeplas et al., 2008). After invasion of *Campylobacter* in human body, it attacks the ileum (small intestine) and large intestine, which leads to inflammatory diarrhea (Van Vliet & Ketley, 2001). It is usually estimated that 90% of *Campylobacter* infections are due to meat consumption and 80% of this is particularly from poultry meat consumption (ICGFI, 1999).

The invasiveness of *C. jejuni* strains plays a vital role in pathogenesis and is often used as a measure of bacterial virulence, reflecting the involvement of multiple bacterial structures and mechanisms in this process (Van Vliet & Ketley, 2001). Flagella, capsular polysaccharide

(CPS), sialylation of the lipooligosaccharides (LOS) outer core or *Campylobacter* invasive antigens (Cia) are the implicated bacterial factors in host cell invasion (Rivera-Amill, Kim, Seshu, & Konkel, 2001; Watson & Galán, 2008). The presence of flagella in *C. jejuni* is important for the initial interactions in the host and ease the colonization in intestinal epithelial cells (Hendrixson & DiRita, 2004; Konkel, Monteville, Rivera-Amill, & Joens, 2001). Misuse of antibacterial agents in food-producing animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant Campylobacter (Sadiq, Tarning, Aye Cho, & Anal, 2017), which has potentially serious impact on food safety in both human and veterinary health (Van Looveren et al., 2001). The *Campylobacter* isolates from quail carcasses in a commercial processing facility were found resistant to various antibiotics, indicating the potential threat for public health (Cox et al., 2018). In Brazil, the frequent presence of *Campylobacter* spp. in retail poultry meat products suggested the need for the monitoring and regulating authorities to develop and implement better prevention and control strategies (Lopes, Landgraf, & Destro, 2018).

Discoveries of new antimicrobial compounds in natural products, especially of plant origin, have become one of the notable substitutes to treatment since they are rich in variety of secondary metabolites such as tannins, alkaloids, anthraquinones, flavonoids, and phenolic compounds with antimicrobial properties (Sadiq, Hanpithakpong, Tarning, & Anal, 2015; Sadiq, Tharaphan, Chotivanich, Tarning, & Anal, 2017; Sriwattanachai, Sadiq & Anal, 2018). One of the large genus of rhizomatous herbs is turmeric *Curcuma* (Zingiberaceae) which can be found in subtropical and tropical district especially in Thailand, Indonesia, India, the Malay Archipelago, Indochina, Indonesia as well as northern Australia. The rhizomes of *Curcuma* and clove *Syzygiumaromaticum* have been usually used in the form of powder as flavors in native dishes and constituent in many traditional medicines to treat various illnesses (Sylvester, Son, Lew, & Rukayadi, 2015).

A very limited literature is available on the prevalence of *C. jejuni* in retail chicken meat available in developing countries like Pakistan. Most of the recent studies on *Campylobacter* spp. reported the prevalence and identification only, without any solution to control the *Campylobacter* spp. Therefore, the aims of the present study were to determine; the prevalence and antibacterial susceptibility of *C. jejuni* by disc diffusion assay in freshly slaughtered and processed raw chicken meat sold at retail outlets; to identify bacterial isolates and determine the virulence factors by PCR and primers; to evaluate the antibacterial effects of natural preservatives that is, clove oil, cinnamon, and turmeric extracts were evaluated against *Campylobacter jejuni* isolates.

2 | MATERIALS AND METHODS

A total of 200 chicken meat samples (100 chilled/processed +100 freshly slaughtered poultry meat) were randomly collected from the retail markets and butcher shops during the period of March–December 2016 from Quetta, Pakistan. Samples were collected in a sterile container containing ice pads and transported to Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), Bacteriology Lab for analysis.

2.1 | Isolation and identification procedures

Primary isolation and presumptive identification of thermophilic *Campylobacter jejuni*, was performed according to Microbiology of food and animal feeding stuffs—Horizontal method for detection of *Campylobacter* spp. (ISO 10272-1, 2006) with some modifications. In brief, approximately 25 g meat was excised from each collected sample, chopped, and aseptically transferred into sterilized stomacher bag, and inoculated with 225 mL enrichment medium Bolton Broth supplemented with (0.02 g/L cefoperazone, 0.02 g/L vancomycin, 0.02 g/L trimethoprim lactate, and 0.01 g/L amphotericin B) and 5% laked horse blood. The samples were then incubated in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) at 37°C for 4–6 hr, then at 41.5°C for 44 hr (± 4 hr) (Guyard-Nicodème et al., 2015). After completion of incubation, the enriched culture was streaked onto modified charcoal cefoperazone deoxycholate agar (mCCDA) plates supplemented with (0.032 g/L cefoperazone and 0.01 g/L amphotericin B) the plates were then incubated at 41.5°C in a microaerobic atmosphere for 44 hr (± 4 hr). Suspected colonies were selected from each mCCDA plates and further streaked onto blood agar no2 plates for confirmation. The plates were incubated in a microaerobic atmosphere at 41.5°C for 24–48 hr. Presumptive identification was performed through Gram reaction, microscopic observation, catalase test, oxidase test, Hippurate hydrolysis test and hydrolysis identification of indoxyl acetate. The presumptively identified isolates were further confirmed by polymerase chain reaction (PCR, Bio-Rad, Hercules, CA). Antibacterial susceptibility and resistance patterns of *Campylobacter* isolates were determined using the disc diffusion assay, following the guidelines of NCCLS (2002a, 2002b) and Huysmans and Turnidge (1997).

