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(RESEARCH ARTICLE)

High-resolution melting curve real-time PCR for rapid differentiation of *Escherichia coli* and *Campylobacter jejuni* in poultry samples

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Abstract

Foodborne pathogens remain a major challenge for public health and food safety worldwide. Rapid and accurate molecular methods are essential for the early detection of bacterial contamination in food products. The present study evaluated the application of real-time PCR combined with melting curve analysis for the rapid differentiation of two major foodborne pathogens, *Escherichia coli* and *Campylobacter jejuni*. A total of 86 chicken carcass swab samples were collected from poultry markets in Baghdad, Iraq. Specific primers targeting the *yhaV* gene of *E. coli* and the *hipO* gene of *C. jejuni* were used in a real-time PCR assay followed by melting curve analysis. Distinct melting temperature profiles were obtained for each species. The mean melting temperature for *E. coli* isolates was **79.75 ± 0.29 °C**, whereas *C. jejuni* isolates showed a higher mean melting temperature of **80.80 ± 0.27 °C**, with a statistically significant difference between the two groups (**$p < 0.01$**). The assay demonstrated high specificity with no secondary melting peaks or nonspecific amplification products. The clear thermal separation between the two pathogens confirms the reliability of melting curve analysis as a rapid molecular discrimination tool. These findings highlight the potential of HRM-based real-time PCR as a rapid screening approach for foodborne pathogens in poultry products and support its application in routine food safety monitoring and epidemiological surveillance programs. This study represents one of the first applications of HRM-based molecular differentiation of foodborne pathogens isolated from poultry in Iraq.

Keywords: *Campylobacter jejuni*; Melting curve; Real-time PCR; qPCR

1. Introduction

Foodborne infections result from the ingestion of contaminated food or beverages. A variety of germs or pathogens can contaminate food, resulting in numerous types of foodborne diseases [1].To date, more than 250 different foodborne illnesses have been described, approximately two-thirds of which are caused by bacteria [2].The majority of foodborne illnesses result from infections induced by diverse bacteria, viruses, and parasites [3].Others are poisonings resulting from deleterious poisons or compounds that adulterate food. Salmonella and *E. coli* have been the most common pathogens in foodborne outbreaks in the United States over the past 15 years, but Vibrio, Campylobacter, and Listeria species are also often responsible[4].Some symptoms of *E. coli*, Salmonella, and Campylobacter are similar, such as bloody or watery diarrhea, stomach pain and cramps, fever, and sometimes vomiting. [5].*Campylobacter jejuni* is one of the main bacterial strains that cause diarrhea in humans. It has been linked to several cases of food poisoning in Several countries around the world. Poultry is the natural host of *Campylobacter jejuni*, and infected birds carry very high loads of the bacteria when they eat raw, undercooked, or improperly prepared chicken, as well as liver and rotisserie chicken. *Campylobacter jejuni* does not grow well in well-refrigerated foods, but it can survive in the refrigerator and grows if contaminated food is left at room temperature. These bacteria are sensitive to heat, so cooking meat and poultry thoroughly can kill it. *Campylobacter jejuni* and *E. coli* account for the highest incidence of foodborne clinical Campylobacteriosis, and therefore, detection and identification of Campylobacter is almost exclusively limited to these two species. [6] found that mPCR-HRM was able to differentiate between *C. coli* and *Campylobacter jejuni*.

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E. coli is often contaminated with undercooked or raw ground beef, mixed vegetable sprouts, salads, cheese, flour, nut butter, and cookies.*E. coli* is a rod-shaped bacterium with numerous harmless strains. However, some strains of *E. coli* (such as *E. coli* O157:H7) can cause serious food poisoning, and recent outbreaks have involved leafy green vegetables and ground beef [5]. The melting points of PCR amplicons can be determined using colored chemical dyes [7] or saturated cross-linking dyes such as Green Plus and Eva Green (Shoute and Loppnow 2018). PCR melting curve analysis is a fast and reliable method, given the widespread use of real-time PCR instruments in medical diagnostics and forensic laboratories [8]. This method can also be adapted to work with other pathogens, such as viruses and bacteria [9], [10]. The aim of this work was to establish a multiplex PCR melting curve analysis for the rapid detection and identification of two predominant food-borne pathogens, i.e., *Escherichia coli* and *Campylobacter jejuni*. We demonstrated the results of specificity, sensitivity and repeatability through single bacterium's DNA testing as well as multiple tests.

