

Presumptive *Campylobacter* occurrence and representative molecular confirmation in poultry and fresh produce from Punjab, India

Nidhi Sharma

nidhisharmaihgi@gmail.com

Lovely Professional University

Naresh Kumar

Lovely Professional University

Article

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Abstract

Campylobacter is a poultry-associated foodborne pathogen, but evidence across poultry and produce matrices is limited in northern India. We surveyed 392 samples collected in four Doaba districts of Punjab from February 2022 to June 2023: 289 poultry-associated matrices or food-contact swabs and 103 spinach or lettuce samples. Presumptive isolates were recovered by selective enrichment and mCCDA culture under microaerophilic conditions and characterized by morphology, motility, oxidase and catalase tests. A representative subset of 24 isolates (18 poultry-associated; six produce) underwent PCR for *Campylobacter* 16S rRNA, *cadF*, *hipO* and *ceuE*. Presumptive *Campylobacter* was recovered from 190 samples (48.5%, 95% CI 43.6–53.4): 144/289 poultry-associated samples (49.8%) and 46/103 produce samples (44.7%; Yates-corrected $\chi^2 = 0.62$, $P = 0.432$). Positivity was 50.0% in spinach and 38.8% in lettuce ($P = 0.344$). All 24 tested isolates were 16S rRNA- and *cadF*-positive; 11 carried *hipO* only, six carried *ceuE* only, one carried both targets, and six remained unresolved to *C. jejuni* or *C. coli*. These findings indicate presumptive contamination in both food chains but do not establish source attribution or human transmission. Expanded quantitative, seasonal, antimicrobial-resistance and genomic surveillance is warranted.

Introduction

Foodborne disease remains a major global public-health challenge. Updated World Health Organization estimates indicate that contaminated food causes approximately 866 million illnesses, 1.52 million deaths and 57.1 million disability-adjusted life years annually [1]. *Campylobacter* spp. are among the principal bacterial hazards within this burden. In the European Union, campylobacteriosis was the most frequently reported zoonosis in 2024, with 168,396 confirmed cases and a notification rate of 55.3 per 100,000 population [2]. Updated United States estimates similarly attribute approximately 1.9 million domestically acquired foodborne illnesses, 13,000 hospitalizations and 197 deaths each year to *Campylobacter* spp. [3].

Thermotolerant *Campylobacter*, particularly *C. jejuni* and *C. coli*, commonly colonize the intestinal tract of poultry without causing overt disease in the host. Contamination can occur during slaughter, defeathering, evisceration, washing, cutting and retail handling, and raw poultry can subsequently contaminate hands, utensils, chopping boards and ready-to-eat foods [4,5]. Human infection usually causes acute gastroenteritis, but can be followed by reactive arthritis, post-infectious irritable bowel syndrome and Guillain-Barré syndrome [6].

Poultry is therefore a central control point, but it is not the only potential vehicle. Leafy vegetables may be contaminated through irrigation water, manure-amended soil, animal faecal deposition, washing water, harvesting containers, transport surfaces or handling [7,8]. Because spinach, lettuce and other salad vegetables may be consumed raw or minimally processed, there may be no final heat step to reduce contamination. The co-location of raw poultry, fresh produce and shared food-contact surfaces in

markets and food outlets creates a plausible interface for environmental or handling-mediated transfer, although detection in different commodities alone cannot establish transmission direction.

Indian surveillance remains fragmented, and routine laboratory diagnosis of human campylobacteriosis is limited in many settings. A recent systematic review and meta-analysis of retail chicken meat and associated environments in India reported a pooled *Campylobacter* prevalence of 18% (95% CI 11–27%) and substantial between-study heterogeneity [9]. The Indian Council of Medical Research Foodborne Pathogen Surveillance and Research Network has begun integrating hospital, market, animal, poultry and environmental surveillance in North-East India [10,11], but region-specific evidence from northern food systems remains limited.