2.2 | DNA extraction

The DNA extraction was performed through phenol–chloroform method as described earlier by Fadl, Nguyen, and Khan (1995), with some modifications. Briefly, 3–4 colonies were added in 1 mL TE buffer in Eppendorf tubes and vortex for 1 min followed by centrifugation at 2,000 × *g* for 4 min. Supernatant was discarded and pellets were resuspended in 474 μL of TE (10 mM Tris–HCL pH 8, 1 mM Na_2EDTA), 25 μL 10% SDS and 1.25 μL proteinase K (20 mg/mL). After incubation at 55°C for 30 min, 500 μL of phenol–chloroform pH 8 (1:1) was added, mixed vigorously and the samples were centrifuged (10,000 × *g*, 4 min). The aqueous phase was transferred to another Eppendorf tube and the DNA was precipitated with 3 M sodium acetate and ice-cold isopropanol for 30 min. The samples were centrifuged at 16,000 × *g* for 10 min and the pellets were washed with 80% ethanol. Finally, the pellets were resuspended in 50 μL TE Buffer. Quantification of extracted DNA was performed on Shimadzu UV/VIS spectrophotometer and stored at 4°C until PCR was performed.

2.3 | Detection of virulence factor

For detection of virulence factor and antibiotic-resistant genes in *Campylobacter jejuni*, specific primers to amplify *mapA*, *cadF*, *flaA*, *virB11*, *racR*, *dnaJ*, *ciaB*, *pldA*, *iamA*, *tetO*, and *gyrA* genes as described earlier (Carvalho et al., 2001; Datta, Niwa, & Itoh, 2003; Denis et al.,

1999; Gibreel et al., 2004; Konkel, Kim, Rivera-Amill, & Garvis, 1999; Oyofo, Thornton, Burr, Pavlovskis, & Guerry, 1992; Zirnstein, Li, Swaminathan, & Angulo, 1999) were synthesized from Macrogen Inc. (Korea) and used for PCR amplification.

For PCR amplification, initial denaturation was set at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing temperature was set for each primer as mentioned in Table 1 followed by extension at 72°C for 1 min and a final extension at 72°C for 5 min. The amplified DNA products were analysed by electrophoresis on 2% (w/v) agarose gel stained with ethidium bromide and visualized by UV illumination.

2.4 | Turmeric and cinnamon extract preparation

Turmeric and cinnamon extracts were obtained as described earlier by Funk, Frye, Oyarzo, Zhang, and Timmermann (2009). Moreover, analytical grade clove oil was purchased from local market.

2.5 | Antibacterial activity of extracts

The antibacterial activity of extracted turmeric, cinnamon, and clove oil was carried by disc diffusion method (Sadiq et al., 2015). About 8 mm diameter discs were impregnated with 10 μg of each turmeric and cinnamon extracts, and 10 μL of clove oil. *C. jenuni* inoculum (0.5 McFarland standard) was spread evenly on the Muller Hinton Agar (MHA) plates with the help of sterilized swabs and discs were placed on the agar surface with the help of sterilized forceps. Plates were incubated at 37°C for 48 hr in microaerophilic condition.

2.6 | Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey test to determine significant group differences ($p < 0.05$) between samples using SPSS statistical software package (SPSS, version 23.0, Chicago, IL).

3 | RESULTS

3.1 | Prevalence and antimicrobial susceptibility of *Campylobacter jejuni*

Out of 200 chicken meat samples, 80 samples (40%) were found contaminated with *Campylobacter jejuni*. Significantly high ($p < 0.05$) contamination rate 48% was observed in freshly slaughtered chicken meat followed by 32% in processed chicken meat (Table 2).

Antimicrobial susceptibility test of 80 *Campylobacter jejuni* isolates revealed that resistance to tetracyclines was significantly higher ($p < 0.05$) compared to other antibiotics. Out of 80 isolates, 60 (75%) isolates were found resistant to tetracycline followed by 31 (38.75%) to ciprofloxacin, 12 (15%) to ampicillin, 8 (10%) to erythromycin, and 2 (2.5%) isolates were found resistant to chloramphenicol (Table 3). Ciprofloxacin and tetracycline resistance genes in isolates were also confirmed by PCR by using *tet*O and *gyr*A specific primers (Figure 1).

Out of 80 isolates tested 42 (52.5%) of the isolates were resistant to either one out of five antibiotics tested, 30 (37.5%) isolates were found resistant to two antibiotics, 4 (5%) isolates were tested found resistant to three or more of three antibiotics testes, and only 4 (5%) were found sensitive to all the antibiotics tested.

3.2 | Detection of virulence factor

All the isolates were tested for their virulence genes through PCR amplification by use of specific primers, out of 80 isolates tested all (100%) were found positive for *cad*F, *fla*A, and *dna*J genes whereas, 72 (90%) for *rac*R, 61 (76.25%) for *cia*B, 43 (53.75%) for *iam*A,

TABLE 1 Primers used in this study with amplicon size and annealing temperature

Target gene	Primers 5′–3′	Amplicon size (bp)	Annealing temperature (°C)	References
*map*A	F-CTATTTTATTTTTGAGTGCTTGTG R-GCTTTATTTGCCATTTGTTTTATTA	589	54	Denis et al., 1999
*cad*F	F-TTGAAGGTAATTTAGATATG R-CTAATACCTAAAGTTGAAAC	400	47	Konkel et al., 1999
*fla*A	F-ATG GGA TTT CGT ATT TG R-GTC AAA CGG GTA GCA ATA CC	450	46.5	Oyofo et al., 1992
*vir*B11	F-TCTTGTGAGTTGCCTTACCCCTTT T R-CCTGCGTGTCCTGTGTTATTTACC C	494	53	Datta et al., 2003
*rac*R	F-GATGATCCTGACTTTG R-TCTCCTATTTTTACCC	584	45	Datta et al., 2003
*dna*J	F-AAGGCTTTGGCTCATC R-CTTTTTGTTCATCGTT	720	46	Datta et al., 2003
*cia*B	F-TTTCCAAATTTAGATGATGC R-GTTCTTTAAATTTTTCATAATGC	1,165	50	Konkel et al., 1999
*pld*A	F-AAGCTTATGCGTTTT R-TATAAGGCTTTCTCCA	913	45	Datta et al., 2003
*iam*A	F-GCGCAAAATATTATCACCC R-TTCACGACTACTATGCGG	518	52	Carvalho et al., 2001
*tet*O	F:AACTTAGGCATTCTGGCTCAC R:TCCCACTGTTCCATATCGTCA	515	56	Gibreel et al., 2004
*gyr*A	F-TTTTTAGCAAAGATTCTGAT R-CAAAGCATCATAAACTGCAA	265	50	Zirnstein et al., 1999

