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Campylobacter Prevalence, Molecular Detection, and Virulence Gene Profiles Isolated from Broilers Slaughtered in The Faith Al-Qasim Slaughterhouse

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Abstract

POULTRY and poultry products, particularly contaminated broiler meat, are widely recognized as primary sources of *Campylobacter* infection in humans.This study aimed to assess the prevalence *Campylobacter jejuni* and *coli*in broilers and to identify genes associated with their pathogenicity. Samples were taken from the ceca of 200 broilers slaughtered at Faith Al-Qasim slaughterhouse between January and June 2022. These samples were inoculated on *Campylobacter* selective agar at 42°C for 48–72 hours. Confirmatory tests included oxidase, catalase, and indoxyl hydrolysis. Then, DNA extracted from pure cultures was subjected to singleplexPCRto detect the *hipO* gene for *C. jejuni* and the*glyA* gene for *C. coli*. Additionally, PCR was used to evaluate the presence of six virulence genes:*aspA*, *cdtA*,*cdtC*, *ciaB*, *pldA*, and *dnaJ*. The overall prevalence of *Campylobacter* spp. was 44.5%. Considering species identification, 55 (61.7%) isolates were *C. jejuni*, 18 (20.22%) isolates *C. coli* and 16 (17.9%) isolates belong to other *Campylobacter* spp. In *C. jejuni* isolates, the most prevalent virulence genes were*cdtA* (87.2%), *cdtC* (85.4%), *aspA* (85.4%), *ciaB* (83.6%), *dnaJ* (81.8%), and *pldA* (76.3%). For *C. coli* isolates, the most frequent virulence factors were *pldA* (88.8%), *aspA* (83.3%), *dnaJ* (83.3%), *ciaB* (83.3%), *cdtA* (77.7%), and *cdtC* (77.7%). This study reveals a significant presence of *Campylobacter* species with high prevalence of critical virulence genes in broiler chickens, highlighting the need for ongoing research and robust food safety programs to mitigate the risks of severe infections and enhance public health.

Keywords:*C. coli*, *C. jejuni*, *Campylobacter* prevalence, PCR, Virulence genes.

Introduction

Campylobacter species (spp.) are gram-negative bacteria that colonize several wild and domestic animals, raw milk, and animal-based food [1]. Currently, the *Campylobacter* genus includes 39 valid published species and 16 subspecies [2]. Poultry, particularly broiler chickens, serve as the primary source of *Campylobacter*, with *Campylobacter jejuni* (*C. jejuni*) being the most common species found in these flocks, followed by *Campylobactercoli* (C. *coli*) and occasionally other species [3].

The environmental conditions in the poultry intestine range from anaerobic in the cecum and ileum to aerobic in the duodenum [4]. *Campylobacter* uses aspartate ammonia-lyase (aspA) as alternative substances like fumarate and succinate as a final electron acceptor instead of oxygen to support its growth in the anaerobic environment conditions and are essential to intracellular survival [5]. The secretion system of *C. jejuni* encodes the *ciaB* gene synthesis protein factor called *Campylobacter* invasion antigen (ciaB), which is involved in protein exportation. It is crucial in intestinal avian species' epithelial cell invasion and colonization [6]. There are three encoded cytotoxic subunit adjacent genes, *cdtA*, *cdtB*, and *cdtC*, called cytolethal distending toxin (*cdt*) [7]. The *cdtB* encodes responsible for the toxicity activity of cdt. In

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contrast, *cdtA* and *cdtC* genes encode binding to protein and host cell internalization which is responsible for the cytotoxic expression and host enterocytes lethal activity [8]. The chaperon protein (heat shock protein) is expressed by the thermotolerance gene (*dnaJ*) [9]. These virulence genes enable the microorganism to cope with colonization and adherence to diverse physiological stresses [10]. Phospholipase A outer membrane (*pldA*) genes play a crucial role in external cell membrane phospholipase synthesis, cecum cell invasion, and colonization [11].

Epidemiological data on the prevalence of *Campylobacter* species and their virulence genes in poultry are very limited in Iraq. Therefore, the present study aimed to assess the prevalence of two *Campylobacter* species, namely *Campylobacter jejuni* and *coli,* and to identify specific genes associated with their pathogenicity in slaughtered broilers.

