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Characterisation of ESBL-producing *Escherichia coli* from ready-to-eat chicken shawarma, chicken sandwich and mayonnaise from Thrissur and Ernakulam districts of Kerala#

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Abstract

This study investigated extended-spectrum β-lactamase (ESBL)-producing Escherichia coli (E. coli) in ready-to-eat (RTE) chicken shawarma, chicken sandwiches and mayonnaise retailed in Thrissur and Ernakulam, Kerala, India. ESBL-producing E. coli in RTE foods represents an important food-safety and antimicrobial-resistance concern because these products are consumed without further cooking, enabling direct consumer exposure. A total of 150 samples (50 per product) from 50 outlets were examined using standard enrichment, culture on MacConkey agar (MCA) and Eosin Methylene Blue (EMB) agar, and biochemical identification, with genus-level molecular characterisation by PCR targeting the 16S rRNA gene. ESBL production was screened by the combined disc diffusion method and genotyped by PCR for bla_{TEM}, bla_{SHV} and bla_{CTX-M}. Overall, 70 out of 150 samples (46.7 per cent) yielded Escherichia spp. isolates, biochemically consistent with E. coli, with a significantly higher occurrence in shawarma (68.0 per cent) than in mayonnaise (40.0 per cent) and chicken sandwiches (32.0 per cent), indicating a clear matrix effect for this complex, highly handled poultry-based RTE product. Among the 70 confirmed isolates, 23 (32.9 per cent) were ESBL producers. ESBL-positive isolates predominantly carried bla_{TEM} (52.17 per cent), followed by bla_{CTX-M} (39.13 per cent) and bla_{SHV} (17.39 per cent), and these genes were frequently detected in combination, supporting the likelihood of multideterminant resistance dissemination within the RTE food chain. Collectively, these findings identify the sampled poultry-based RTE foods as potential vehicles for ESBL-producing E. coli and underscore the need for strengthened hygiene and process control at retail, routine microbiological monitoring, and One Health–aligned antimicrobial stewardship.

Keywords: *Escherichia coli, ready-to-eat foods, extended-spectrum β-lactamase*

Food safety remains fundamental to public health and economic productivity, yet the burden of foodborne disease remains high in many low- and middle-income countries (LMICs) because of weak regulation, limited infrastructure and large informal food markets. The World Health Organisation estimates that contaminated food causes around 600 million illnesses and 420,000 deaths annually (WHO, 2024). In India, foodborne illness remains under-recognised, with 2,700

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outbreaks and over 570 deaths reported between 2009 and 2018 (Bisht *et al*., 2021). In Kerala, improved surveillance has documented 3,394 food poisoning cases and two deaths in 2024 alone, indicating that outbreaks persist even where reporting systems are robust (Directorate of Health Services, Government of Kerala, 2025). RTE foods are of particular concern as they are consumed without further cooking and are often associated with poor refrigeration, unhygienic handling and limited microbiological oversight. In Kerala, rapid urbanisation, food tourism and a vibrant street-food culture have driven increased consumption of RTE items such as chicken shawarma, chicken sandwiches and mayonnaise, which are especially risky due to vertical stacking and uneven heating of meat, frequent handling of sauces, raw vegetables and use of raw-egg mayonnaise. Among foodborne pathogens, *E. coli* serves both as an indicator of faecal contamination and an important cause of diarrhoeal disease (WHO, 2018). The emergence of antimicrobial-resistant (AMR) *E. coli*, particularly ESBL producers, further limits therapeutic options and facilitates dissemination of resistance along the food chain (Castanheira *et al*., 2021). These factors underscore the need for evidence on high- risk RTE foods in Kerala. Accordingly, the present study characterised ESBL-producing *E. coli* from chicken shawarma, chicken sandwiches and mayonnaise in Thrissur and Ernakulam.

Materials and methods

Study area and sample collection

This study was conducted in Thrissur and Ernakulam districts of Kerala, India. A total of 50 retail outlets (25 per district) were selected, and from each outlet one sample each of chicken shawarma, chicken sandwich and mayonnaise was collected, yielding 150 RTE food samples. Sampling was carried out over six months from March to August 2025. Immediately after collection, samples were transported to the laboratory in insulated ice-cooled containers and maintained under chilled conditions. All samples were processed within 4 h of receipt.

Processing of food samples and isolation of E. coli

All food samples were examined for the presence of *E. coli* following the protocol of Meng *et al.* (2001) with minor modifications. Approximately 25 g of each sample was aseptically weighed and homogenised with 225 mL of Tryptone Soya Broth (TSB) in a laboratory stomacher for 120 seconds. The homogenates were pre-enriched at 37°C for 18 hours. Following enrichment, a loopful of each culture was streaked onto MCA and incubated at 37°C for 18 hours. Lactose-fermenting colonies exhibiting typical pink pigmentation were selected and subcultured onto EMB agar. Colonies showing the characteristic green metallic sheen on EMB were regarded as presumptive *E. coli* and were identified using standard morphological and biochemical tests (Barrow and Feltham, 1993).