The Doaba region of Punjab, comprising Jalandhar, Hoshiarpur, Kapurthala and Shaheed Bhagat Singh Nagar districts, combines intensive food retail activity, poultry handling, irrigated vegetable production and organized and informal market systems. We therefore assessed presumptive *Campylobacter* occurrence in poultry-associated matrices, food-contact swabs and fresh leafy produce collected across these four districts. The objectives were to estimate aggregate and source-specific positivity, compare spinach and lettuce, characterize presumptive isolates phenotypically, and molecularly confirm a representative subset using genus-, species- and adhesion-associated gene targets. Because PCR was not performed on every presumptive isolate and no human isolates or source-attribution analyses were included, the study was designed to describe contamination patterns rather than prove foodborne transmission.

Results

Presumptive occurrence by sample source

Among 392 analyzable samples, 190 yielded isolates with selective-culture and phenotypic characteristics consistent with thermotolerant *Campylobacter*, corresponding to an overall presumptive occurrence of 48.5% (95% CI 43.6–53.4). Positivity was 144/289 among poultry-associated matrices and food-contact swabs (49.8%, 95% CI 44.1–55.6) and 46/103 among fresh-produce samples (44.7%, 95% CI 35.4–54.3). The absolute difference was 5.1 percentage points and was not statistically significant (Yates-corrected $\chi^2 = 0.62$, df = 1, P = 0.432; Table 1). These estimates describe presumptive culture positivity; they should not be interpreted as PCR-confirmed prevalence because molecular testing was conducted on a subset.

Table 1
Presumptive *Campylobacter* occurrence by source category

Source category	Examined, n	Presumptive positive, n	Occurrence, %	95% CI
Poultry-associated	289	144	49.8	44.1–55.6
Fresh produce	103	46	44.7	35.4–54.3
Total	392	190	48.5	43.6–53.4
Wilson confidence intervals are shown. The source comparison used a two-sided Pearson chi-square test with Yates continuity correction: $\chi^2 = 0.62$, df = 1, P = 0.432.				

Occurrence in spinach and lettuce

Within the 103 produce samples, 27/54 spinach samples were presumptively positive (50.0%, 95% CI 37.1–62.9), compared with 19/49 lettuce samples (38.8%, 95% CI 26.4–52.8). The 11.2-percentage-point difference was not statistically significant (Yates-corrected $\chi^2 = 0.89$, df = 1, P = 0.344; Table 2). No inference about the origin of produce contamination can be made because irrigation water, soil, retail surfaces and paired poultry–produce samples were not tested as linked transmission sets.

Table 2
Presumptive *Campylobacter* occurrence in spinach and lettuce

Vegetable	Examined, n	Presumptive positive, n	Occurrence, %	95% CI
Spinach	54	27	50.0	37.1–62.9
Lettuce	49	19	38.8	26.4–52.8
Total	103	46	44.7	35.4–54.3
Wilson confidence intervals are shown. The vegetable comparison used a two-sided Pearson chi-square test with Yates continuity correction: $\chi^2 = 0.89$, df = 1, P = 0.344.				

Phenotypic characterization

After incubation on modified charcoal cefoperazone deoxycholate agar (mCCDA), presumptive colonies were described as grey to greyish-white, moist, smooth or translucent, with flat to slightly raised morphology. Gram-stained preparations showed slender curved, comma-shaped, S-shaped or spiral Gram-negative rods. Wet mounts showed rapid darting or corkscrew-like motility, and the isolates were oxidase- and catalase-positive. Concordance across selective growth, cellular morphology, motility and biochemical reactions was used to define presumptive thermotolerant *Campylobacter* positivity.

PCR confirmation of the representative subset

Twenty-four presumptive isolates were selected for molecular testing: 18 from poultry-associated positives and six from produce positives. All 24 amplified the expected 816-bp *Campylobacter* 16S rRNA target and the 400-bp *cadF* target (Table 3; Figs. 1 and 2). Among the poultry-associated isolates, eight

carried *hipO* without *ceuE*, four carried *ceuE* without *hipO*, one carried both species-associated targets and five were positive only for 16S rRNA and *cadF*. Among the produce isolates, three carried *hipO* only, two carried *ceuE* only and one was positive only for 16S rRNA and *cadF*. Thus, across the tested subset, 11 isolates were classified as *C. jejuni*-target positive, six as *C. coli*-target positive, one showed co-detection and six were unresolved by the two species targets. These subset counts are descriptive and cannot be extrapolated as species prevalence among all 190 presumptive positives.