Material and Methods

Sampling

The study was carried out at the Microbiology and Parasitology Department, Zoonosis unit, Faculty of Veterinary Medicine, Al-Qadisiyah University. The samples were collected randomly from 20 farms during the slaughtering procedure. The study was designed to collect (200) cecal swab samples from the broiler carcass during the evisceration process at the Faith Al-Qaseem slaughterhouse in Babylon from mid-January 2022 to June 2022.

Isolation and Bacterial Identification:

The collected cecal swabs were transferred by a Cary Blair C&S ® collection vial of 15 ml transport broth (hardy company, USA; medical chemical corporation), transported directly to the laboratory, and streaked by disposable sterile swabs onto Campylobacter age base ® (Criterion, Hardy diagnostic). Media enriched with 5-10 % (vol/vol) human blood and Campylobacter selective supplement ® (karmali, oxoid), composed of Sodium pyruvate, Cefoperazone, Vancomycin, and Cycloheximide (Hardy diagnostic company). The plates were then incubated at 42 ℃ for 48hours under microaerophilic conditions (85% N2, 5% O2, and 10% CO2), generated by CampyloGen TM kit ® (Oxoid CN0035) for 48 hours, and sealed in the anaerobic jar (oxoid, UK). The morphology of *Campylobacter* colonies was flat or convex entire irregular edges, mucoid, grayish color and nonhemolytic. Furthermore, subcultures used the same media, and all positive colonies were tested by catalase, oxidase, and indoxyl hydrolysis tests [12]. Then, genomic DNA was extracted by using Bioneer extraction DNA kit ®. The DNA extraction procedure was done according to company instructions catalogue. The isolated DNA was checked with a Nano-drop spectrophotometer (Thermo-scientific, Germany). DNA Quality was checked by running the isolated DNA on 1% agarose gel electrophoresis, followed by visualization under Ultraviolet (UV- illumination) using a UV-Transilluminator (Cleaver Scientific, UK) and stored at -20℃ until use (Applied Biosystem, USA). The genus *Campylobacter* was identified using the primer pairs for 16S rRNA gene as presented in Table 1. For species identification, the primers pairs for the *hipO* gene and the *glyA* gene were used for detection of *C. jejuni*, and *C. coli*, respectively (Table 1) [14, 15].

Virulence genes molecular characterization:

The virulence genes *aspA*, *cdtA*, *cdtC*, *ciaB*, *pldA*, *dnaJ* were surveyed in the present study using specific primers (Table 1). The PCR amplification conditions were conducted according to [18].

Statistical analysis

The percentage value was used for evaluation the results of prevalence *Campylobacter* isolates and occurrence of virulence related genes.

Results

Eighty-nine *Campylobacter* isolates (44.5%) were recovered from 200 cecal samples collected from slaughtered broilers. All isolates were phenotypically identified (Figs. 1,2) and then were subjected for molecular identification using genus-specific 16S rRNA primer pairs as presented in Fig. 3.

According to PCR amplification results of *hipO* gene and the *glyA* gene, 55 out of 89 isolates were identified as *C. jejuni* (61.7%), and 18 isolates were as *C. coli* (20.22%) as showed in Fig.4 and 5, while 16 of 89 were belong to other *Campylobacter* species.

The PCR amplification results of six different virulence genes (*cdtA, cdtC, aspA, dnaJ, ciaB, PldA*)examined in the current study were presented in Figs 6, 7, 8, 9,10, 11, respectively.

Table 2 shows the distribution of these virulence determinants among *C. jejuni* and *C. coli*. The most prevalent virulence genes in *C. jejuni* isolates were *cdtA* (87.2%), *cdtC* (85.4%), *aspA* (85.4%), *ciaB* (83.6%), *dnaJ* (81.8%), and *pldA* (76.3%). In contrast, the most frequent virulence factors in *C. coli* isolates were *pldA* (88.8%), *aspA* (83.3%), *dnaJ* (83.3%), *ciaB* (83.3%), *cdtA* (77.7%), and *cdtC* (77.7%).