TABLE 2 Prevalence of *Campylobacter jejuni* in chicken meat

Type of samples	No. of samples	*C. jejuni* positive samples	Percentage %
Freshly slaughtered	100	48	48
Processed/chilled	100	32	32
Total	200	80	40

21 (26.25%) for *pld*A, and 9 (11.25%) for *vir*B11 genes were found positive (Figures 2–4 and Table 4).

3.3 | Antibacterial activity of extracts

Antibacterial activity of turmeric (10 μg/disc) and cinnamon (10 μg/disc) extract and clove oil (10 μL/disc) were tested by disc diffusion method against all the isolates of *Campylobacter jejuni*. Turmeric extract did not show any zone of inhibition (ZI) against any of the isolate tested. Out of 80 isolates 34 (42.5%) were found resistant to cinnamon extract (ZI = 0), ZI < 15 mm was observed against 18 (22.55%) isolates and ZI >15 mm was found against 28 (35%) isolates. Out of 80 isolates 11 (13.75%) were found resistant to clove oil, ZI < 15 mm was found against 33 (41.25%) of the isolates and ZI >15 mm ZI was observed against 36 (45%) of the isolates tested (Table 5).

TABLE 3 Antibiotic susceptibility of the *Campylobacter jejuni* isolates to the selected antibiotics through disc diffusion method

	Number of *Campylobacter jejuni* isolates			
Antimicrobial agents	Sensitive (S)	Intermediate (I)	Resistant (R)	% of resistant isolates
*Tetracycline (30 μg/disc)	12	8	60	75%
R: ≤ 14 mm				
I: 15–18 mm				
S: ≤ 19 mm				
*Ciprofloxacin (5 μg/disc)	20	29	31	38. 75%
R: ≤ 15 mm				
I: 16–22 mm				
S: ≤ 21 mm				
*Ampicillin (25 μg/disc)	51	17	12	15%
R: ≤ 13 mm				
I: 14–16 mm				
S: ≤ 17 mm				
*Erythromycin (15 μg/disc)	56	16	8	10%
R: ≤ 13 mm				
I:14–22 mm				
S: ≤ 23 mm				
*Chloramphenicol (30 μg/disc)	37	41	2	2.5%
R: ≤ 11 mm				
I: 12–22 mm				
S: ≤ 23 mm				

*Interpretations adopted in this study for the breakpoints of disc diffusion criteria were suggested by NCCLS (2002a, 2002b) and Huysmans and Turnidge (1997).

4 | DISCUSSION

4.1 | Prevalence and antimicrobial susceptibility of *Campylobacter jejuni*

The contamination rate of *Campylobacter jejuni* was found 40% in both freshly slaughtered and processed/packaged chicken meat samples. Guyard-Nicodème et al., 2015 reported 64.5% prevalence of *C. jejuni* in chicken meat. Some research studies reported lower prevalence of *C. jejuni* in chicken meat than the current study such as; 19.56% (de Moura et al., 2013), 13.7% (Ma et al., 2017), and 13.3% (Hara-Kudo et al., 2013).

In this study, 60 (75%) *C. jejuni* isolates were found resistant to tetracycline, the results corroborate with those reported by Nguyen et al. (2016), whereas, Ma et al., 2017 reported high rate (77.4%) of tetracycline resistance in *C. jejuni* isolates. *C. jejuni* isolates (38.75%) were found resistant to ciprofloxacin, this result was in agreement with previous reports (Gblossi Bernadette et al., 2012).

All the isolates of *C. jejuni* were found sensitive to chloramphenicol and this result was in accordance with Guyard-Nicodème et al. (2015), whereas, Ma et al., 2017 reported 19.4% resistance of *C. jejuni* to ciprofloxacin.

Multidrug resistance of *Campylobacter* isolates was reported earlier by Usha et al. (2010), in commercially produced poultry and retail broiler chicken. Cox et al. (2018) reported that all the *Campylobacter* isolates from quail carcasses were found resistant to tetracyclines. Lopes et al. (2018 reported that *Campylobacter* isolates from retail poultry products were mainly resistant to nalidixic acid (77.8%), ciprofloxacin (72.2%), tetracycline (16.7%), and streptomycin (16.7%). The increased incidence of multidrug-resistant *Campylobacter* in meat products indicated the dire need for the development of regulations, policies, and preventive measure to control the MDR *Campylobacter* and ensure the public health and safety. The developing counties like Pakistan are lacking in policies and monitoring programs to control the MDR *Campylobacter*, therefore, this study can be used as a reference for regulatory authorities to identify the potential threats associated with *Campylobacter* spp. and seek possible solutions.

4.2 | Virulence factors

The *Campylobacter* isolates were subjected to detection of putative virulent genes. Virulence genes involved in adherence and colonization (*Fla*A, *cad*F, *rac*R, and *dna*J) and invasion (*cia*B, *iam*A, and *pld*A) were observed frequently in all the *Campylobacter* isolates whereas, plasmid-borne invasion associated gene (*vir*B11) was not detected in any of the isolates.

