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RESEARCH ARTICLE

Molecular Detection of *C. jejuni* in Chicken Meat Samples in the Qadisiyah Province, Iraq

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ABSTRACT

Chicken meat is viewed as the essential wellspring of disease with Campylobacter spp. in people. A sum of 50 chicken meat (thigh and bosom meat) tests from naturally butchered chicken at retail outlets in business sectors in the AL -Qadisiyah region, Iraq from January to May 2018. The outcomes exhibit a high commonness rate of *C. jejuni* in chicken thigh meat tests 23 out of 50 tests (46 %), trailed by chicken bosom meat 12 out of 50 tests (24 %). RT -PCR focusing on the species particular harmfulness quality cad f and hip O quality particular for *C. jejuni* .

Key words: - Molecular, detection, Campylobacter .

INTRODUCTION

Campylobacter spp. are a noteworthy reason for bacterial gastroenteritis around the world (1). The moderately low infective measurements, the conceivably genuine squeal (1), and additionally the relationship between certain Campylobacter destructiveness qualities and the example of clinical contamination (2), affirm the significance of this zoonotic disease as a noteworthy wellbeing peril. Customary symptomatic strategies using a mix of culture and biochemical testing require that speculated stool examples are refined on particular agar at 42 °C under microaerophilic conditions for up to 72 h before a negative report is issued. Just culture plates with settlements demonstrating trademark Campylobacter morphology and oxidase inspiration are accounted for as *Campylobacter spp*. Advance recognizable proof to the species level requires different tests, including development temperature inclinations, anti -infection affectability to cephalothin and nalidixic corrosive, and biochemical tests. The sodium hippurate hydrolysis response is the main biochemical test used to separate *Campylobacter jejuni* and *Campylobacter coli*. The expanded and monotonous nature of these techniques has invigorated research into atomic demons trative methodologies. A few laborers have explored the use of multiplex PCR for Campylobacter recognition and speciation (3, 4) with these conventions being connected to confines from unadulterated societies. In any case, the utilization of multiplex PCR on bacterial provinces implied that ordinary societies were as yet required for the underlying

recognizable proof. To assess the multiplex approach straightforwardly on stool examples (5) falsely spiked stool examples with microscopic organisms. Be that as it may, just two reports have depicted the immediate use of a multiplex convention on stools acquired from patients with enteritis (3) and both utilized ground works focusing on the ceuE quality. Different mixes of family particular and species specific qualities, and in addition mixes of species-particular successions of ceuE or lpxA qualities, have been connected in multiplex conventions (6 ,7). The general point of the present work was to explore the commitment of chicken as potential wellsprings of *C. jejuni* contaminations in people at Wasit Province. This point was accomplished by utilizing regular and atomic devices to research the event of *C. jejuni* in chicken examples.

MATERIALS AND METHODS

Samples Collection

An aggregate of 50 tests of chicken meat (thigh and breast meat) tests were acquired from naturally butchered chicken at retail outlets in business sectors in the AL -Qadisiyah region , Iraq between January to May 2018. Twenty - five grams from each chiseled chicken meat (thigh and bosom) were aseptically exchanged to a sterile blender containing 225 ml of Preston improvement juices for homogenization of the example (8).

Biochemical Iden tification of Bacteria

0.1 ml of the juices was streaked onto adjusted Campylobacter particular agar base Cefoperazone Char coal Desoxycolate Agar (mCCDA) (Oxoid, CM 0739). The plates were then brooded at 42ºC for 48 hours under microaerophilic conditions . Suspected provinces were purged on blood agar plates and subjected to biochemical ID utilizing catalase test, oxidase test, urea hydrolysis test, hydrogen sulfide (H2S) generation, citr ate usage test and fast hippurate hydrolysis test (9).

Molecular Identification of Bacteria

DNA extraction from the biochemically recognized disengages was performed by the producer rules utilizing Bacterial DNA Extraction Kit (Spin -section) (BioTeke Corporation, China). The continuous test based PCR (qPCR) responses wer e utilized independently for the affirmation of *C. jejuni* biochemically distinguished secludes. Species - particular preliminaries and Taq Man test sets focusing on hipO quality particular for *C. jejuni* (3). The groupings of hipO ground works and test are: Cj -F1 forward: 5' - TGCTAGTGAGGTTGCAAAAGAATT -3', Cj-R1 switch: 5' - TCATTTCGCAAAAAAATCCAAA -3' and Cj-FAM probe: 5’ -ACGATGATTAAATTCACAATTTTTTTCGCCAAA -3’. (Table-1).