Genus-level characterisation by 16S rRNA PCR

All biochemically confirmed isolates were subjected to PCR targeting the 16S *rRNA* gene to confirm affiliation to the genus *Escherichia*. The primer sequences, expected amplicon sizes, and corresponding references are presented in Table 1. Genomic DNA extracted by snap-chilling was used as template (50 ng/µL) in a 25 µL reaction mixture containing 2.5 µL of 10× PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 1 µL of dNTP mix (10 mM each), 0.5 µL of Taq DNA polymerase (5 U/µL) and 1 µL each of forward and reverse primers (10 µM), and 15.5 µL of nuclease-free distilled water. Amplification was performed under standard cycling conditions comprising initial denaturation, 30–35 cycles of denaturation, annealing at 50°C and extension, followed by a final extension step. Amplicons were resolved by agarose gel electrophoresis and visualised under UV transillumination.

Phenotypic detection of ESBL production

Phenotypic detection of ESBL production among *Escherichia* spp. isolates was performed using the combined disc diffusion (clavulanate synergy) method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. Isolates were initially screened using third-generation cephalosporins, namely cefotaxime (30 µg) and ceftazidime (30 µg). Isolates showing reduced inhibition zones were further tested using cefotaxime (30 µg) and ceftazidime (30 µg), alone and in combination with clavulanic acid (10 µg). An increase of ≥5 mm in zone diameter for either antimicrobial agent tested in combination with clavulanate compared to the cephalosporin alone was interpreted as indicative of ESBL production. Confirmed ESBL-producing isolates were preserved for subsequent molecular characterisation.

Molecular detection of ESBL-encoding genes

All phenotypically confirmed ESBL-producing isolates were examined for the presence of ESBL-encoding genes bla_{TEM}, bla_{SHV} and bla_{CTX-M} using uniplex PCR assays. Gene-specific primer sequences, expected amplicon sizes and references are presented in Table 1. PCR amplification was performed in a 25 µL reaction mixture containing 2.5 µL of 10× PCR buffer, 1.8 µL of 25 mM $MgCl_2$, 0.25 µL of dNTPs (10 mM each), 0.5 µL of Taq DNA polymerase (5 U/µL) and 1 µL each of forward and reverse primers (10 µM), and 15.95 µL of nuclease-free distilled water, with minor optimisation of reagent concentrations for individual targets. Cycling conditions consisted of initial denaturation, followed by 30-35 amplification cycles of denaturation, annealing at 56°C and extension, with a final extension step. Amplicons were analysed by agarose gel electrophoresis and visualised under UV transillumination.

Table 1. Primers used PCR

Genes	Primer	Length (bp)	Primer sequence	Size (bp)	Reference
16S*rRNA*	F	21	5'-ATCAACCGAGATTCCCCCAGT -3'	231	Sundong-bo *et al.* (2011)
	R	20	5'-CACTATCGGTCAGT CAGGAG -3'		
bla_{CTX-M}	F	17	5'-CGCTTTGCGATGTGCAG-3'	550	Ahmed *et al.* (2004)
	R	17	5'ACCGCGATATCGTTGGT-3'		
bla_{SHV}	F	22	5'GATGAACGCTTTCCCCATGATG-3'	214	Yazdi *et al.* (2012)
	R	21	5'-CGCTGTTATCGCTCATGGTAA-3'		
bla_{TEM}	F	20	5'-ATGAGTATTCAACATTTCCG-3'	847	Yazdi *et al*. (2012)
	R	20	5'-GTCACAGTTACCAATGCTTA-3'		

Statistical analysis

All data were analysed using SPSS (version 24.0). Differences in the occurrence of *Escherichia* spp., ESBL production and ESBL-encoding genes across sample types were assessed using the Chi-square (χ^2) test. Occurrence of ESBL-encoding genes bla_{TEM}, bla_{SHV} and bla_{CTX-M} among isolates was evaluated using Cochran's Q test. Where significant differences were detected, post-hoc pairwise comparisons were performed using Bonferroni-adjusted McNemar tests. A p-value of < 0.05 was considered statistically significant.

Results and discussion

Occurrence of E. coli across sample types

In the present study, *E. coli* occurrence was determined using culture-based isolation, biochemical characterisation, and genus-level characterisation was performed by the detection of 16S *rRNA* gene. Overall, *E. coli* was detected in 70 out of 150 food samples (46.7 per cent), indicating substantial hygienic compromise in these foods.

