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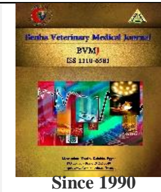
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Original Paper

Virulent genes of Campylobacter jejuni contaminating chicken cuts and giblets

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ABSTRACT

The objective of this examination was to decide the predominance of Campylobacter spp. in some chicken cuts and giblets items by both ordinary and sub-atomic techniques. 120 chicken item tests (25g each) were gathered from different general stores at Menofia legislature of chicken breast, thigh, liver and gizzard. Most examples were defiled with Campylobacter species and chicken liver demonstrated the most elevated pollution (56.67%) trailed by gizzard (53.33%), thigh (30%) and breast (23.33%). *Campylobacter jejuni* was the most segregated serotype (6.67%), (13.33%),(16.67%) and (23.33%) in breast, thigh, gizzard and liver ,individually. hipO quality was identified in secluded *C. jejuni*. Harmfulness qualities cdtA, cdtB and cdt C, cdtA and cdtC, cdtB and cdtC, CdtA, CdtB and CdtC were 30% , 15%, 25%, 10%, 15% and 5% of the detached *C. jejuni* strains, separately. At long last, the use of atomic techniques is better than regular strategies in identification of Campylobacter serotypes.

1. INTRODUCTION

Chicken meat industry is the greatest provider of worthy creature protein with high meat yield, low shrinkage in cooking and extraordinary wellspring of amino acids, nutrients and minerals (Oulkeiret al., 2017). Campylobacter has been recuperated from chicken remains, poultry meat parts and supplies in handling plants overall (García-Sánchez et al. 2017). Campylobacter is a zoonotic microbe and is the primary driver of human bacterial gastroenteritis on the planet (Humphrey and O'Brien, 2007 and Tam and Rodrigues, 2012). The regularly detailed pathogenic species is *C. jejuni* representing over 90% of the cases, trailed by *C. coli* speaking to 7% of the diseases, with the remainder of cases being principally *C. lari* and *C. fetus* (Moore and Corcoran, 2005). Human *C. jejuni* and *C. coli* contaminations don't contrast with respect to clinical indications and length of ailment. Notwithstanding, patients tainted with *C. coli* are in general more seasoned than those with *C. Jejuni* (Karenlampi and Rautelin, 2007). The incubation period is 2 to 5days, and the disease brings about an intense self-restricting gastrointestinal sickness normally settled in multi weeks, described by mellow to extreme watery/wicked loose bowels, fever, queasiness, discomfort and stomach torment (Blaser, 1997). In created nations the greater part of the human cases happen from pre-summer until summer (Kovats and Edwards, 2005). Death rate is inadequately characterized however low, with passing regularly restricted to immuno-traded off patients or those experiencing another extreme sickness, for example, entrails malignant growth (Allos, 2001). There is impressive epidemiological proof that the main danger factor related with human Campylobacter contamination is the presence of this living being in chicken (Sheppard-Dallas et al., 2009). Notwithstanding immediate corpse pollution, intestinal substance sully machines, working surfaces, defensive apparel and worker's hands expanding the open door for cross-tainting of without campylobacter cadavers (FAO and WHO, 2002).

Campylobacter species are overall significant reason for bacterial gastroenteritis (Moore et al., 2005). As a matter of fact, *C. jejuni* and *C. coli* are liable for 90% and 10% of human enteric disease cases, individually (Lastovica, 2006). Campylobacter contaminations in people are typically described without help from anyone else restricting watery/bleeding the runs, abdominal cramps, queasiness and fever; in any case, extreme neurological sequelae, bacteremia and other extra intestinal complexities may grow rarely (Blaser and Engberg, 2008). The recognizable proof and segregation of *C. jejuni* and *C. coli* is viewed as risky on the grounds that it just relies upon a solitary phenotypic test dependent on the hydrolysis of hippurate (Steinhauserova et al., 2001). Subsequently, atomic distinguishing proof strategies have been portrayed as an option to the off base, tedious, biochemical phenotypic techniques (LaGier et al., 2004). Various traditional PCR measures focusing on an assortment of qualities, for example, hipO, glyA, cadI, ceuE and mapA have been recorded (On and Jordan, 2003). Along these lines, the objective of this investigation is recognizable proof of Campylobacter species by sub-atomic and regular strategies in some chicken cuts and offal's.