2. Materials and Methods

2.1. Sample Collection

The DNA of both *C. jejuni* and *E. coli* strain MG1655, was obtained from the (ATCC, Manassas, VA). Eighty-six random chicken swab samples were collected from obtained from broiler carcasses sold in diverse districts of Baghdad, Iraq between April to October 2024. The gathered chicken carcases were also used for the study. Polymerase chain reaction (PCR) testing and HRM curve analysis were also carried out. All samples were immediately transported to the Food Contamination Laboratory at the Iraqi Scientific Research Council, incubated at a cold temperature (below 4°C), and stored in a refrigerator until the microbes were isolated [11].

2.2. DNA Extraction

The Bacterial Genomic DNA Isolation Kit (Noragen bioteck, Canada) was used to get total DNA from bacterial isolates. The company included instructions on how to obtain DNA. Using a NanoDrop Spectrophotometer: ND-1000 (YangMing Company, Taiwan) at a wavelength of 260 nm, the concentration of the isolated DNA was changed to 50 µg/ml. The purity of the extracted DNA samples, as measured by the 260/280 ratio, was between 1.81 and 1.94.

2.3. PCR primer

The multiplex assay made use of previously described PCR primers for *Escherichia coli*, [12], and *Campylobacter jejuni* (Staji, Birgani and Raeisian 2018). Table 1 lists the sequences of primer and gene names. In order to assess the primers' specificity in silico, the NCBI basic tool was used. For the PCR experiments, the primers derived from Integrated DNA Technologies (IDT- USA.) They were then mixed with nuclease-free water, measured using a biophotpmeter from (eppendrof plus,Germany)

Table 1 PCR assays utilized primers and genes.

Species	Gene name	Forowerd primer	rev
E. coli	yhaV	TATACATATGGATTTTCCACAAAGG	CTCGAGTCAATGGGTTTCTTC
C. jejuni	hipO gene	GAA GAG GGT TIG GGT GGT G	AGC TAG CTT CGC ATA ATA ACT TG

2.4. Analysis of Campylobacter spp. isolates with qPCR-HRM

The qPCR-HRM test was done using the approach that [12]. set up. The primers used in this study were designed to find the hipO gene in *C. jejuni* and the yhaV gene in *E. coli*. The PCR mixture (20 µL) contained 7.5 µL of SYBR Green Master Mix (Thermo Fisher Scientific, USA), 1 µL (10 pmol) of Forowerd and reverse primers for each gene (Integrated DNA Technologies (IDT)-USA), 3 µL of nuclease-free water, and 5 µL of DNA template. A no-template control (NTC) containing nuclease-free water instead of DNA template was included in every qPCR run to monitor potential contamination and non-specific amplification. The smart sycler thermal cycler (cepheid, USA) was used to do the qPCR-HRM amplification.The amplification process was carried out according to a variant of the approach described by [12]. The first stage was an initial denaturation at 95°C for 3min. Polymerase chain reaction was performed in a final volume of 20 µL, denatured at 95°C for 5 minutes after the first cycle and then subjected to 40 cycles of amplification, consisting of a denaturation step at 95°C for 3 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 20 seconds. High-resolution melting analysis was conducted from 70 °C to 90 °C with temperature increments of 0.1 °C to ensure precise discrimination of melting profiles. Fluorescence data were acquired continuously during the melting phase to

generate high-resolution melting profiles. We performed the melting curve behavior analysis and PCR reaction using Q-Rex software, obtained from Q-Rex software Qiagen GmbH (Germany).

3. Results

3.1. Amplification profiles of qPCR

Real-time PCR amplification curves demonstrated efficient and consistent amplification for all tested samples. No amplification signal was detected in the no-template control (NTC), confirming the absence of contamination and the high specificity of the assay.