Each qPCR measure utilizing preliminaries and tests particular for C. jejuni , was done by (Quant iTect® Probe RT - PCR units Qiagen). Each qPCR response contained 12.5 µl of 2x QuantiTect Probe RT-PCR Master Mix (containing HotStart Taq® DNA polymerase, QuantiTect Probe RT -PCR support [Tris -Cl, KCl, (NH4)2SO4, 8 mM MgCl2], dNTP blend including dUTP, ROX ™ detached reference color and 8 mM MgCl2), 0.1 units AmpErase [Uracil N - glycosylase] (Qiagen), 500 nM of pertinent groundworks and 500 nM of important test and 5 µl DNA format. Nuclease free water was added to a last volume of 25 µl. Non layout DNA and positive controls of C. jejuni . The response conditions were 50ºC for two minutes to actuate UNG, 95ºC for 15 min then 40 cycles at 94ºC for 15 sec and 60ºC for 60 sec took after by plate read for fluorescence securing.

RESULT

Prevalence of *C. jejuni* in Chicken Samples

The commonness rates of *C. jejuni* in chicken meat tests gathered from business sectors in the AL -Qadisiyah domain. The event of *C. jejuni* was recognized by bacteriological examination and biochemical examination. The outcomes exhibit a high commonness rate of *C. jejuni* in chicken thigh meat tests 23 out of 50 tests (46 %), trailed by chicken breast meat 12 out of 50 tests (24 %) (figure -1**).**

Molecular Identification

The molecular confirmation by real time PCR was connected just to bacteriology and biochemically *C. jejuni* detaches. The outcomes exhibit that rate of *C. jejuni* in chicken thigh meat tests 6 out of 23 tests (26 %), trailed by chicken breast meat 2 out of 12 tests (16 %) (figure -2).

Statistical Analysis

The measurable investigation was performed utilizing SAS (Statistical Analysis System- rendition 9.1) (10).

DISCUSSION

Campylobacter species, basically *C. jejuni* is perceived as imperative bacterial specialists of gastroenteritis in human (11, 12) and household creatures particularly poultry, domesticated animals and partner creatures (13). Poultry and poultry items are viewed as a typical and principle wellspring of Campylobacter disease to people (14). A world study evaluated the pollution of chickens with *Campylobacter spp*. to be around 58% (15). Oven corpses could be cross - tainted with *Campylobacter spp*. by fecal substance or ingest (16), so the utilization of undercooked poultry items and direct contact with live poultry or their defecation are the conceivable hazard pathways for human contaminations (17). In this study ground works against two qualities of *Campylobacter spp* . counting cad F (family particular harmfulness quality), hip O (hippuri case quality for *C. jejuni*) were utilized. These qualities and ground works have been considered autonomously and provided details regarding by different specialists and every one of them, particularly the cad F quality, are exceedingly moderated among detaches of various sources (18, 19,20, 21, 22). The objectives of this review w ere to distinguish and to separate *C. jejuni* and to decide the recurrence of Campylobacter gastroenteritis in chicken meat in the Qadisiyah Province, Iraq utilizing PCR. Poultry are presented to *Campylobacter spp*. Right off the bat at cultivates level because of deficient biosecurity measure, optional at showcase outlets because of defilement of remains amid gutting and singing, thirdly amid capacity (23). Nations utilizing pluck-shop based markets have higher defilement rates of *Campylobacter spp* . from poultry than nations utilizing current handling plants (24). Manual butchering and gutting lead to fecal pollution of cadavers, which thusly might b e in charge of expanded quantities of *Campylobacter spp*. in poultry meat (24). The danger of chicken meat sullied with *Campylobacter spp*. isn't just because of the utilization, yet in addition because of the exchange of the microbes display in chicken parts to hands, kitchen utensils and to other nourishment either straightforwardly or by means of cutting sheets (25). The outcomes point to the appropriateness of the PCR based assays as touchy techniques for fast and direct recognition and concurrent speciation of Campylobacter spp. The outcome demonstrates that *Campylobacter spp*. were disengaged from chicken thigh m eat tests 23 out of 50 tests (46 %), trailed by chicken bosom meat 12 out of 50 tests (24 %) are Similar to (26) and (25) detailed the confinement of *Campylobacter spp*. from 31% of bosom meat and 47.9% of chicken legs, individually. The recognizable proof and separation of *C. jejuni* and *C. coli* is viewed as dangerous in light of the fact that it just relies upon a solitary phenotypic test in light of the hydrolysis of hippurate (27). Hence, atomic distinguishing proof strategies have been portrayed as a con trasting option to the off base, tedious, biochemical phenotypic techniques (3). Be that as it may, the ongoing improvement of constant PCR expelled the