Discussion

The primary objective of this study was to examine the rate of *Campylobacter* infection and identify the profile of virulence genes. The identification criteria based on cultural characteristics, biochemical tests and molecular

identification matched those reported in previous studies [19]

The overall *Campylobacter* prevalence was 44.5% from 200 cecal swabs obtained from slaughtered broilers. In accordance with the present results, previous studies have demonstrated similar prevalence rate of *Campylobacter* isolated from chicken in Iran [20]. Relatively, lower prevalence (35.84%) was demonstrated in chicken carcasses in slaughterhouses from South of Brazil [21]. In Ireland and Argentina, higher prevalence (63% &66%, respectively) was found in cecal samples of chickens in slaughterhouse [22, 23]

The prevalence *of C. jejuni* and *C. coli* among the examined samples was 61.7% and 20.22%, respectively. These findings agree with the findings of other studies, in which the occurrence of *C. jejuni* was higher than *C. coli* [21, 23, 22]. *A possible explanation for these results may be the more sensitive nature of C. coli to stress conditions during slaughtering process of broilers* [23].

Many previous studies have demonstrated that virulence genes involved in adhesion (*dnaJ*), invasion (*pldA* and *ciaB*), cytotoxin production (*cdtA* and *cdtC*), and that important for growth of *Campylobacter* under severely oxygen-limited conditions in the avian or mammalian gut (*aspA*) play a significant role in the development of *Campylobacter* infections [24]. It is interesting to note that the *C. jejuni* isolates showed higher prevalence to four virulence genes namely (*cdtA*, *cdtC*, *aspA*, and *PldA*), while *C. coli* strains showed higher prevalence of *dnaJ* and *ciaB*. Overall, these genes were detected in majority current *Campylobacter* spp. isolated from the examined samples. Consequently, consuming raw or undercooked poultry meat can result in campylobacteriosis. Therefore, studies should be focusing on the virulence characteristics of *Campylobacter* in food of animal origin, which are pivotal for public health and food safety programs [18].

Campylobacter cannot metabolize exogenous sugars but has alternative enzymatic and transport pathways for amino acid catabolism, such as proline, serine aspartate, and glutamate amino acid [25, 26]. In this study, the occurrence of *aspA* gene was 85.4% of *C. jejuni* and 77.7% of C. *coli* isolates. Campylobacter colonization in poultry hosts' guts is likely to grow under microaerobic conditions and utilize alternative electron acceptors. The expression of the *aspA* gene is essential for *C. jejuni*'s pathogenicity and ability to colonize and persist in the host [27].

The high percentage of the *cdtA* (85.4% and 83.3%) in *C. jejuni* and *C. coli* respectively, and *cdtC* (87.2%, 77.7%) in *C.jejuni* and C. coli, these findings in agreement with a previous study mentioned that most of chicken Campylobacter isolates had *cdt* genes [28], however, lower prevalence was reported by others [29]. It is argued that three *cdt* genes in a cluster are more potent for releasing functional cytotoxin [30, 31].

The CiaB protein is involved in the translocation of *Campylobacter* into target host cells for cell invasion and colonization in chicken cecum [32]. The current study found a high prevalence of the invasion-related *ciaB* gene in both *C. jejuni* and *C. coli*, highlighting the clinical importance of these isolates. However, a study conducted in China reported a low prevalence of this gene, suggesting a reduced invasive ability of their *Campylobacter* isolates [33].

In the present study, *dnaJ* gene was present in 88.8% of *C. coli* isolates and 76.3% of *C. jejuni* isolates. These results differ from other published studies, which have demonstrated a lower prevalence of *dnaJ* gene among *C. coli* and *C. jejuni* [34, 35, 36].

The *pldA* gene is a critical virulence factor for colonizing *Campylobacter* species in the intestinal epithelial cells of chickens, which encodes proteins associated with elevation ability of microorganism invasion [37]. Majority of investigated *C. jejuni* and *C. coli* isolates were positive for *pldA* gene. These results agreed with [38] who found comparable positive results of *pldA* gene in *C. jejuni* isolates recovered from chickens. while, lower prevalence of *pldA* gene was reported by [39].

Campylobacter virulence factors vary globally according to geographic areas and seasons. The high prevalence of surveyed virulence genes in the present study may be due to genetic variation, seasonal variables, and sampling and isolation methods [40]. The current research pays excellent attention to this microorganism's possible high public health risks and zoonosis, especially with a high percentage of virulent factors.