Across sample types, *E. coli* occurrence differed significantly, with the highest positivity in chicken shawarma (68.0 per cent), followed by mayonnaise (40.0 per cent) and chicken sandwich (32.0 per cent) (Table 2). The strong association between sample type and positivity ($\chi^2 = 51.604$, $p < 0.05$) confirmed a clear matrix effect, indicating greater vulnerability of composite, high-handling RTE foods compared with simpler matrices.

Comparable matrix-linked patterns have been reported elsewhere. In Al Qassim, Saudi Arabia, *E. coli* was more frequent in chicken shawarma (38.67 per cent) than in chicken burgers (29.33 per cent) (Abalkhail, 2023). In Tiruchirappalli, India, mixed vegetable salads and fried items showed higher positivity than meat products (Jenifer and Sathiyamurthy, 2020), while in Mymensingh, Bangladesh, salad vegetables exhibited greater contamination than RTE meat and milk products (Ema *et al*., 2022), supporting that risk differentials are driven by matrix composition and preparation practices rather than uniform background prevalence.

Overall, the pattern in the present study was most consistent with contamination pathways intensified by extensive manual handling (slicing, mixing, assembling) and incorporation of raw vegetables in composite RTE foods, increasing opportunities for transfer from hands and food-contact surfaces. The marked gradient across foods more plausibly reflects matrix-specific exposure to hygiene lapses at preparation surfaces and time–temperature deviations during preparation and holding, particularly affecting composite and emulsion-type products.

Occurrence of ESBL-producing E. coli

Phenotypic screening of the confirmed *E. coli* isolates for ESBL production was carried out using the combined disc diffusion (clavulanate synergy) method and interpreted according to CLSI. (2024). Overall, ESBL production was detected in 23 of 70 *E. coli* isolates recovered from RTE chicken shawarma, chicken sandwich and mayonnaise, corresponding to an occurrence of 32.86 per cent.

Table 2. Distribution of *E. coli* across different sample types

Sample type	Total no. of samples	Samples positive for *E. coli*	
		No.	Per cent (%)
Chicken shawarma	50	34	68.0[a]
Chicken sandwich	50	16	32.0[b]
Mayonnaise	50	20	40.0[b]
Total	150	70	46.7
Chi-square test: $\chi^2 = 51.604$, $p < 0.05$*			

Values bearing the different superscript within the column differ significantly, while bearing the same superscript within the column do not differ significantly; * $p < 0.05$

Similar levels of ESBL-producing isolates have been documented in street-vended and RTE foods elsewhere. Giri *et al*. (2021) reported ESBL-producing *E. coli* in 25.42 per cent of isolates from Indian street foods, while Zurita *et al*. (2020) detected cefotaxime-resistant coliforms in 22.6 per cent of street foods in Ecuador, with ESBL-producing *E. coli* harbouring bla_{CTX-M} variants confirmed in 20 samples. Likewise, Yaici *et al*. (2017) identified ESBL/AmpC-producing Enterobacteriaceae in 21 of 200 street-vended sandwiches in Algeria, including 18 *E. coli* isolates harbouring ESBL genes.

Collectively, these findings indicated that ESBL-producing *E. coli* were now commonly associated with RTE foods across diverse settings. The relatively high occurrence of ESBL-producing isolates (32.86 per cent) in the present work lies at the upper end of reported ranges and likely reflects several context-specific risk factors. Meat-rich fillings, prolonged holding and repeated heating of shawarma, and the addition of raw vegetables and sauces without further cooking facilitate survival and introduction of resistant strains. Inadequate sanitation, contaminated water and poor hand hygiene further promote cross-contamination, suggesting that shawarma and sandwich outlets may act as community reservoirs for ESBL-producing *E. coli*, highlighting the need for strict hygiene monitoring and control measures.

Occurrence of ESBL-producing E. coli isolates across sample types

Analysis revealed that 23 of the 70 *E. coli* isolates (32.9 per cent) were ESBL producers, with the highest proportion detected in chicken sandwiches (43.8 per cent), followed by shawarma (32.4 per cent) and mayonnaise (25.0 per cent) (Table 3). However, the variation among product types was not statistically significant ($\chi^2 = 1.424$, $p = 0.491$), indicating that although chicken sandwiches numerically harboured more ESBL-producing isolates, all three RTE matrices contributed comparably to the dissemination of resistant *E. coli*.

This distribution indicated chicken meat as a key upstream source of ESBL-producing *E. coli* in RTE foods. When compared with studies, Mathew *et al*. (2025) reported multidrug-resistant *E. coli* in raw chicken meat from Kerala, reinforcing poultry as a significant source of resistance determinants. Similarly, Gazal *et al*. (2021) detected ESBL/AmpC-producing *E. coli* at multiple points along the poultry processing chain, including carcasses, equipment and processing environments, highlighting persistent contamination and opportunities for transfer to cooked products. Beyond source contamination, product-specific handling and preparation practices likely shaped the observed ESBL distribution.