All the isolates tested were found positive for *fla*A, *dna*J, and *cad*F genes. *fla*A gene plays an important role in *Campylobacter* pathogenesis and *cad*F gene is an important virulence factor-encoding *Campylobacter* adhesion to fibronectin. The prevalence of *fla*A, *dna*J, and *cad*F genes in all the isolates indicated the pathogenic potential of *Campylobacter* (Frazao *et al*., 2017). The *cad*F gene involvement in *Campylobacter* colonization was clearly shown by Ziprin *et al*. (2001) in a chicken model.

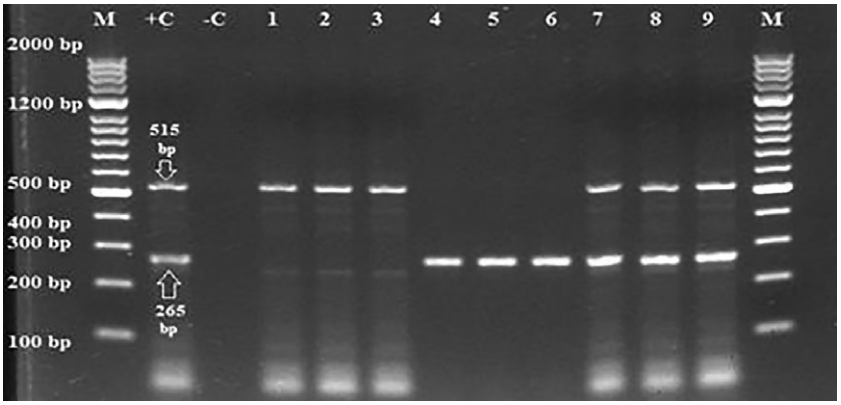

FIGURE 1 Agarose gel (2%) electrophoresis shows amplification fragments of gyrA (265 bp) and tetO (515 bp) in *Campylobacter jejuni* isolates. Lanes 1, 2, and 3 positive amplifications of *tet*O. Lanes 4, 5, and 6 positive amplifications of *gyr*A. Lanes 7, 8, and 9 positive amplifications of both *tet*O and *gyr*A genes in *Campylobacter jejuni* isolates. +C: Positive control, −C: Negative control, M: Molecular markers

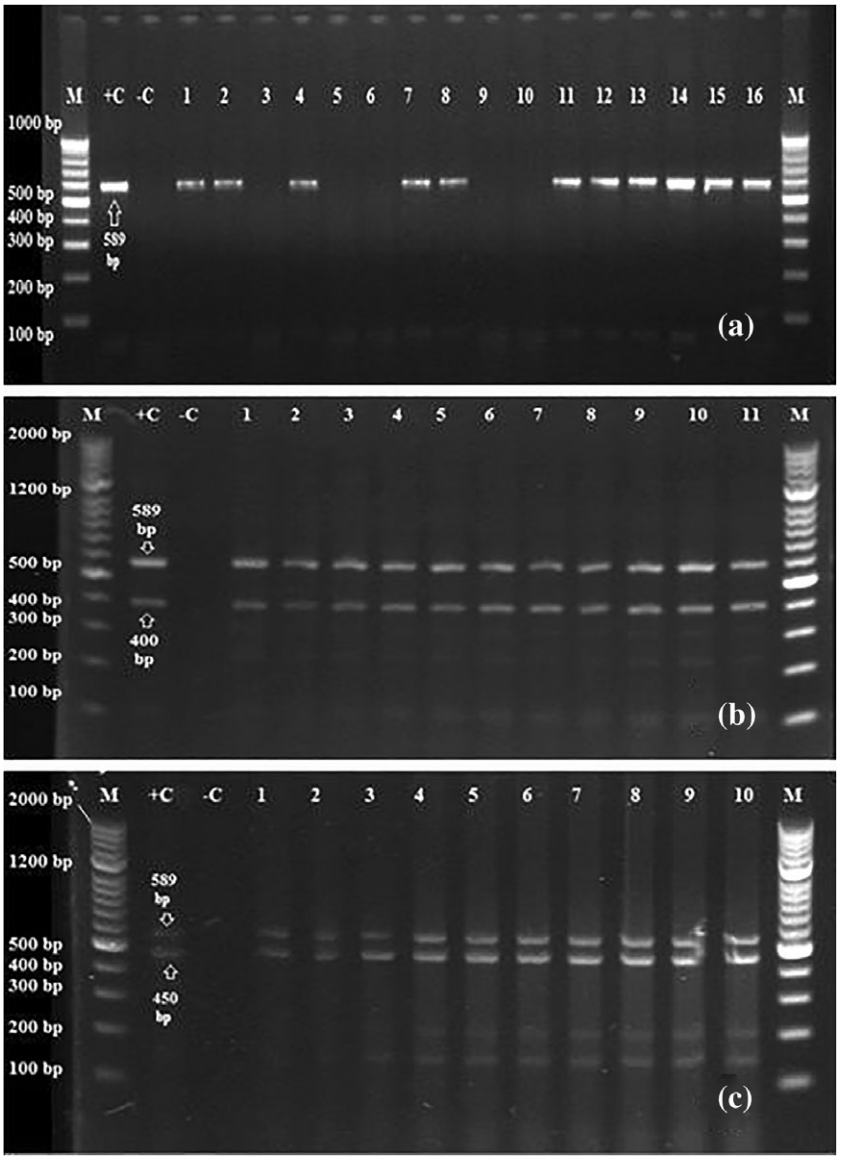

FIGURE 2 Agarose gel (2%) electrophoresis shows (a) amplification fragments of *Campylobacter jejuni*. Lanes 1, 2, 4, 7, 8, 11, 12, 13, 14, 15, and 16 positive amplifications of *Campylobacter jejuni* (*mapA* 589 bp), +C: Positive control, −C: Negative control, M: Molecular markers. (b) Positive amplification of *cadF* gene (400 bp) and mapA (589 bp) in *Campylobacter jejuni* isolates. (c) Positive amplification of *flaA* genes (450 bp) and *mapA* (589 bp) in *Campylobacter jejuni* isolates. +C: Positive control, −C: Negative control, M: Molecular markers