Table 3
PCR marker patterns in the representative subset of presumptive isolates

Source	Tested, n	16S rRNA +	<i>cadF</i> +	<i>hipO</i> only	<i>ceuE</i> only	Both targets	Genus/ <i>cadF</i> only
Poultry-associated	18	18	18	8	4	1	5
Fresh produce	6	6	6	3	2	0	1
Total	24	24	24	11	6	1	6
“Only” indicates absence of the other species-associated target. The 16S rRNA and <i>cadF</i> targets were detected in every tested isolate. These data describe the selected subset and are not species-prevalence estimates for all presumptive positives.							

Discussion

This study identified a high proportion of presumptive *Campylobacter*-positive samples across poultry-associated matrices, food-contact swabs and leafy vegetables in the Doaba region of Punjab. The similar aggregate proportions in the poultry-associated and produce groups indicate that both sampled food chains contained substantial contamination signals. The interpretation must, however, remain bounded by the diagnostic design: culture and phenotype were applied to all samples, whereas molecular confirmation was restricted to 24 representative isolates. The primary prevalence estimates are therefore presumptive rather than fully PCR-confirmed.

Poultry-associated samples showed 49.8% positivity, consistent with the established role of poultry as a reservoir for thermotolerant *Campylobacter* [4,5]. The estimate is higher than the pooled Indian retail-chicken estimate reported by Ayoub et al. [9], but direct comparison is limited because the present category combined multiple poultry-associated matrices and contact surfaces, laboratory protocols differed, and the meta-analysis documented marked heterogeneity. The present dataset also lacked sample-type counts and site-level clustering information needed to determine whether intestinal contents, meat, feathers, knives or chopping boards drove the aggregate result. Future analyses should preserve these strata and model shop, market and district as hierarchical sampling units.

Fresh produce showed 44.7% presumptive positivity, including 50.0% in spinach and 38.8% in lettuce. Leafy vegetables can acquire enteric organisms from water, soil, animals, containers and handling [7,8,12]. Their irregular surfaces, retained moisture and consumption without cooking may increase

exposure relevance. Nevertheless, the produce result is unusually high and should be independently confirmed using molecular testing of all isolates, direct PCR with appropriate inhibition controls, and quantitative culture or most-probable-number methods. The current study did not test paired irrigation water, soil, wash water or retail-contact surfaces, and it did not establish transfer from poultry to produce. The findings therefore identify a food-safety signal, not a demonstrated transmission pathway.

The representative PCR results support the presence of thermotolerant *Campylobacter* within both source groups. *hipO* and *ceuE* are commonly used for identification of *C. jejuni* and *C. coli*, respectively [13,14], while *cadF* encodes a fibronectin-binding adhesin involved in host-cell attachment [15,16]. Detection of *cadF* in all tested isolates indicates the presence of this adhesion-associated marker, but it does not demonstrate clinical virulence. Pathogenicity depends on multiple traits, including motility, chemotaxis, invasion, toxin production, stress tolerance, dose and host susceptibility [6,17]. Broader panels including *flaA*, *ciaB*, *peb1* and *cdtABC*, together with whole-genome sequencing, would provide stronger characterization.

Six representative isolates were positive for the genus and *cadF* targets but negative for both species targets, and one poultry-associated isolate showed both *hipO* and *ceuE*. These patterns may reflect non-*jejuni*/non-*coli* species, mixed cultures, target mismatch, DNA quality or assay limitations. The co-detection result should be re-examined from a purified colony and, ideally, confirmed by sequencing. Culture-based recovery itself can be affected by oxygen exposure and stress-induced viable-but-nonculturable states [18,19]. Species assignment should therefore not rely on a limited target panel when isolates give discordant patterns.