Results of this study were described by descriptive analysis for all data extracted. This study examined the melting curve patterns and peak melting temperatures in comparison to those for *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560, used as positive control references. We had already found the best temperatures for positive controls, which were 79.5°C±0.3°C for *E. coli* and 80.6°C ± 0.3°C for *C. jejuni (*figure 1*)*. All collected data were classified based on The *C. jejuni* isolates exhibited melting curve patterns that were distinct from the positive control, with a melting peak temperature of 79.5 °C±0.3°C. In contrast, the *e. coli* isolates displayed melting curve patterns that diverged from the positive control, featuring melting peaks at 81.4°C±0.3°C. [13]. [14] showed that HRM curves may be differentiated based on substantial differences in curve design and/or Tm, with a 0.3°C difference being significant.

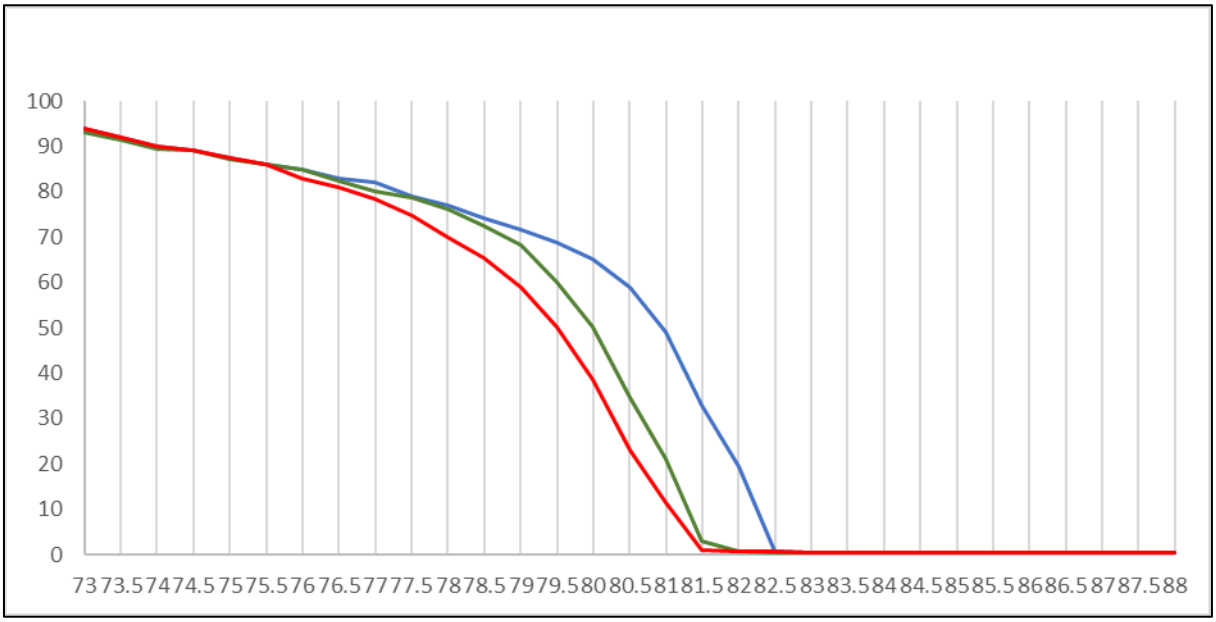

Figure 1 Analysis of conventional and calibrated melting curves for two reference strains of *E. coli* and *C. jejuni* bacteria Analysis of conventional and calibrated high-resolution melting curves for the product of yhaV gene amplification in red and hipO in blue using polymerase chain reaction (PCR) technology

3.2. Melting curve analysis

Melting curve analysis revealed distinct thermal profiles for the two bacterial species. The *Escherichia coli* isolates exhibited melting peaks within the range of 79.55–79.83 °C, whereas Campylobacter jejuni isolates showed higher melting peaks ranging from 80.42–80.87 °C. The fluorescence graphs showed a steady decline as the temperature went higher and then a rapid drop when each species reached its own thermal melting point (Tm). All isolates demonstrated thermal consistency within their individual groups, as shown by peak clustering and the absence of tails, secondary peaks, or nonspecific products, therefore confirming accurate molecular amplification and a unique amplicon for each reaction.