2. MATERIAL AND METHODS

2.1. Collection and preparation of samples:

An aggregate of 120 random examples of fresh chicken cut and giblets were gathered from chicken butchering shops

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with various disinfection levels in Menofia government. The gathered examples were characterized into Fresh chicken meat was comprised of 30 chicken breast, 30 chicken thigh. Chicken giblets included 30 chicken livers and 30 chicken gizzards. The examples were acquired in their packaging as offered to the shoppers and moved straightforwardly to the lab in a fridge without undue delay where they were ready for bacteriological assessment.

2.2. Appraisal of campylobacter tally:

2.1.1. Arrangement of tests in enhancement stock:

Accurately 25 grams of each example were aseptically put to sterile stomacher sack containing 225ml Bolton particular advancement stock for homogenization at that point enhanced (sallam, 2001).

2.2.2. Confinement on particular plating media (Bolton et al., 1984)

Loopfulls from the recently hatched stock societies were streaked on adjusted Campylobacter Charcoal Deoxycholate Agar (CCDA, Biolife Italiana) base plates enhanced with 10 mg/L of amphotericin B and 32 mg/L of cefoperazone (Biolife, Italiana). The immunized plates were hatched for 48 h at 42˚C under microaerophillic condition (5% O2, 10% CO2 and 85% N2) utilizing CampyGen sachets (Vandepitte and Verhaegen, 2003).

2.3. Identification of hypothetical settlements:

2.3.1. Microscopically assessment (ISO, 1995):

By a stage contrast magnifying instrument, for trademark, twisting or bended slim poles with a wine tool like motility.

2.3.2. Gram staining according to *ISO,* (*1995):*

*2.3.3. Biochemical distinguishing proof followin*g OIE, (2008):

Catalase test, Oxidase test, Triple sugar iron test, Lead acetic acid derivation strip, Growth within the sight of 1% glycine, Nitrate decrease, Hippurate hydrolysis test, Nalidixic corrosive opposition and Growth temperature resistance were done.

2*.3.4. Serological recognizable proof* of Campylobacter species by Latex Agglutination Kit:It was done by Oyarzabal et al., (2007).

2.4. Polymerase Chain Reaction (PCR):

2.4.1. Groundwork groupings utilized for PCR ID framework:

Utilization of PCR for 23S rRNA quality as corroborative apparatus for location and recognizable proof of Campylobacter species was done. Besides, multiplex PCR was utilized for ID and portrayal of cytological distending poisons spoke to by cdtA, cdtB and cdtC as harmfulness qualities of *Campylobacter jejuni*

2.4.2. DNA Extraction utilizing QIA amp pack (Ehsannejad et al., 2015).

2.4.3. DNA intensification.

2.4.3.1. Intensification response of 23S rRNA (Wang et al., 2004).The intensification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany).

2.4.3.2. Amplification of the chose destructiveness qualities (Carvalho et al., 2013):Precisely, 40 µl of PCR combination were utilized. All responses contained appropriate concentrations of 3 preliminary sets, 0.2 mM every one of dATP, dCTP, dGTP and dTTP, 1 × Ex Taq DNA polymerase cradle, and 1.0 U of Ex Taq DNA polymerase in a 40-mL response volume.