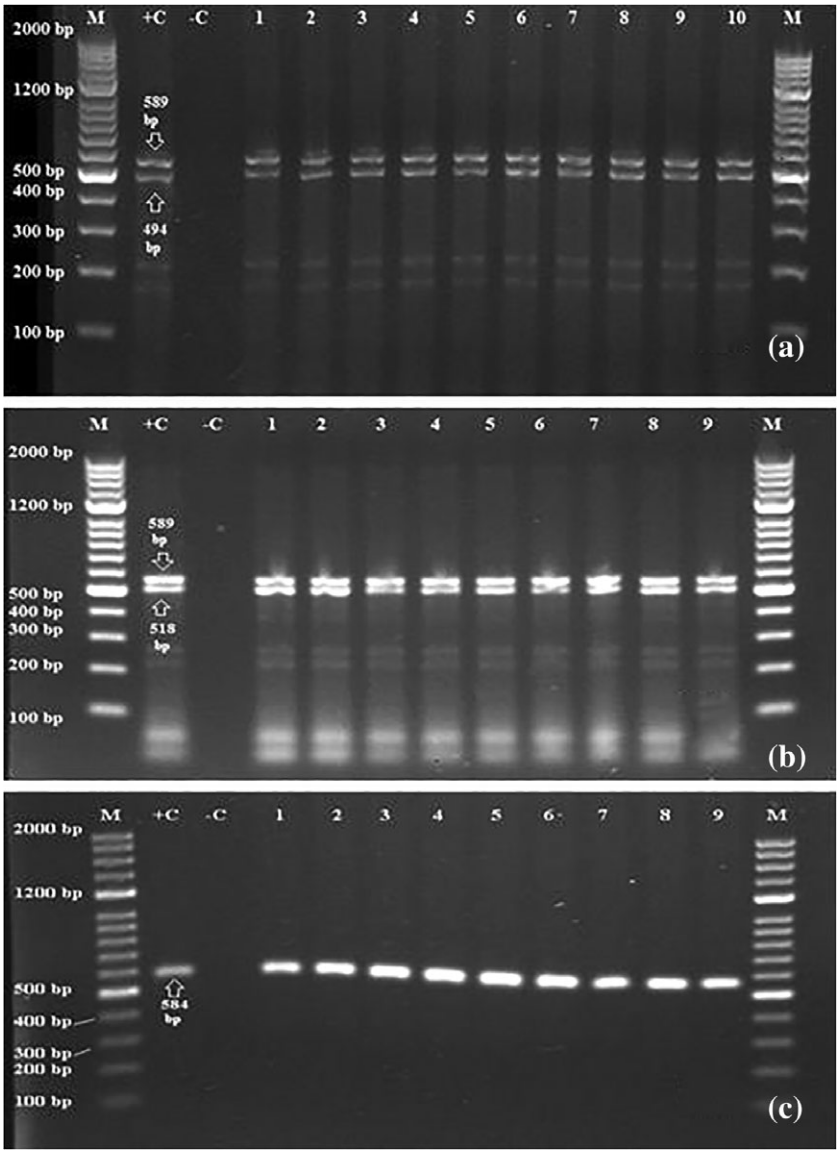

FIGURE 3 Agarose gel (2%) electrophoresis shows (a) positive amplification of *virB11* gene (494 bp) and *mapA* (589 bp) in *Campylobacter jejuni* isolates. (b) Positive amplification of *iamA* gene (518 bp) and *mapA* genes (589 bp) in *Campylobacter jejuni* isolates. (c) Positive amplification of *racR* genes (450 bp) in *Campylobacter jejuni* isolates. +C: Positive control, −C: Negative control, M: Molecular markers

The *rac*R gene is responsible for colonization in the chicken intestinal tract and it is involved in the temperature-dependent signaling pathway (Brás, Chatterjee, Wren, Newell, & Ketley, 1999). In this study, 90% of the isolates tested were found positive for *rac*R gene.

C. jejuni secretes a protein which is essential for the invasion of cultured epithelial cells, called *Cia*B (Konkel et al., 1999). *Campylobacter* isolates (76.5%) were found positive for *cia*B gene, whereas, Frazão, Medeiros, da Silva Duque, & Falcão, 2017 reported all *Campylobacter* isolates positive for *cia*B gene.

*pld*A gene encoding for outer membrane phospholipase A and involved in host cell invasion was investigated and found positive for 26.25% of the *Campylobacter* isolates. Previous studies reported a high prevalence of *pld*A gene in *Campylobacter* isolates such as 100% for *pld*A gene was reported by Frazão et al., 2017. Relatively low prevalence (11.25%) of *vir*B11 gene was found in *C. jejuni* which was in accordance with previous reports (González-Hein, Huaracán, García, & Figueroa, 2013; Wieczorek, Szewczyk, & Osek, 2012).

4.3 | Antibacterial activity of extracts

To overcome the emergence of MDR bacteria, it is essential to ensure the limited use of synthetic preservatives and antibiotics in animal farming. Therefore, the use of natural preservatives can be an effective alternative approach to overcome the emergence of MDR bacteria (Fernández, Agüero, & Jagus, 2018). Plant-derived natural antimicrobials have been used since ages for the preservation of foods (Sadiq et al., 2015). In this study, the antibacterial activity of turmeric, cinnamon extracts, and clove oil was investigated against *Campylobacter jejuni* isolates.