The results have practical implications for food handling. Poultry interventions should focus on hygienic evisceration, separation of clean and dirty operations, sanitation of knives and chopping boards, temperature control and prevention of raw-juice contact with ready-to-eat foods. Produce risk management requires safe irrigation and washing water, clean containers and surfaces, and separation from raw poultry during retail and household preparation. These measures are consistent with a One Health approach, but the present study did not evaluate intervention effectiveness.

The study has several limitations. It was cross-sectional and cannot describe seasonality, persistence or direction of transmission. The sample design was reported only at aggregate level, preventing district-, site- and matrix-specific estimates or adjustment for clustering. PCR was performed on 24 of 190 presumptive positives, so molecular confirmation and species-marker distributions cannot be generalized to all positives. No quantitative bacterial load, antimicrobial susceptibility, genome sequence, source-attribution model, human clinical sample or linked environmental-water assessment was included. Exact enrichment-broth details and full PCR reaction and cycling parameters were not present in the supplied manuscript and must be completed for reproducibility. Original uncropped gel images should accompany any cropped or composite presentation. These limitations do not negate the observed contamination signal, but they materially constrain causal and prevalence claims.

In conclusion, presumptive *Campylobacter* was frequently recovered from both poultry-associated and fresh-produce samples collected in the Doaba region. Representative PCR supported genus identity and detected *C. jejuni*-, *C. coli*- and *cadF*-associated targets in both source groups. The study supports strengthened surveillance and hygiene across poultry and produce chains, while highlighting the need for complete molecular confirmation, quantitative microbiology, antimicrobial-resistance testing, seasonal sampling and genomic source attribution before transmission relationships can be inferred.

Methods

Study design and setting

A cross-sectional microbiological survey was conducted from February 2022 to June 2023 in Jalandhar, Hoshiarpur, Kapurthala and Shaheed Bhagat Singh Nagar districts of the Doaba region of Punjab, India. Sampling sites included poultry farms, poultry shops, local vendors, vegetable markets and food outlets. The study evaluated food and food-contact samples and did not include human participants, human biological material or experimental infection of live animals.

Sample categories and inclusion criteria

The final analyzable dataset comprised 392 samples: 289 poultry-associated samples and 103 fresh-produce samples. Poultry-associated matrices included whole chicken, intestinal contents, breast meat, thigh meat, feathers, chopping-board swabs and knife swabs. Produce samples consisted of spinach (n = 54) and lettuce (n = 49) selected because they are frequently consumed raw or minimally processed. Records with incomplete laboratory outcomes or essential metadata were excluded from analysis. The 392-sample dataset was used consistently for the analyses reported here.

Sample collection and transport

Approximately 25 g of poultry meat or produce was collected aseptically into sterile sampling bags. Food-contact surfaces were sampled using sterile swabs pre-moistened with buffered peptone water. Samples were assigned traceability codes incorporating district, source, sample type and collection date. Poultry, produce and swab samples were packaged separately, transported in insulated boxes at approximately 4°C and processed preferably within 4–6 h. Samples not processed immediately were held at 4°C and analyzed within 24 h; freezing was avoided.

Selective enrichment, culture and presumptive identification

Solid samples (25 g) were transferred to 225 mL of buffered peptone water or a selective enrichment broth to prepare a 10^{-1} suspension and were homogenized. Swabs were suspended and vortexed in sterile diluent or enrichment broth. Enriched material was streaked onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated at 41.5–42°C for 24–48 h under microaerophilic conditions

approximating 5% O₂, 10% CO₂ and 85% N₂. The approach was based on standard culture principles for thermotolerant *Campylobacter* [20,21].

Suspect colonies were purified by repeated re-streaking. Fresh cultures were examined after Gram staining and by wet-mount microscopy. Slender curved or spiral Gram-negative rods with rapid darting or corkscrew motility were considered compatible with *Campylobacter*. Oxidase activity was recorded when dark-purple colour developed within 10–30 s after exposure to tetramethyl-*p*-phenylenediamine reagent. Catalase activity was recorded from immediate effervescence after exposure to 3% hydrogen peroxide. Isolates showing compatible selective growth, morphology, motility and positive oxidase and catalase reactions were classified as presumptive thermotolerant *Campylobacter*.