Conclusion

The findings of this study highlight the significant presence of Campylobacter species, particularly *C. jejuni* and *C. coli*, in broiler chickens slaughtered at local slaughterhouse. Molecular detection revealed a high prevalence of critical virulence genes, including *cdtA*, *cdtC*, *aspA*, *ciaB*, *dnaJ*, and *pldA*, which are essential for the bacteria's pathogenicity. These findings indicate a potential for severe infections resulting from these isolates, underscoring the clinical importance of monitoring these virulence factors. This study emphasizes the critical need for continued research on *Campylobacter* virulence characteristics in food animals, especially poultry, to enhance consumer

safety and public health. Implementing robust food safety programs and monitoring systems is crucial to mitigate the risks posed by *Campylobacter* contamination in poultry products. Understanding the distribution and impact of virulence genes can inform better control strategies and reduce the incidence of campylobacteriosis from poultry consumption.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication and or funding of this manuscript.

Ethical of approval

Ethical approval was not required in this study. However, samples were collected as per the standard sample collection procedure.

TABLE 1. Target genes, primer sequences forward and reverse, amplicon sizes, annealing temperatures and references used in present study.

Target gene	Primer sequence (5´→3´)	Products size bp	Annealing temp. ℃	References
16S r RNA	F -AGAGTTTGATCCTGGCTCAG- R - GGTTACCTTGTTACGACTT'	1500	55	13
hipO	F- ACTTCTTTATTGCTTGCTG R- GCCACAACAAGTAAAGAAGC	323	59	14
glyA	F- GTAAAACCAAAGCTTATCGTG R- TCCAGCAATGTGTGCAATG	126	59	15
aspA	F-GGTATGATTTCTACAAAGCGAGA R-ATAAAAGACTATCGTCGCGTG	500	53	14
cdtA	F-CCTTGTGATGCAAGCAATC R-ACACTCCATTTGCTTTCTG	370	49	16
cdtC	F-CGATGAGTTAAAACAAAAAGATA R-TTGGCATTATAGAAAATACAGTT	182	48	16
ciaB	F-TGCGAGATTTTTCGAGAATG R-TGCCCGCCTTAGAACTTACA	527	54	17
PldA	F-AAGAGTGAGGCGAAATTCCA R-GCAAGATGGCAGGATTATCA	384	46	10
dnaJ	F-ATTGATTTTGCTGCGGGTAG R-ATCCGCAAAAGCTTCAAAAA	177	50	17

TABLE 2. Number and percentage of virulence genes distribution among *C. jejuni* and *C. coli*.

Virulence Factor	*C. jejuni* no. (%)	*C. coli* no. (%)
cdtA	(47) 85.4%	(15) 83.3%
cdtC	(48) 87.2%	(14) 77.7%
aspA	(47) 85.4%	(14) 77.7%
dnaJ	(42) 76.3%	(16) 88.8%
ciaB	(45) 81.8%	(15) 83.3%
PldA	(46) 83.6%	(15) 83.3%

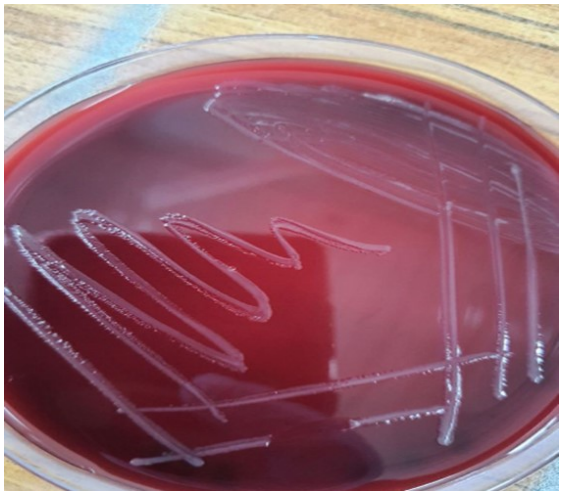

Fig. 1. The colonies on Campylobacter blood agar appeared as small colonies, mucoid, grayish, convex, glistening, non-hemolytic after 48 hours of incubation micro-aerobically.

Fig. 2. Biochemical tests of Campylobacter. a) catalase; b) oxidase; c) indoxyl test.