Murali, Kumar-Phillips, Rath, Marcy, and Slavik (2012) evaluated different medicinal plants including lemon, green tea, and turmeric

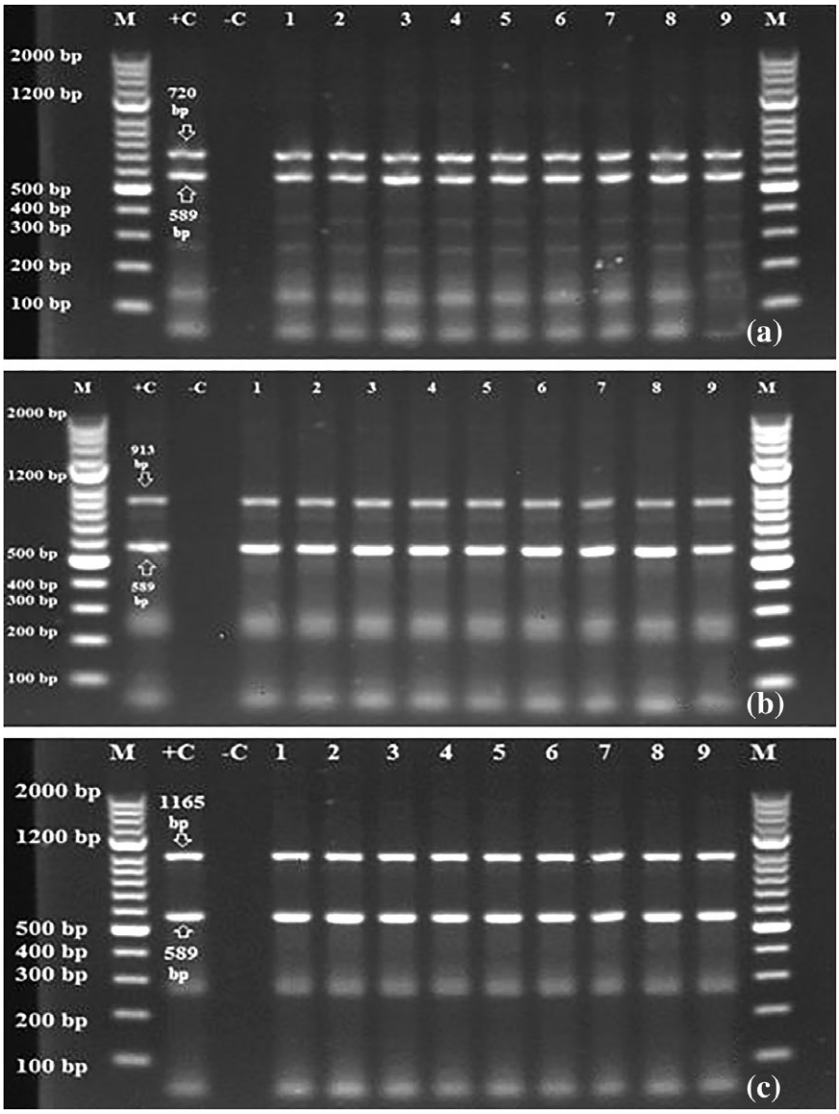

FIGURE 4 Agarose gel (2%) electrophoresis shows (a) positive amplification of *dnaJ* gene (720 bp) and *mapA* (589 bp) in *Campylobacter jejuni isolates*. (b) Positive amplification of *pldA* gene (913 bp) and *mapA* (589 bp) in *Campylobacter jejuni* isolates. (c) Positive amplification of *ciaB* genes (1,165 bp) and *mapA* genes (589 bp) in *Campylobacter jejuni* isolates. +C: Positive control, −C: Negative control, M: Molecular markers

TABLE 4 Detection of virulence genes in *Campylobacter jejuni* isolates

	Genes detection n (%)								
Total isolates	*mapA*	*cadF*	*flaA*	*dnaJ*	*racR*	*ciaB*	*iamA*	*pldA*	*virB11*
80[a]	80[a] (100%)	80[a] (100%)	80[a] (100%)	80[a] (100%)	72[b] (90%)	61[c] (76.25%)	43[d] (53.75%)	21[e] (26.25%)	9[f] (11.25%)

Different superscript letters (a–f) indicate the significant ($p < 0.05$) differences between observations.

against foodborne pathogens and found that lemon and green tea extracts were more effective against *Campylobacter* and other foodborne pathogens, but turmeric extract alone had no effect against these pathogens. Sheeladevi (2012) tested the antibacterial activity of clove oil against foodborne pathogens and reported 23.53 mm zone of inhibition against *C. jejuni*.

TABLE 5 Antibacterial activity of turmeric and cinnamon extract and clove oil against C.jejuni isolates

	Zone of inhibition			
Name of spices	00 mm	<15 mm	>15 mm	Total isolates
Turmeric extract	80 (100%)	00 (00%)	00 (00%)	80
Cinnamon extract	34 (42.5%)	18 (22.55%)	28 (35%)	80
Clove oil	11 (13.75%)	33 (41.25%)	36 (45%)	80

5 | CONCLUSION

High prevalence of *Campylobacter* was found in chicken mat samples collected from the local market of Quetta, Pakistan. Many bacterial isolates were found resistant to various commercially available antibiotics. All the bacterial isolates carried *cad*F, *fla*A, and *dna*J genes responsible for the virulence of *Campylobacter*. The presence of virulence genes can be a potential threat for transmission of resistance. Thus, food safety regulations, strict control system implementation, and limitation in the application of antibiotics in animal farming, agriculture, and human are required in urgent.