3.3. Statistical analysis of melting temperatures

In *E. coli* isolates, the mean melting temperature was 79.66 ± 0.12 °C and 80.63 ± 0.21 °C in *C. jejuni* isolates ($p<0.001$).

Both groups were significantly different from one another ($F = 68.85$, $p < 0.001$) and one-way ANOVA analysis proved melting temperature to be a reliable differentiating parameter. ROC analysis showed strong diagnostic performance for separating the two bacterial species with an AUC of 1.00.

For all species, first derivative (−dF/dT) curves exhibited distinct and pronounced peaks. The group for C. jejuni had sharper and larger peaks than that for E. coli. This showed a larger difference in temperature shifts for the two populations and further implied that Melting Curve analysis may be used to diagnose different food-borne pathogens as they differ significantly in laboratory evaluations.

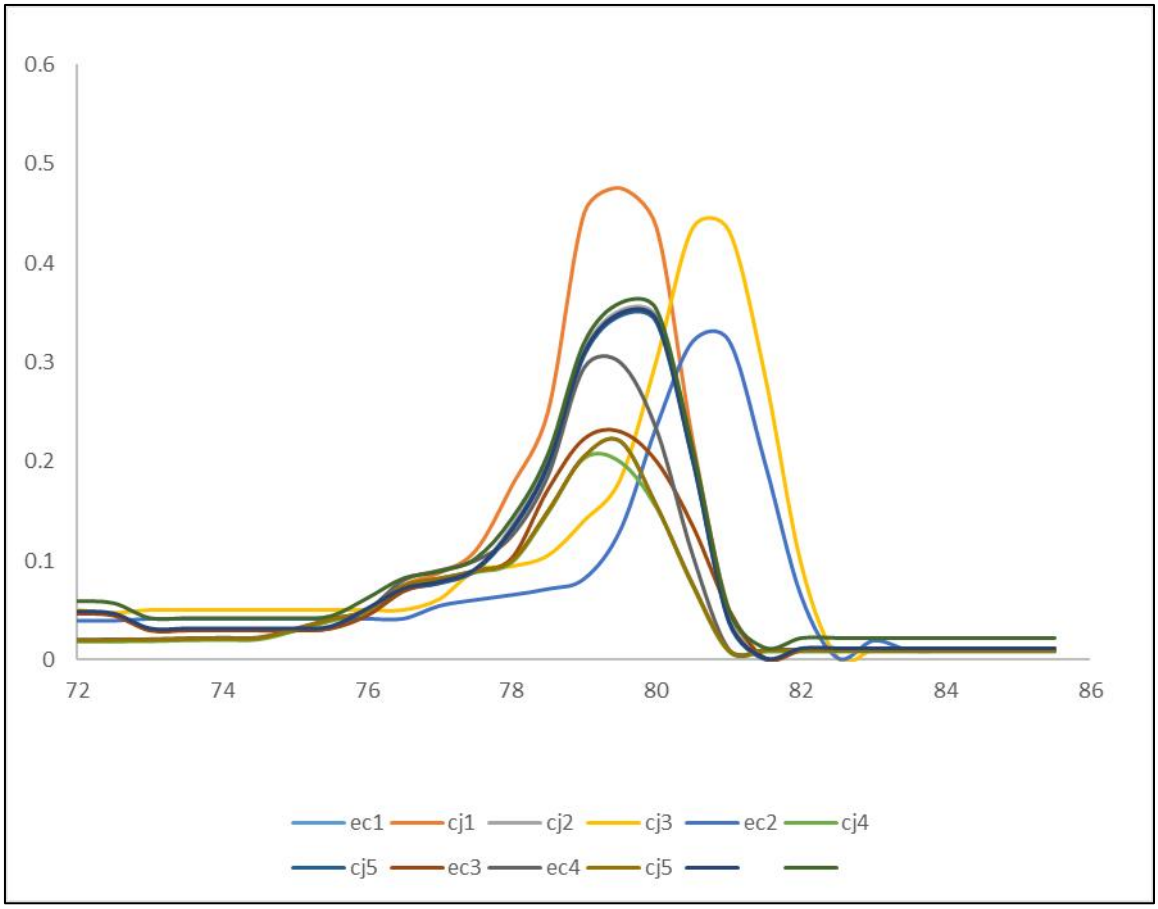

Figure 2 Analysis of the conventional melting curve for *E. coli* and *C. jejuni* species of polymerase chain reaction (PCR) amplification products detected from studied chicken isolates