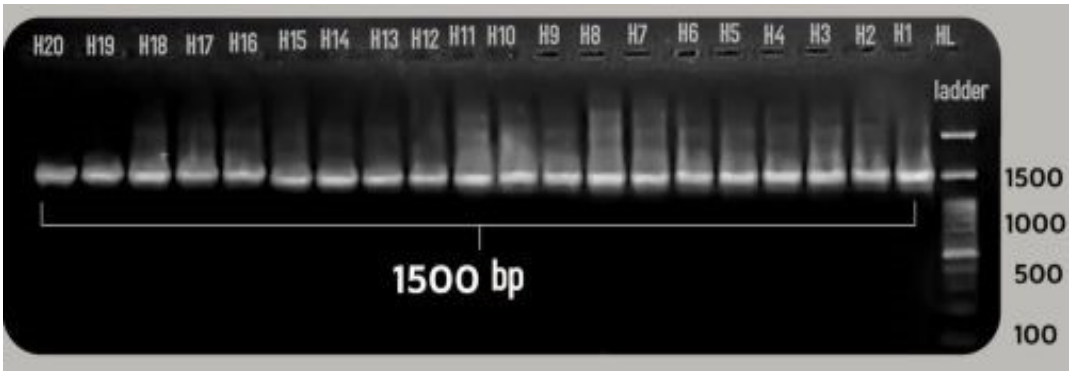

Fig. 3. Red-Safe stained agarose gel (90 volts per 42 minutes) of PCR results for detection of *Campylobacter* genus-specific 16S rRNA gene. Lane M: safe-green™ 100bp Opti-DNA Marker (ABM, Canada); Lanes numbered according to strain designation code. Lanes: HL, H1-H20 shows positive results with 16S rRNA gene (1500 bp).

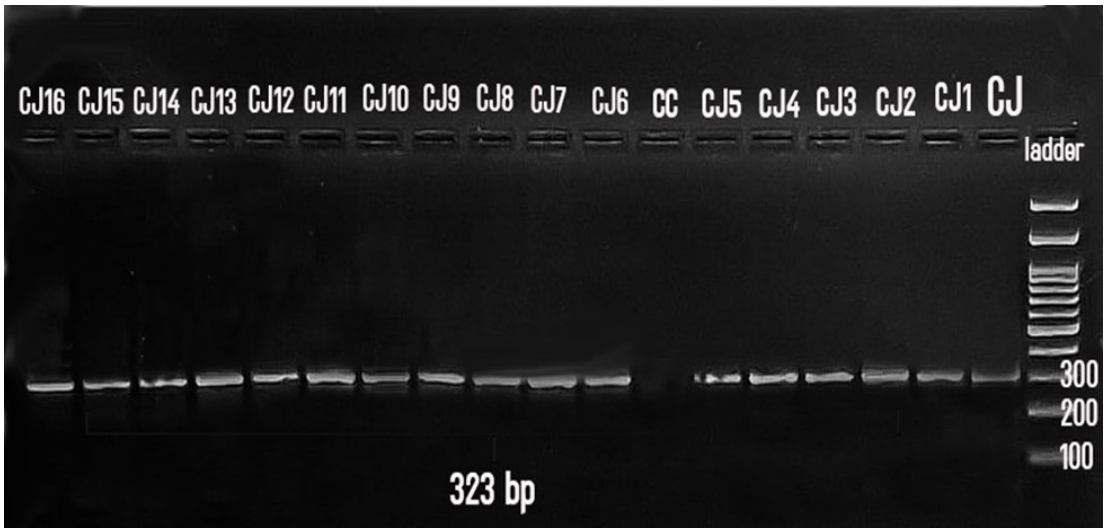

Fig. 4. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *C. jejuni*, *hipO* gene. Lane M: safe-green™ 100bp Opti-DNA Marker (ABM, Canada); Lanes numbered according to isolate designation code. Lanes: CJ, CJ-CJ16 shows positive results with *C. jejuni*, *hipO* gene (CJ primer, 323 bp).

Fig. 5. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of C. coli, *glyA*gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC22, show positive results with PCR products *C. coliglyA* gene (CC primers) 126 bp.