need to control PCR items after enhancement to diminish cross-sullying (3). The single duplicate quality hip O quality (benzoglycine amidohydrolase) is in charge of the hippurate movement which separates *C. jejuni* from other *Campylobacter spp*. (28). The outcome demonstrate that rate of *C. jejuni* in chicken thigh meat tests 6 out of 23 tests (26 %), trailed by chicken bosom meat 2 out of 12 tests (16 %) were affirmed by qPCR. These outcomes fortify the speculation that inspite of the fact that hippurate hydrolysis test is generally used to separate *C. jejuni* from different species, *C. jejuni* hippurate negative strains and false positive strains have been disengaged (29). Moreover (28) and (3) announced that around 10% of *C. jejuni* segregates neglect to hydrolyze hippurate under research facility conditions, bringing about misclassification of these secludes a s C. coli. Also, the hippurate hydrolysis measure is reliant upon the inoculums size of the bacterium, which implies that the examiner can't recognize low level of hippuricase item (30). In this manner, the discovery of the quality by PCR rather than the ph enotypic identification of the hippuricase item are viewed as a solid elective strategy for the segregation of *C. jejuni* separates (31).

CONCLUSION

Poultry dealing with amid butcher and destruction significantly affects the danger of poultry meat sullying instead of capacity temperature.

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REFERENCES

1. Moore JE, Corcoran D, Dooley JS, Fanning S, Lu cey B, Matsuda, M, McDowell, DA, Me´ graud F, Miller BC and other Authors. 2005. Campylobacter. Vet Res 36, 351 –382.
2. Al-Mahmeed A, Senok AC, Ismaeel AY, Bindayna KM, Tabbara, KS and Botta, GA. 2006. Clinical relevance of virulence genes in Campylobacter jejuni isolates in Bahrain. J Med Microbiol 55, 839 –843.
3. LaGier MJ, Joseph LA, Passaretti TV, Musser KA and Cirino, NM. 2004. A real-time multiplexed PCR assay for rapid detection and differentiation of Campylobacter jejuni and Campylobacter coli. Mol Cell P robes 18, 275–282.
4. Klena JD, Parker CT, Knibb K, Ibbitt JC, Devane PM, Horn ST, Miller WG and Konkel ME. 2004. Differen tiation of Campylobacter coli, Campylobacter jejuni, Campylobacter lari, and Campylobacter upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid A gene lpxA. J Clin Microbiol 42, 5549 –5557.
5. Persson S and Olsen KE. 2005. Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. J Med Microbi ol 54, 1043–1047.
6. Jensen AN, Andersen MT, Dalsgaard A, Baggesen DL and Nielsen EM. 2005. Development of real -time PCR and hybridization methods for detection and identification of thermophilic Campylobacter spp. in pig faecal samples. J ApplMicrobiol 99, 292–300.
7. Gebreyes WA, Thakur S and Morrow WE. 2005. Campylobacter coli: prevalence and antimicrobial resistan ce in antimicrobial -free (ABF) swine production systems. J Antimicrob Chemother 56, 765 –768.
8. Kiss I. 1984. Testing methods in food microbiology. A kademiaKiado, Budapest, Hungary.
9. Nachamkin I. 1999. Campylobacter and Arcobacter. In Muray, P. R., Baron, E. J. et al.(Eds). Manual o f clinical microbiology, p. 716 -726. Washington, D. C: ASM press.
10. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010
11. Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J.1999. Identification of the enteropathogens Campylobacter jejuni and Campylobacter coli based on the cadF virulence gene and its product. J Clin Microbiol. 37 (3):510–7.
12. Persson S, Olsen KE. 2005. Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. J Med Microbiol. 54(Pt 11):1043 –7.