ORCID

Ali Akbar https://orcid.org/0000-0002-0376-1425

REFERENCES

Akbar, A., & Anal, A. K. (2013). Prevalence and antibiogram study of salmonella and *Staphylococcus aureus* in poultry meat. *Asian Pacific Journal of Tropical Biomedicine*, *3*(2), 163–168.

Brás, A. M., Chatterjee, S., Wren, B. W., Newell, D. G., & Ketley, J. M. (1999). A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization. *Journal of Bacteriology*, *181*(10), 3298–3302.

Carvalho, A. C., Ruiz-Palacios, G. M., Ramos-Cervantes, P., Cervantes, L. E., Jiang, X., & Pickering, L. K. (2001). Molecular characterization of invasive and noninvasive *Campylobacter jejuni* and *Campylobacter coli* isolates. *Journal of Clinical Microbiology*, *39*(4), 1353–1359.

Centre for Disease Control and Prevention (CDC). (2013). Incidence and trends of infection with pathogens transmitted commonly through food – Foodborne diseases active surveillance network, 10 U.S. sites, 1996–2012. *MMWR. Morbidity and Mortality Weekly Report*, *62*, 283–287.

Chanseyha, C., Sadiq, M. B., Cho, T. Z. A., & Anal, A. K. (2018). Prevalence and analysis of antibiotic resistant genes in Escherichia coli and salmonella isolates from green leaf lettuce. *Chiang Mai Journal of Science*, *45*, 1–13.

Cox, N. A., Cosby, D. E., Thippareddi, H., Ritz, C. W., Berrang, M. E., Jackson, J. S., … Ukidwe, M. S. (2018). Incidence, species and antimicrobial resistance of naturally occurring Campylobacter isolates from quail carcasses sampled in a commercial processing facility. *Journal of Food Safety*, *38*(2), e12438.

Datta, S., Niwa, H., & Itoh, K. (2003). Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *Journal of Medical Microbiology*, *52*(4), 345–348.

de Moura, H. M., Silva, P. R., Silva, P. H. C. D., Souza, N. R., Racanicci, A. M. C., & Santana, Â. P. (2013). Antimicrobial resistance of *Campylobacter jejuni* isolated from chicken carcasses in the Federal District, Brazil. *Journal of Food Protection*, *76*(4), 691–693.

Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G., & Colin, P. (1999). Development of am-PCR assay for simultaneous identification of *Campylobacter jejuni* and C. coli. *Letters in Applied Microbiology*, *29*(6), 406–410.

Fadl, A. A., Nguyen, A. V., & Khan, M. I. (1995). Analysis of *Salmonella enteritidis* isolates by arbitrarily primed PCR. *Journal of Clinical Microbiology*, *33*(4), 987–989.

Fernández, M. V., Agüero, M. V., & Jagus, R. J. (2018). Green tea extract: A natural antimicrobial with great potential for controlling native microbiota, *Listeria innocua* and *Escherichia coli* in fresh-cut beet leaves. *Journal of Food Safety*, *38*(1), e12374.

Frazão, M. R., Medeiros, M. I. C., da Silva Duque, S., & Falcão, J. P. (2017). Pathogenic potential and genotypic diversity of *Campylobacter jejuni*: A neglected food-borne pathogen in Brazil. *Journal of Medical Microbiology*, *66*(3), 350–359.

Funk, J. L., Frye, J. B., Oyarzo, J. N., Zhang, H., & Timmermann, B. N. (2009). Anti-arthritic effects and toxicity of the essential oils of turmeric (*Curcuma longa* L.). *Journal of Agricultural and Food Chemistry*, *58*(2), 842–849.

Gblossi Bernadette, G., Eric Essoh, A., Solange, E., Natalie, G., Souleymane, B., Lamine Sébastien, N., & Mireille, D. (2012). Prevalence and antimicrobial resistance of thermophilic campylobacter isolated from chicken in Côte D'ivoire. *International Journal of Microbiology*, *2012*, 1–5.

Gibreel, A., Tracz, D. M., Nonaka, L., Ngo, T. M., Connell, S. R., & Taylor, D. E. (2004). Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. *Antimicrobial Agents and Chemotherapy*, *48*, 3442–3450. https://doi.org/10.1128/AAC.48.9.3442-3450.2004

González-Hein, G., Huaracán, B., García, P., & Figueroa, G. (2013). Prevalence of virulence genes in strains of *Campylobacter jejuni* isolated from human, bovine and broiler. *Brazilian Journal of Microbiology*, *44*(4), 1223–1229.

Guyard-Nicodème, M., Rivoal, K., Houard, E., Rose, V., Quesne, S., Mourand, G., & Chemaly, M. (2015). Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. *International Journal of Food Microbiology*, *203*, 8–14.

Hara-Kudo, Y., Konuma, H., Kamata, Y., Miyahara, M., Takatori, K., Onoue, Y., & Ohnishi, T. (2013). Prevalence of the main food-borne pathogens in retail food under the national food surveillance system in Japan. *Food Additives & Contaminants: Part A*, *30*(8), 1450–1458.

Hendrixson, D. R., & DiRita, V. J. (2004). Identification of *Campylobacter jejuni* genes involved in commensal colonization of the chick gastrointestinal tract. *Molecular Microbiology*, *52*(2), 471–484.

Huysmans, M. B., & Turnidge, J. D. (1997). Disc susceptibility testing for thermophilic campylobacters. *Pathology*, *29*, 209–216.

ICGFI – International Consultative Group on Food Irradiation, 1999. *Safety of poultry meat: from farm to table.* Retrieved from http://www-naweb.iaea.org/nafa/fep/public/poultrymeat.pdf.

ISO 10272-1. (2006). *Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of Campylobacter spp. Part 1: Detection method*. Geneva, Switzerland: ISO.

Konkel, M. E., Kim, B. J., Rivera-Amill, V., & Garvis, S. G. (1999). Bacterial secreted proteins are required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Molecular Microbiology*, *32*(4), 691–701.