4. Discussion

The present study demonstrates that melting curve analysis combined with real-time PCR provides a highly sensitive and reliable approach for differentiating *Escherichia coli* and *Campylobacter jejuni*. The statistically significant difference in melting temperatures ($p < 0.001$) confirms that even small thermal variations (~1 °C) are sufficient for accurate species discrimination. No amplification is observed and no secondary peaks are present affirming specificity and robustness of the assay Such results are in line with studies identifying that temperature differences (between 0.5–2 °C) are sufficient for differentiating between bacteria with HRM [15].

In addition, the ROC analysis (AUC = 1.00) demonstrated the potential applicability of the method for fast screening in food safety laboratories and epidemiological monitoring systems.

The negative and progressive reduction of the fluorescence curves after an evident regression point, demonstrated that all isolates had common thermal characteristics and that the initial derivative peaks were clustered in specific ranges. These findings are consistent with those of [15] who showed that *C. jejuni*'s differentiation from *E. coli* and Salmonella via HRM hinge on similar temperature ranges, and the sameness in peak among a species is important determinant of certainty of genetic differentiation. The data are consistent with previous HRM works, especially the reports by [16]

who used hipO to discriminate *C. jejuni* from C. coli at a 1–2°C temperature difference and that of [17] and validated that the differentiation of Tm for Campylobacter with hsp60 yields a consistent thermal signature for various species within the genus. [18] found that hipO is a stable molecular target associated with high-specificity thermal peak, even in the current LAMP-HRM methods. The fact that the curves in the EC and CJ groups are similar demonstrates that the target gene regions are stable and that heat did not modify them. This demonstrates that the DNA extraction was done appropriately and that the reaction conditions were the same each time. Results show Melting Curve analysis serves as a rapid and efficient alternative to conventional approaches requiring post-PCR procedures or electrophoresis, It is also helpful for discovering germs that may make people sick in laboratories that test food and in hospitals [19]. This also means that Multiplex-qPCR approaches will probably be able to determine the difference between more pathogens in one response in the future [20]. This makes the method a reliable and helpful option for molecular diagnostics that are employed all around the world.

5. Conclusion

This study shows that HRM curve analysis coupled with real-time PCR is a quick and robust method for the differentiation of Escherichia coli and *Campylobacter j*ejuni isolates from poultry samples. The HRM-qPCR method reported compares favorably with conventional microbiological methods because it offers a faster, simpler, and more convenient system that eliminates the need for post-PCR processing or gel electrophoresis. This study may facilitate the building of relevant knowledge in food safety surveillance and public health protection by enabling rapid detection of bacterial contamination from poultry products.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] PCR and high-resolution melt curve analysis. PloS one, 10(9), e0138808 .Bintsis, T. (2017) Foodborne pathogens. AIMS Microbiol, 3, 529-56

[2] Ma, X., L. Bo & X. Zhou (2024) Knowledge, attitude, and practice toward foodborne disease among Chinese college students: a cross-sectional survey. Frontiers in Public Health, 12, 1435486.

[3] Olaimat, A. N., A. O. Taybeh, A. Al-Nabulsi, M. Al-Holy, M. m. M. Hatmal, J. Alzyoud, I. Aolymat, M. H. Abughoush, H. Shahbaz, A. Alzyoud, T. Osaili, M. Ayyash, K. M. Coombs & R. Holley (2024) Common and Potential Emerging Foodborne Viruses: A Comprehensive Review. 14, 190.

[4] Duan, K., G. Pang, Y. Duan, H. Onyeaka & J. Krebs (2025) Current research development on food contaminants, future risks, regulatory regime and detection technologies: A systematic literature review. Journal of Environmental Management, 381, 125246.

[5] Sell, J., & Dolan, B. (2018). Common gastrointestinal infections. Primary Care: Clinics in Office Practice, 45(3), 519-532.