3. RESULTS

As shown in table (1) results revealed that the incidence of Campylobacter spp. was positive for all samples. The highest incidence was found in chicken liver and gizzard (56.67%), (53.33%) followed by thigh and breast (30%), (23.33%), respectively. It is evident from results recorded in table (2) that the incidence of *C. jejuni* was (6.67%), (13.33%), (16.67%) and (23.33%) in breast, thigh, gizzard and liver, respectively. Table (3) showed Latex Agglutination test for confirmatory identification of *C. jejuni* was (23.33), (16.67), (13.33) and (3.33) breast, thigh, gizzard and liver, respectively.Table (4) and photo (1 & 2) showed the occurrence of virulence genes of *C. jejuni* strains isolated from the examined samples of chicken cuts and giblets. Virulence genes *cdtA, cdtB* and *cdt C, cdtA* and *cdtB, cdtA* and *cdtC, cdtB* and *cdtC* were present at a rate of 44.4%, 11.1%, 16.7%, 27.8% of the examined of strain of *C. Jejuni,* respectively.

4. DISCUSSION

Chickens having up to 100% asymptomatic transporters of Campylobacter in their intestinal lots and may hold up to 109 microorganisms for each 25 g, which quickly spread among different chickens. This much surpasses the human irresistible portion (Humphrey et al., 2007).

Table 1 Incidence of Campylobacter species in the examined samples of chicken cuts and giblets.

Chicken cuts and giblets	No. of ex. samples	No.	%
Breast	30	7	23.33
Thigh	30	9	30
Gizzard	30	16	53.33
Liver	30	17	56.67
Total	120	49	40.83

Table 2 Incidence of *Campylobacter jejuni* isolated from the examined samples of chicken cuts and giblets (n=30).

Identified strains	Chicken cuts and giblets							
	Breast		Thigh		Gizzard		Liver	
	No.	%	No.	%	No.	%	No.	%
Campylobacter jejuni	2	6.67	4	13.33	5	16.67	7	23.33

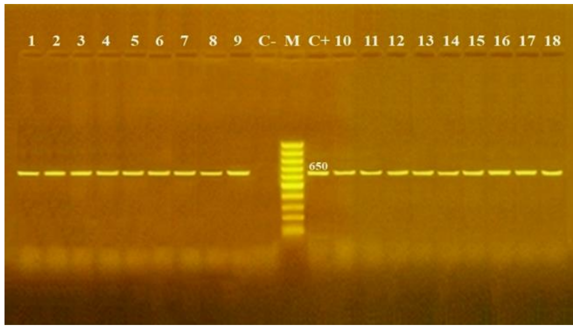
Table 3 Latex Agglutination test for confirmatory identification of *Campylobacter jejuni* isolated from the examined samples of chicken cuts and giblets (n=30).

Chicken cuts and giblets	Latex Agglutinating Kit Observation	Latex Agglutinating	
		No.	%
Breast	+ve agglutination clumps	7	23.33
Thigh	+ve agglutination clumps	5	16.67
Gizzard	+ve agglutination clumps	4	13.33
Liver	+ve agglutination clumps	2	3.33
Total (120)		18	15

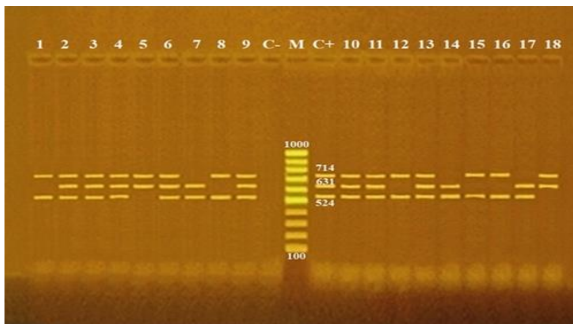
N.B. % was calculated according to total number of samples

Table 4 Occurrence of virulence genes of *C. jejuni* strains isolated fromthe examined samples of chicken cuts and giblets (n= 18).

Virulence genes	No.	%
cdtA, cdtB and *cdtC*	8	44.4
cdtA and *cdtB*	2	11.1
cdtA and *cdtC*	3	16.7
cdtB and *cdtC*	5	27.8
Total	18	100



Photograph 1 Agarose gel electrophoresis of PCR of *23S rRNA* (650 bp)as confirmatory gene for detection of *Campylobacter* species. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *for 23S rRNA* gene. Lane C-: Control negative. Lanes from 1 to 18: Positive *Campylobacter* strains for *23S rRNA* gene.