13. Aquino MH, Regua Mangia AH, Filgueiras AL, Teixeira LM, Ferreira MC, Tibana A. 2001. Use of a multiplex PCR-based assay to differentiate *Campylobacter jejuni* and *Campylobacter coli* strains isolated from human and animal sources. Vet J. 163(1):102–4.
14. Humphrey T, O'Brien S and MadSen M. 2007. Campylobacter spp. as zoonotic pathogens: a food production perspective. International Journal of Food Microbiology 117: 237 –257.
15. Suzuki H and Yamamoto S. 2009. Campylobacter contamination in retail poultry meats and by -products in the world: a literature survey. The Journal of Veterinary Medical Science 71 (3): 255 –261.
16. Mead GC, Hudson WR and Hinton MH. 1995. Effects of changes in processing to improve hygiene control on contamination of poultry carcasses with Campylobacter. Epidemiology and Infection 115: 495 –500.
17. Anderson J, Horn BJ Gilpin BJ. 2012. The prevalence and genetic diversity of Campylobacter spp. in domestic backyard poultry in Centerbury, New Zealand. Zoonosis and Public Health 59 (1): 52 -60.
18. Abu-Halaweh M, Bates J, Patel BK. 2005. Rapid detection and differentiation of pathogenic Campylobacter jejuni and Campylobacter coli by real -time PCR. Res Microbiol. 156(1):107 –14.
19. Al Amri A, Senok AC, Ismaeel AY, Al -Mahmeed AE, Botta GA. 2007. Multiplex PCR for direct identification of Campylobacter spp. in human and chicken stools. J Med Microbiol. 56(Pt 10):1350 –5.
20. Nayak R, Stewart TM, Nawaz MS. 2005. PCR identification of Campylobacter coli and Campylobacter jeju ni by partial sequencing of virulence genes. Mol Cell Probes. 19(3):187 –93.
21. Linton D, Lawson AJ, Owen RJ, Stanley J. 1997. PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples. J Clin Microbiol. 35(10):2568–72.
22. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Pri ce L, et al. 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(12):4744 –7.
23. Ellis-Iversen J, Jorgensen F, Bull S, Powell L, Cook A J and Humphrey TJ. 2009. Risk factors for Campylobacter colonization during rearing of broiler flocks in Great Britain. Preventive Veterinary Medicine 89 (3-4): 178-184.
24. Parkar SFD, Sachdev D, deSouza N, Kamble A, Suresh G, Munot H, Hanagal D, Shouche Y and Kapadnis B. 2013. Prevalence, seasonality and antibiotic susceptibility of thermophilic Campylobacter spp. in ceca and carcasses of poultry birds in the "live -bird market". African Journal of Microbiological Research 7 (21): 2442 -2453.
25. Guyard-Nicodème M, Tresse O, Houard E, Jugiau F, Courtillon C, El Manaa K, Laisney M and Chemaly M. 2013. Characterization of Campylobacter spp. transferred from naturally contaminated chicken legs to cooke d chicken slices via a cutting board. International Journal of Foo d Microbiology 164 (1): 7 –14.
26. Luu QH, Tran TH, Phung DC and Nguyen TB. 2006. Study on the prevalence of Campylobacter spp. from chicken meat in Hanoi, Vietnam. Annals of the New York Academy of Science 1081: 273 –275.
27. Steinhauserova I, Ceskova J, Fojtikova K and Obrovska I. 2001. Identification of thermophilic Campylobacter spp. by phenotypic and molecular methods. Journal of Applied Microbiology 90: 470 –5.
28. Englen MD, Ladely SR and Fedorka -Cray PJ. 2003. Isolation of Campylobacter and identification by PCR. Methods in Molecular Biology 216: 109 –121.
29. Nayak R, Stewart TM and Nawaz MS. 2005. PCR identification of Campylobacter coli and Campylobacter j ejuni by partial sequencing of virulence genes. Molecular and Cellular Probes19 (3): 187 -193.
30. Linton D, Lawson AJ , Owen RJ and Stanley J. 1997. PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples. Journal of Clinical Microbiology 35: 2568–2572.
31. Slater ER and Owen RJ. 1997. Res triction fragment length polymorphism analysis shows that the hippuricase gene of Campylobacter jejuni is highly conserved. Letters in Applied Microbiology 25: 274 –278.

Table 1: List of Primers that used in PCR amplification

PCR	Primer	Sequence	
Cycle	hip O	F	TGCTAGTGAGGTTGCAAAAGAATT
		R	TCATTTCGCAAAAAAATCCAAA