[6] Banowary, B., V. T. Dang, S. Sarker, J. H. Connolly, J. Chenu, P. Groves, M. Ayton, S. Raidal, A. Devi, T. Vanniasinkam & S. A. Ghorashi (2015) Differentiation of Campylobacter jejuni and Campylobacter coli Using Multiplex-PCR and High-Resolution Melt Curve Analysis. PLoS One, 10, e0138808.

[7] Mao, F., W. Y. Leung & X. Xin (2007) Characterization of EvaGreen and the implication of its physicochemical properties for qPCR applications. BMC Biotechnol, 7, 76.

[8] Bogiel, T., R. Górniak, W. Ambroziak, S. Zieliński, D. A. Zieja & P. Kanarek (2024) High-Resolution Melting PCR as a Fast and Simple Molecular Biology-Based Method for the Identification of Hypervirulent Clostridioides difficile Strains Directly in Stool Samples. 12, 2228.

[9] Tavares, E. R., de Lima, T. F., Bartolomeu-Gonçalves, G., de Castro, I. M., de Lima, D. G., Borges, P. H. G., ... & Yamada-Ogatta, S. F. (2023). Development of a melting-curve-based multiplex real-time PCR assay for the simultaneous detection of viruses causing respiratory infection. Microorganisms, 11(11), 269.

[10] Ghorbani, J., F. B. Hashemi, F. Jabalameli, M. Emaneini & R. Beigverdi (2022) Multiplex detection of five common respiratory pathogens from bronchoalveolar lavages using high resolution melting curve analysis. BMC Microbiol, 22, 141.

[11] Banowary, B., Dang, V. T., Sarker, S., Connolly, J. H., Chenu, J., Groves, P., ... & Ghorashi, S. A. (2015). Differentiation of Campylobacter jejuni and Campylobacter coli using multiplex-PCR and high-resolution melt curve analysis. PloS one, 10(9), e0138808.

[12] de Boer, P., H. Rahaoui, R. J. Leer, R. C. Montijn & J. M. B. M. van der Vossen (2015) Real-time PCR detection of Campylobacter spp.: A comparison to classic culturing and enrichment. Food Microbiology, 51, 96-100.

[13] Merchant-Patel, S., P. J. Blackall, J. Templeton, E. P. Price, S. Y. Tong, F. Huygens, P. M. J. A. Giffard & e. microbiology (2010) *Campylobacter jejuni* and *Campylobacter coli* genotyping by high-resolution melting analysis of a flaA fragment. 76, 493-499.

[14] Erali, M., K. V. Voelkerding & C. T. Wittwer (2008) High resolution melting applications for clinical laboratory medicine. Exp Mol Pathol, 85, 50-8.

[15] Reese, K. R. & K. M. Elkins (2022) Simultaneous Detection of Foodborne Pathogens Using a Real-Time PCR Triplex High-Resolution Melt Assay. 2, 453-459.

[16] Syarifah, I. K., H. Latif, C. Basri & P. Rahayu (2020) Identification and differentiation of Campylobacter isolated from chicken meat using real-time polymerase chain reaction and high-resolution melting analysis of hipO and glyA genes. Vet World, 13, 1875-1883.

[17] Young, P., P. Tarce, S. Adhikary, J. Connolly, T. Crawshaw & S. A. J. P. o. Ghorashi (2021) Evaluation of high-resolution melt curve analysis for rapid differentiation of Campylobacter hepaticus from other species in birds. 16, e0251328.

[18] Liu, Y., M. Xu, J. Wang, Y. Cao, T. Wang, L. Mu & C. Niu (2022) Detection of Campylobacter jejuni Based on a Real-Time Fluorescence Loop-Mediated Isothermal Amplification Method. Biomed Res Int, 2022, 3613757.

[19] Choi, W., Y. Yamaguchi, J.-W. Lee, K.-M. Jang, M. Inouye, S.-G. Kim, M.-H. Yoon & J.-H. Park (2017) Translation-dependent mRNA cleavage by YhaV in Escherichia coli. FEBS letters, 591.

[20] Staji, H., S. F. Birgani & B. J. A. o. m. Raeisian (2018) Comparative clustering and genotyping of Campylobacter jejuni strains isolated from broiler and turkey feces by using RAPD-PCR and ERIC-PCR analysis. 68, 755-762.