DNA extraction and PCR

A representative subset of 24 presumptive isolates was selected for PCR: 18 from 144 poultry-associated positives and six from 46 produce positives. DNA was prepared from purified colonies by boiling. Bacterial growth was suspended in 100 µL nuclease-free water, heated at 95–100°C for 10 min, chilled on ice and centrifuged at 10,000–12,000 rpm for 5 min. The supernatant was used as DNA template. Targets were a genus-associated 16S rRNA fragment, the adhesion-associated *cadF* gene, *hipO* for *C. jejuni* and *ceuE* for *C. coli* (Table 4) [13–15,22]. Amplicons were resolved on 1.5–2.0% agarose gels and visualized with a gel-documentation system. A 100-bp DNA ladder and no-template negative control were included. The supplied figures also identify positive amplification patterns in the tested isolates.

Table 4
Primers used for molecular confirmation and species-associated identification

Target	Primer sequence (5'–3')	Amplicon	Interpretation	Reference
16S rRNA	F: GGATGACACTTTTCGGAGC R: CATTGTAGCACGTGTGTC	816 bp	Campylobacter genus-associated	[22]
cadF	F: TTGAAGGTAATTTAGATATG R: CTAATACCTAAAGTTGAAAC	400 bp	Adhesion-associated marker	[15]
hipO	F: ACTTCTTTATTGCTTGCTGC R: GCCACAACAAGTAAAGAAGC	323 bp	C. jejuni-associated	[13]
ceuE	F: AATTGAAAATTGCTCCAACATG R: TGATTTTATTATTTGTAGCAGCG	462 bp	C. coli-associated	[14]

Image acquisition and processing

Gel images were supplied as exported image files. Figure 1 contains two juxtaposed gel segments separated by a visible vertical boundary. No quantitative comparisons were made between segments. Figure 2 shows one gel image with a molecular marker and two negative-control lanes. Only global resizing for page layout was applied in this manuscript; band content was not retouched. Original, uncropped gel images with gel edges visible, acquisition settings and any exposure or contrast adjustments should be supplied as Supplementary Fig. S1 in accordance with journal image-integrity requirements.

Statistical analysis

Positivity was calculated as the number of presumptive positive samples divided by the number examined. Wilson 95% confidence intervals were calculated for the overall, source-specific and vegetable-specific proportions. Two-by-two comparisons used Pearson's chi-square test with Yates continuity correction because all expected cell counts exceeded five. Tests were two-sided, the significance threshold was $\alpha = 0.05$, and exact P values are reported. The poultry-associated versus produce comparison used $n = 392$ sample records; the spinach versus lettuce comparison used $n = 103$. No adjustment for clustering by collection site or for multiple comparisons was possible because site-level cluster identifiers and a prespecified family of confirmatory comparisons were not available. The reported statistics were independently reproduced in Python 3.13.5 using SciPy 1.17.0 and statsmodels 0.14.6.

Declarations

Ethics statement

Formal human- or animal-research ethics approval was not applicable because the study analyzed retail food products and environmental food-contact swabs and involved no human participants, identifiable personal data, human biological material, live-animal procedures or experimental infection.

Use of generative artificial intelligence

A large language model was used for language editing, structural reorganization and consistency checking during manuscript preparation. It was not used to generate experimental observations or replace author verification of the data, analyses, references or scientific interpretation. All authors remain accountable for the submitted work.

Funding Declaration

This research was conducted as a part of the corresponding author's Ph.D. research work and did not receive any specific funding from public, commercial, or not-for-profit funding agencies.

Additional information

Supplementary information: Original uncropped gel images with gel edges, molecular markers and lane labels visible should be supplied as Supplementary Fig. S1. Any cropping, lane juxtaposition and global image adjustment must be described in the accompanying legend.