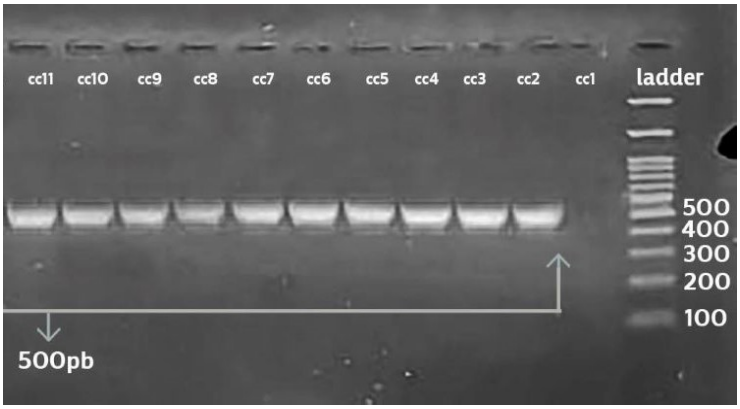

Fig. 6. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *aspA* gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *aspA* gene (500 bp).

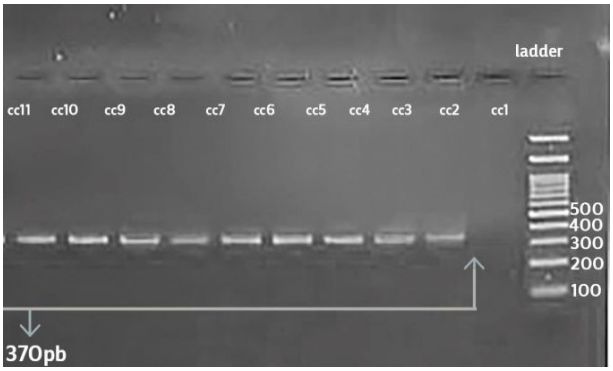

Fig. 7. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *cdtA* gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *cdtA* gene (370 bp).

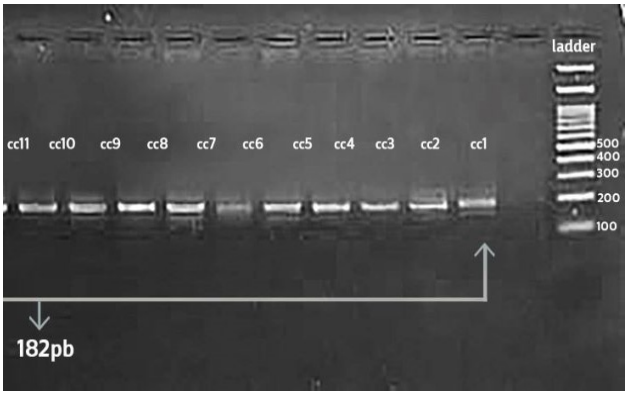

Fig. 8. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *cdtC* gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *cdtC* gene (182 bp).

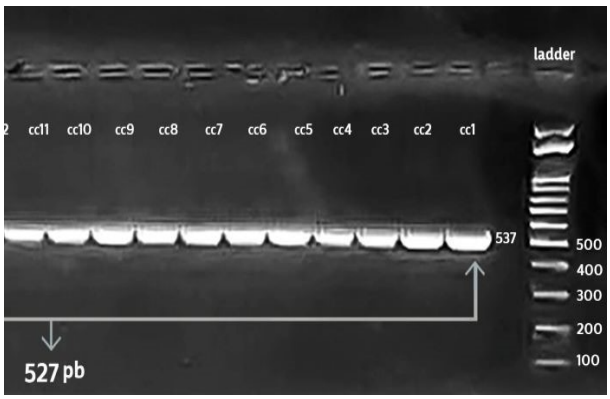

Fig. 9. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *ciaB* gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *ciaB* gene (527 bp).

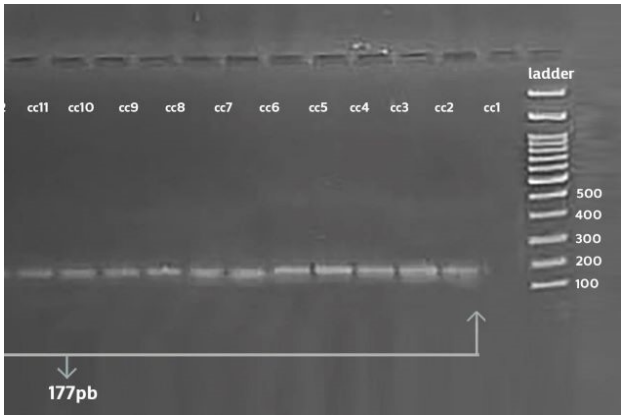

Fig. 10. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *dnaJ* gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *dnaJ* gene (177bp).