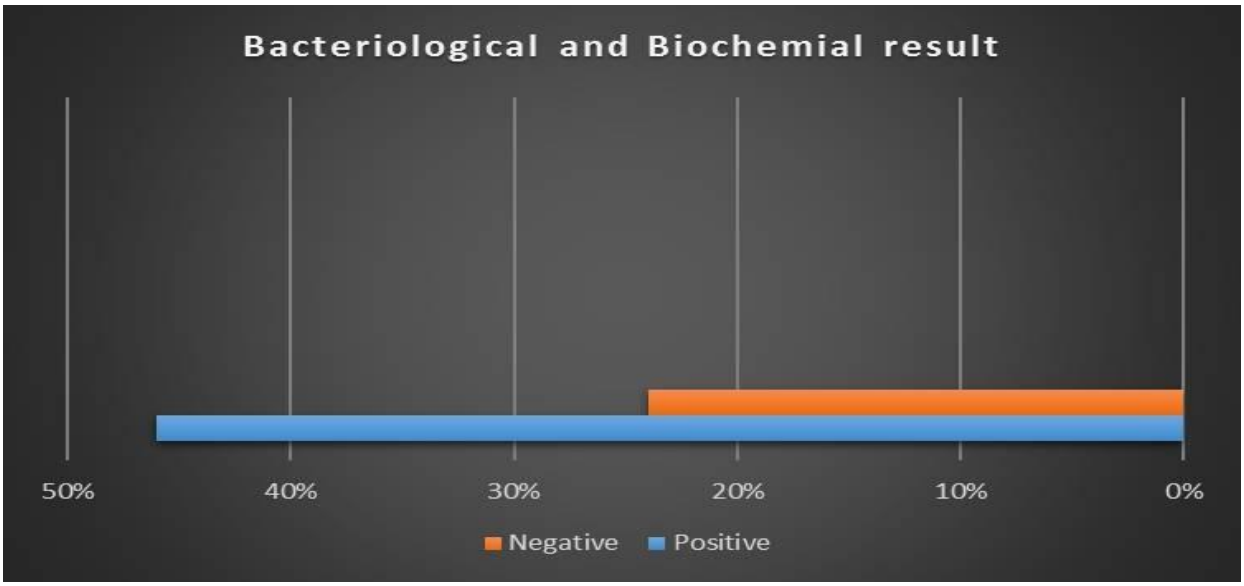

Figure 1: - The Result of Bacteriological and Biochemical Test

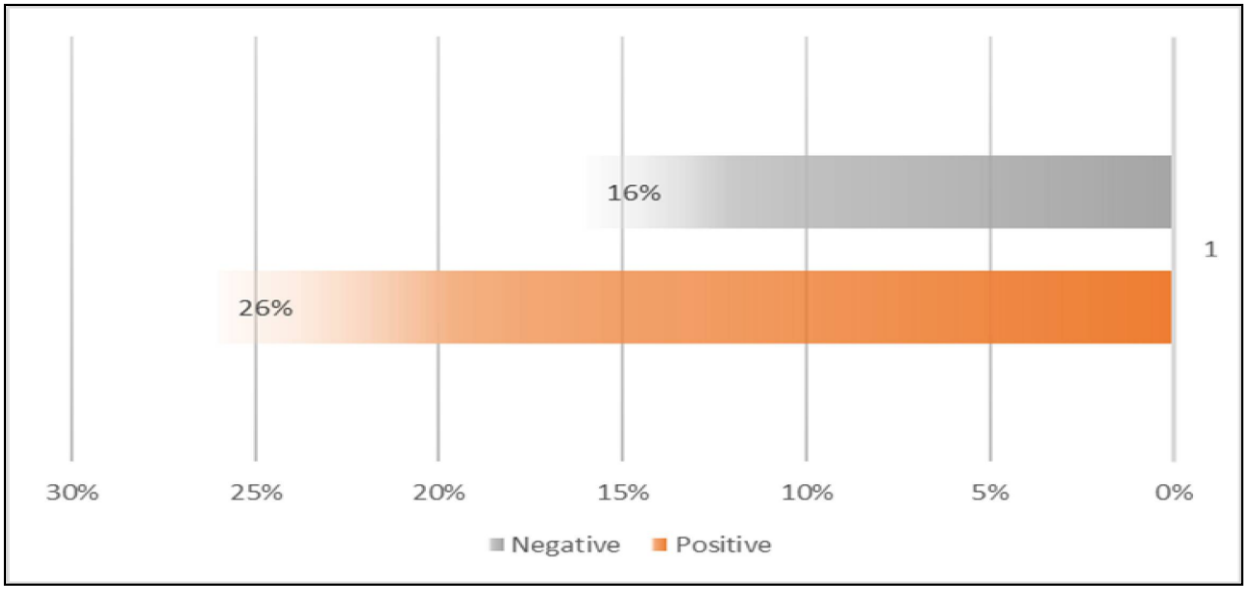

Figure 2: - The Result of Molecular Test