Photograph 2 Agarose gel electrophoresis of multiplex PCR for cytological distending toxins *cdtA* (631 bp), *cdtB* (714 bp) and *cdtC* (524 bp) as virulence genes for characterization of *Campylobacter jejuni.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *C. jejuni* for *cdtA, cdtB* and *cdtC* genes. Lane C-: Control negative. Lanes 2, 3, 4, 6, 9, 10, 11 & 13: Positive *C. jejuni* for *cdtA, cdtB &cdtC* genes. Lanes 5 & 18: Positive *C. jejuni* strains for *cdtA* and *cdtB* genes.Lanes 7, 14 & 17: Positive *C. jejuni* strains for *cdtA* and *cdtC* genes. Lanes 1, 8, 12, 15 & 16: Positive *C. jejuni* strains for *cdtB* and *cdtC* genes.

As appeared in table (1) results delineated that liver and gizzard indicated the higher occurrence of Campylobacter contamination than different examples. It may allude to the first intestinal pollution during fowl destruction (Moore et al., 2005). Our outcome was higher than that of Khalifa et al. (2013) (36%) and El-Tras et al. (2015) (23.5%); the pervasiveness contrasts can be credited to confinement strategies, test types and size not withstanding occasional and provincial varieties (Allos, 2001; Omara et al., 2015). Results in table (2) the distinguishing proof of Campylobacter strains confined from inspected tests indicated that *C. jejuni, C. coli* and *C. butzieri* were distinguished in the pace of 28%, 20% and 12% in breast separately, *C. jejuni*, *C. coli* and *C. lari* were distinguished in pace of 24% 16% and 8%, separately in breast. In thigh the pace of disengagement of *C. jejuni*, *C. coli*, C. *lari* and *C. cinaedi* were 40 %16%, 8 % and 8% individually. Concerning thighs the pace of disconnection of *C. jejuni,C. coli*, *C.lari* and *C. upsaliens* were 36%, 12% and 8 % individually Livers were debased with *C. jejuni*, *C. coli*, *C. lari* and *C. cinaedi* in pace of 48 %, 20 %, 8%and 4 % separately . Gizzards were contaminated with *C. jejuni, C. coli* and *C. lari* in rate, of 44 %, 24 % and 12% individually. Among the zoonotic Campylobacter species, for example *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*, the previous two species are answerable for by far most of the human food borne contaminations, representing 90% and 5-10% of cases (Mikulić et al., 2016). The zoonotic *C. jejuni* is perhaps the most poultry holding microorganisms, with high general wellbeing risk ordinarily connected with chicken, arrangement to the prevalent degrees of human utilization (Humphrey et al., 2007). Photograph (1) demonstrated the presence of hipO gen in separated *C. jejuni*. Polymerase chain response (PCR) focusing on hipO quality was utilized already for ID of *C. jejuni* in chickens; meat and human examples (Khalifa et al., 2013). It is the primary Campylobacter genome to be sequenced was *C. jejuni* by Parkhill et al. (2000). Utilization of sub-atomic instruments, for example, PCR may assist with maintaining a strategic distance from a portion of the constraints of current techniques, where the hipO quality is explicit for *C. jejuni* strains (Sinha et al., 2004). Table (3) and photographs (1&2) indicated the Occurrence of destructiveness qualities of *C. jejuni* strains confined from the inspected tests of chicken meat and giblets as cdtA, cdtB and cdt C, cdtA and cdtB, cdtA and cdtC, cdtB and cdtC were available in 44.4% , 11.1%, 16.7%, 27.8%| separately. These qualities are included essentially in attachment and intrusion and they alluded to as harmfulness factors starting here onwards (Chansiripornchai and Sasipreeyajan, 2009). All in all, the most noteworthy frequency of Campylobacter strains was established in chicken**.**