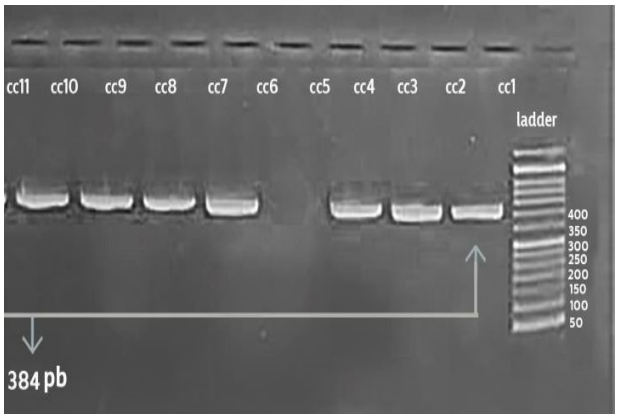

Fig. 11. Red-Safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *phlA*gene. Lane M: safe-green™ 100bp OptiDNA Marker; Lanes numbered according to strain designation code. Lanes: CC1-CC11, show positive results with PCR products *phlA* gene (384 bp).

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انتشار العطيفة، الكشف الجزيئي، وملامح جينات الفوعة، المعزولة من دجاج اللحم المذبوح في مجزرة فيض القسيم

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الملخص

من المعروف على نطاق واسع أن الدواجن ومنتجاتها، وخاصة لحوم الدجاج اللاحم الملوثة، هي المصادر الرئيسية لعدوى العطيفة لدى البشر في جميع أنحاء العالم.. هدفت هذه الدراسة تقييم انتشار العطيفة الصائميةوالقولونية، وتحديد جينات معينة مرتبطة بقدرتهما المرضية.. أخذت عينات منالأعور من اجمالي 200 دجاجة مذبوحة في مسلخ فيض القسيم من منتصف يناير إلى يونيو 2022. تم تلقيح العينات على وسط العطيفة الانتقائي الصلب وحضن عند 42 درجة مئوية لمدة 48-72 ساعة.تم إجراء الفحوصات التأكيدية والتي تضمنت اختبارات الأوكسيدازوالكاتالاز وتحلل الإندوكسيل. بعد ذلك، تم إخضاع عينات الحمض النووي المستخلصة من المزارع النقية لتفاعلات تفاعل البوليميراز المتسلسل الفردي للكشف عن جين hipO لتحديد العطيفة الصائميةوجين glyA لتحديد العطيفة القولونيةاضافة الى ذلك، تم استخدام تفاعل البوليميراز المتسلسل لتقييم توزيع ستة جينات فوعة، وهي*aspA*، *cdtA*، *cdtC*، *ciaB*، و *pldA*. كانت نسبة انتشار أنواع العطيفة 44%. وبالنظر إلى تحديد الأنواع، كانت 55 (61.7%) عزلة من العطيفة الصائمية، و18 (20.22%) عزلة من العطيفة القولونية و16 (17.9%) عزلة تنتمي إلى أنواع أخرى من العطيفة. كانت أكثر جينات الفوعة انتشاراً في عزلاتالعطيفة الصائمية هي*cdtA*(87.2%)، *cdtC* (85.4%)، *aspA* (85.4%)، *ciaB* (83.6%)، *dnaJ* (81.8%)، وpldA(76.3%).بينما، كانت عوامل الفوعة الأكثر شيوعاً في عزلاتالعطيفة القولونية هي*pldA* (88.8%)، *aspA* (83.3%)، *dnaJ* (83.3%)، *ciaB* (83.3%)، *cdtA* (77.7%)، و*cdtC*(77.7%). تظهر هذه الدراسة عن وجود كبير لأنواع العطيفة مع انتشار مرتفع لجينات الفوعة الحرجة في دجاج اللحم، مما يبرز الحاجة إلى استمرار البحث وبرامج سلامة الغذاء النشطة لتقليل مخاطر الاصابات الشديدة وتعزيز الصحة العامة.

الكلمات الدالة: العطيفة القولونية، العطيفة الصائمية، انتشار العطيفة، تفاعل البوليمراز المتسلسل، جينات الفوعة.