Konkel, M. E., Monteville, M. R., Rivera-Amill, V., & Joens, L. A. (2001). The pathogenesis of *Campylobacter jejuni*-mediated enteritis. *Current Issues in Intestinal Microbiology*, *2*(2), 55–71.

Lopes, G. V., Landgraf, M., & Destro, M. T. (2018). Occurrence of Campylobacter in raw chicken and beef from retail outlets in São Paulo, Brazil. *Journal of Food Safety*, *38*, e12442.

Ma, H., Su, Y., Ma, L., Ma, L., Li, P., Du, X., … Lu, X. (2017). Prevalence and characterization of *Campylobacter jejuni* isolated from retail chicken in Tianjin, China. *Journal of Food Protection*, *80*(6), 1032–1040.

Murali, N., Kumar-Phillips, G. S., Rath, N. C., Marcy, J., & Slavik, M. F. (2012). Effect of marinating chicken meat with lemon, green tea and turmeric against foodborne bacterial pathogens. *International Journal of Poultry Science*, *11*(5), 326.

National Committee for Clinical Laboratory Standards. (2002a). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard M31–A2* (p. 23). Wayne, PA: National Committee for Clinical Laboratory Standards.

National Committee for Clinical Laboratory Standards. (2002b). *Performance standards for antimicrobial susceptibility testing, 12th informational supplement M100–S12*. Wayne, PA: National Committee for Clinical Laboratory Standards.

Nguyen, T. N. M., Hotzel, H., El-Adawy, H., Tran, H. T., Le, M. T. H., Tomaso, H., & Hafez, H. M. (2016). Genotyping and antibiotic resistance of thermophilic *Campylobacter* isolated from chicken and pig meat in Vietnam. *Gut Pathogens*, *8*(1), 19.

Oyofo, B. A., Thornton, S. A., Burr, D. H., Pavlovskis, O. R., & Guerry, P. (1992). Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. *Journal of Clinical Microbiology*, *30*(10), 2613–2619.

Rivera-Amill, V., Kim, B. J., Seshu, J., & Konkel, M. E. (2001). Secretion of the virulence-associated Campylobacter invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. *The Journal of Infectious Diseases*, *183*(11), 1607–1616.

Sadiq, M. B., Hanpithakpong, W., Tarning, J., & Anal, A. K. (2015). Screening of phytochemicals and in vitro evaluation of antibacterial and antioxidant activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. *Industrial Crops and Products*, *77*, 873–882.

Sadiq, M. B., Tarning, J., Aye Cho, T. Z., & Anal, A. K. (2017). Antibacterial activities and possible modes of action of *Acacia nilotica* (L.) Del. Against multidrug-resistant *Escherichia coli* and *Salmonella*. *Molecules*, *22*(1), 47.

Sadiq, M. B., Tharaphan, P., Chotivanich, K., Tarning, J., & Anal, A. K. (2017). In vitro antioxidant and antimalarial activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. *BMC Complementary and Alternative Medicine*, *17*(1), 372.

Sheeladevi, A. (2012). Antibacterial activity of plant essential oils against food borne bacteria. *International Journal of Pharmaceutical & Biological Archive*, *3*(5), 1106–1109.

Sriwattanachai, S., Sadiq, M. B., & Anal, A. K. (2018). Synergistic antifungal effects of thyme essential oil and Lactobacillus plantarum cell-free supernatant against Penicillium spp. and in situ effects. *Journal of Food Processing and Preservation*, *42*(1), e13400.

Sylvester, W. S., Son, R., Lew, K. F., & Rukayadi, Y. (2015). Antibacterial activity of Java turmeric (*Curcuma xanthorrhiza* Roxb.) extract against *Klebsiella pneumoniae* isolated from several vegetables. *International Food Research Journal*, *22*(5), 1770–1776.

Usha, M. R., Fauziah, M., Tunung, R., Chai, L. C., Cheah, Y. K., Farinazleen, M. G., & Son, R. (2010). Occurrence and antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* in retail broiler chicken. *International Food Research Journal*, *17*(2), 247–255.

Van Looveren, M., Daube, G., De Zutter, L., Dumont, J. M., Lammens, C., Wijdooghe, M., & Goossens, H. (2001). Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium. *Journal of Antimicrobial Chemotherapy*, *48*(2), 235–240.

Van Vliet, A. H. M., & Ketley, J. M. (2001). Pathogenesis of enteric Campylobacter infection. *Journal of Applied Microbiology*, *90*(S6), 45–56.

Vandeplas, S., Marcq, C., Dubois Dauphin, R., Beckers, Y., Thonart, P., & Théwis, A. (2008). Contamination of poultry flocks by the human pathogen *Campylobacter spp.* and strategies to reduce its prevalence at the farm level. *Biotechnologie, Agronomie, Société et Environnement*, *12*(3), 317–334.

Watson, R. O., & Galán, J. E. (2008). *Campylobacter jejuni* survives within epithelial cells by avoiding delivery to lysosomes. *PLoS Pathogens*, *4*(1), e14.

Wieczorek, K., Szewczyk, R., & Osek, J. (2012). Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. *Veterinární Medicína*, *57*(6), 293–299.

Zirnstein, G., Li, Y., Swaminathan, B., & Angulo, F. (1999). Ciprofloxacin resistance in *Campylobacter jejuni* isolates: Detection of gyrA resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *Journal of Clinical Microbiology*, *37*, 3276–3280.

Ziprin, R. L., Young, C. R., Byrd, J. A., Stanker, L. H., Hume, M. E., Gray, S. A., … Konkel, M. E. (2001). Role of Campylobacter jejuni potential virulence genes in cecal colonization. *Avian diseases*, 549–557.

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