5. REFERENCES

1. Allos, B. M. 2001. Campylobacter jejuni Infections: update on emerging issues and trends. Clinical Infectious Diseases 32(8): 1201-1206 .
2. Asakura, M.; Samosornsuk, W.; Hinenoya, A.; Misawa, N.; Nishimura, K.; Matsuhisa, A. and Yamasaki, S. 2008. Development of a cytolethal distending toxin (cdt) gene-based species- species multiplex PCR assay for the detection and identification of Campylobacter jejuni, Campylobacter coli and Campylobacter fetus. FEMS Immunol. Med. Microbiol. 52: 260-266 .
3. Blaser, M. J. 1997. Epidemiologic and clinical features of Campylobacter jejuni infections. Journal of Infectious Diseases 176 Suppl 2: S103-105 .
4. Bolton, F.J.; Coates, D. and Hutchinson, D.N. 1984: The ability of Campylobacter supplements to neutralize photochemically induced toxicity and hydrogen peroxide. J. of Appl. Bacteriol., 56:151-157 .
5. Carvalho A.; Ruiz-Palacios G.; Ramos- Cervantes, P.; Cervantes L.; Jiang, X. and Pickering L. 2001. Molecular characterization of invasive and noninvasive Campylobacter jujeni and Campylobacter coli isolates. J. Clin. Microbiol., 39:1353-1363 .
6. Chansiripornchai, N. and Sasipreeyajan, J. 2009. PCR detection of four virulence- associated genes of Campylobacter jejuni isolates from Thai broilers and their abilities of adhesion to and invasion of INT-407 cells. J Vet Med Sci.; 71: 839–844
7. Ehsannejad, F.; Sheikholmolooki A.; Hassanzadeh, M.; Shojaei Kavan, R.; Soltani, M. 2015: Detection of cytolethal distending toxin (cdt) genes of Campylobacter jejuni and coli in fecal samples of pet birds in Iran. Iranian J. Vet. Med., 9 (1): 49-56 .
8. EL-Tras, W. F.; Holt, H. R.; Tayel, A. A.; EL- Kady, N .N. 2015: Campylobacter infections in children exposed to infected backyard poultry in Egypt. Epidemiol. Infect. 143(2):308-315 .
9. FAO and WHO 2002: Risk assessment of Campylobacter spp. in broiler chickens and Vibrio spp. in seafood.Rome.
10. FDA (Food and Drug Administration) 1998: FDA Bacteriological Analytical Manual. Chapter 7, Campylobacter, Hunt, J. M., Abeyta, C. & Tran, T. 8th ed. (revision A), 23 pages.UK
11. Haan, C. P. A.; Kivisto, R. I.; Hakkinen, M.; Corander, J. and Hanninen, M. L. 2010. Multilocus sequence types of Finnish bovine Campylobacter jejuni isolates and their attribution to human infections. BMC ,. Microbiol. 10:200 .doi: 10.1186/1471-2180-10-200.
12. Humphrey, T. and O'Brien, O. S. 2007. Campylobacters as zoonoticpathogens: a food production perspective. International Journal of Food Microbiology 117(3): 237-257 .