Competing interests

The authors declare no competing interests.

Author Contribution

N.S. wrote the main manuscript text and prepared figures and tables. All authors reviewed the manuscript.

Data Availability

All aggregate data supporting the reported analyses are contained within this article. The de-identified sample-level laboratory dataset and original uncropped gel images are held by the study investigators and will be made available by the corresponding author on reasonable request, subject to applicable institutional data-governance requirements.

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Figures

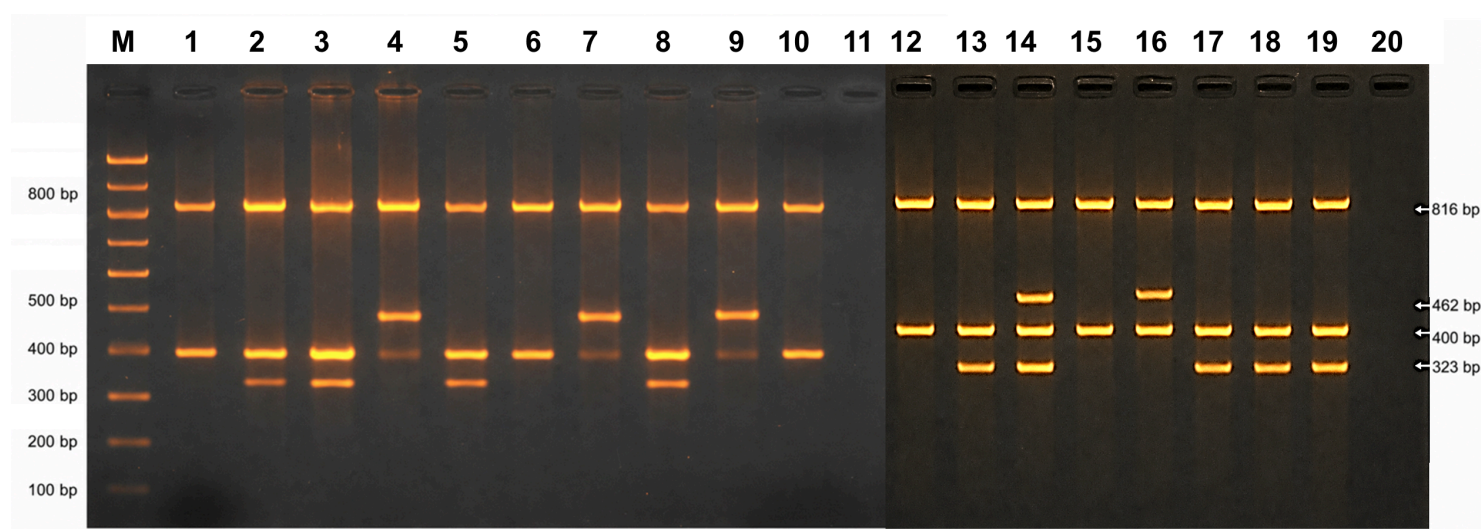


Figure 1

PCR profiles of representative poultry-associated isolates. The supplied figure contains two juxtaposed gel segments separated by a visible vertical boundary. M, 100-bp molecular marker. Lanes 1, 6, 10, 12 and 15 show 816-bp 16S rRNA and 400-bp *cadF* products. Lanes 2, 3, 5, 8, 13, 17, 18 and 19 additionally show a 323-bp *hipO* product. Lanes 4, 7, 9 and 16 additionally show a 462-bp *ceuE* product. Lane 14 shows both 323-bp and 462-bp products. Lanes 11 and 20 are no-template negative controls. Original uncropped gels must be provided as Supplementary Fig. S1.

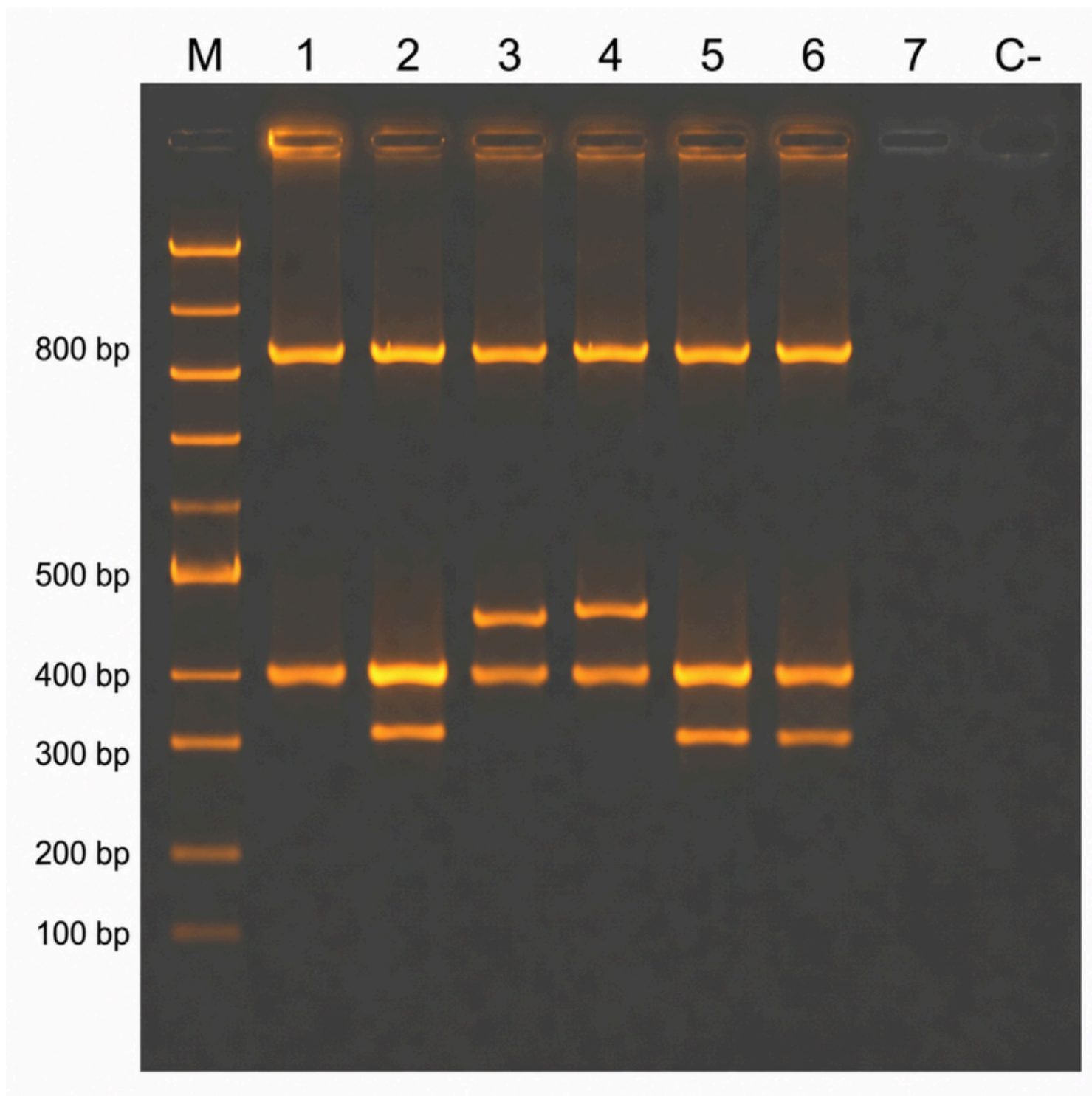


Figure 2

PCR profiles of representative fresh-produce isolates. M, 100-bp molecular marker. Lane 1 shows 816-bp 16S rRNA and 400-bp *cadF* products. Lanes 2, 5 and 6 additionally show a 323-bp *hipO* product. Lanes 3 and 4 additionally show a 462-bp *ceuE* product. Lane 7 and C- are negative-control lanes without visible amplification. Original uncropped gel data must be provided as Supplementary Fig. S1.