13. ISO (International Standards Organization) 1995: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of thermotolerant Campylobacter. ISO 10272: 1995 (E) International Standards Organization, Geneva.
14. Karenlampi, R. and Rautelin , H. 2007. Longitudinal study of Finnish Campylobacter jejuni and C. coli isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. Applied & Environmental Microbiology 73(1): 148-155 .
15. Khalifa, N. O.; Jehan- Afify, S. A. and Nagwa- Rabie, S. 2013. Zoonotic and Molecular Characterizations of Campylobacter jejuni and Campylobacter coli Isolated from Beef Cattle and Children. Glob. Vet. 11(5):585-591
16. LaGier, M. J.; Joseph, L.A.; Passaretti, T. V.; Musser, K. A. and Cirino, N. M. 2004. A real-time multiplexed PCR assay for rapid detection and differentiation of Campylobacter jejuni and Campylobacter coli. Mol. Cell Probes. 18(4):275-282.
17. Mikulić, M.; Humski, A.; Njari, B.; Ostović, M. and Duvnjak , S. 2016. Prevalence of thermotolerant Campylobacter spp. in chicken meat in Croatia and multilocus sequence typing of a small subset of Campylobacter jejuni and Campylobacter coli isolates. Food Technol. Biotechnol. 54(4): 475-481 .
18. Moore, J. E.; Corcoran, D.; Dooley, J. S.; Fanning, S.; Lucey, B.; Matsuda, M.; McDowell, D. A.; Mégraud, F.; Millar, B. C.; O'Mahony, R.; O'Riordan, L.; O'Rourke, M.; Rao, J. R.; Rooney, P.J.; Sails, A. and Whyte, P.2005. Campylobacter. Vet. Res. 36(3):351- 382 .
19. OIE “Office International des Epizooties” 2008. Validation and quality control of polymerase chain reaction methods used for the diagnosis of infectious diseases, in manual of diagnostic tests and vaccines for terrestrial animals (mammal, birds and bees), 6th ed. Paris: Office International des Epizooties: 46- 55 .
20. On, S. L. and Jordan, P. J. 2003. Evaluation of 11 PCR assays for species-level identification of Campylobacter jejuni and Campylobacter coli. J. Clin. Microbiol., 41:330-336 .
21. Oxoid 2006. Oxoid Manual 9th Edition2006, Compiled by E. Y. Bridson .
22. Oyarzabal, O.; Backert, S.; Nagaraj, M.; Miller, R.; Hussain, S. and Oyarzabal, E. 2007. Efficacy of supplemented buffered peptone water for the isolation of Campylobacter jejuni and C.coli from broiler retail products. J. Microbiol. Methods69:129-136 .
23. Parkhill, J.; Wren, B. W.; Mungall, K.; Ketley, J. M.; Churcher, C.; Basham, D.; Chillingworth ,T.; Davies, R.M.; Feltwell, T.; Holroyd, S. and Jagels, K. 2000. The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hyper variable sequences. Nature 403:665-668.
24. Sallam, K.I. 2001. Campylobacter contamination in retailed chicken carcasses from Mansoura, Egypt, and its relation to public health. Alex. J. Vet. Sci., 17 (1).
25. Sheppard, S. K. and Dallas, J. F. 2009. Campylobacter genotyping to determine the source of human infection." Clinical Infectious Diseases 48(8): 1072-1078 .
26. Shimaa- Omara, T.; El Fadaly, H. A.and Barakat, A. M. A . 2015: Public Health Hazard of Zoonotic Campylobacter jejuni Reference to Egyptian Regional and Seasonal Variations. Res. J. Microbiol. 10(8):343-354 .
27. Sinha, S.; Prasad, K. N.; Pradhan, S.; Jain , D. and Jha, S. 2004. Detection of preceding Campylobacter jejuni infection by polymerase chain reaction in patients with Guillain-Barré syndrome. Trans. R. Soc. Trop. Med. Hyg. 98:342-346 .
28. Steinhauserova, I.; Ceskova, J.; Fojtikova, K. and Obrovska, I. 2001. Identification of thermophilic Campylobacter spp. by phenotypic and molecular methods. J. Appl. Microbiol., 90:470-475 .
29. Tam, C. C. and Rodrigues, L. C. 2012. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice." Gut 61(1): 69-77 .
30. Vandepitte, J. and Verhaegen, J. 2003. Basiclaboratory procedures in clinical bacteriology. Second edition, WHO, Switzerland, Pp. 42- 43 .
31. Wang, G.; Clark, C.; Taylor, T.; Pucknell, C.; Barton, C.; Price, L.; Woodward, D. and Rodgers, F. 2002. Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C.upsaliensis, and C. fetus subsp. fetus. J. Clin. Microbiol., 40:4744–4747.