The relatively higher ESBL proportion in chicken sandwiches can be attributed to the assembly of cooked chicken with raw vegetables, sauces and bread under conditions of repeated manual handling, increasing the risk of post-cooking contamination from food handlers, utensils and preparation surfaces. In contrast, shawarma meat undergoes prolonged high-temperature roasting that may reduce bacterial load, while mayonnaise typically involves fewer handling steps. Nevertheless, the lack of a statistically significant difference underscores that ESBL contamination was not confined to a single matrix, emphasising the need for uniform hygiene and control measures across all RTE food categories.

Occurrence of ESBL-encoding genes among E. coli isolates

The phenotypically confirmed isolates were subjected to molecular screening by PCR targeting three β-lactamase genes, namely bla_{TEM}, bla_{SHV} and bla_{CTX-M}. The PCR assays for the individual genes were standardised in the laboratory to obtain the expected amplicon sizes of 847 bp, 214 bp and 550 bp, respectively. The amplified products were resolved by submarine agarose gel electrophoresis (Figs. 1-3).

In this study, molecular characterisation of ESBL-producing *E. coli* showed that bla_{TEM} was the most frequent gene (52.17 per cent), followed by bla_{CTX-M} (39.13 per cent) and bla_{SHV} (17.39 per cent) (Table 4). Cochran's Q test demonstrated a statistically significant difference in the overall distribution of ESBL-encoding genes among the isolates ($Q = 8.167$; $p = 0.017$). The post-hoc analysis revealed that bla_{TEM} occurred significantly more frequently than bla_{SHV}, whereas bla_{CTX-M} exhibited an intermediate distribution pattern and did not differ significantly from bla_{TEM}.

Table 3. Occurrence of ESBL-producing *E. coli* isolates across sample types

Sample type	No. of *E. coli* isolates	ESBL-producing isolates	
		No.	%
Chicken shawarma	34	11	32.4
Chicken sandwich	16	7	43.8
Mayonnaise	20	5	25.0
Total	70	23	32.9
Chi-square (χ^2) = 1.424; p = 0.491^{ns}			

ns: nonsignificant

Comparable patterns have been documented in food and animal sources. In raw milk from Wayanad, Akarsh *et al*. (2019) reported *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ in 56.66, 40.00 and 26.66 per cent of *E. coli* isolates, respectively. Giri *et al*. (2021) likewise found *bla*$_{TEM}$ (40.68 per cent) to be the most prevalent β-lactamase gene, followed by *bla*$_{CTX-M}$ (32.20 per cent) and *bla*$_{SHV}$ (10.17 per cent) in ESBL-producing *E. coli* from Indian RTE street foods. In contrast, broiler-farm isolates from the Philippines were dominated by *bla*$_{CTX-M}$, with high prevalence and complex co-existence patterns of *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$, indicating CTX-M–driven ESBL epidemiology in that production system. (Gundran *et al.,* 2019)

Collectively, the *bla*$_{TEM}$-dominant ESBL profile observed in the present RTE poultry foods can be interpreted as a selection-driven ESBL reservoir persisting in the food chain, while *bla*$_{CTX-M}$ lineages were increasingly reported as the dominant ESBL family across One-Health compartments (humans, animals, and food-associated niches) (Castanheira *et al*., 2021). The intermediate *bla*$_{CTX-M}$ frequency alongside low *bla*$_{SHV}$ detection is consistent with the broader epidemiological transition in which CTX-M-type ESBLs have expanded rapidly via conjugative plasmids, progressively displacing older TEM/SHV-centred patterns in many settings (Rossolini *et al*., 2008; Castanheira *et al*., 2021). Evidence from broiler production also demonstrates high *bla*$_{CTX-M}$ prevalence with frequent co-carriage of *bla*$_{CTX-M}$ + *bla*$_{TEM}$ ± *bla*$_{SHV}$, supporting the plausibility that poultry supply chains can introduce CTX-M-positive *E. coli* into downstream retail foods (Gundran *et al*., 2019). Finally, the mixed matrix typical of shawarma/sandwich outlets—cooked chicken handled alongside raw salads and mayonnaise/sauces—can act as a convergence point for ESBL-bearing *E. coli*, as ESBL producers have been documented in high-handling RTE components such as salads/condiments and chicken in street-food settings (Giri *et al*., 2021).

Occurrence of ESBL-encoding genes across sample types

Across sample types, ESBL gene profiles showed *bla*$_{TEM}$ as the most prevalent determinant (12 isolates), followed by *bla*$_{CTX-M}$ (nine isolates) and *bla*$_{SHV}$ (four isolates). *bla*$_{TEM}$ gene was detected in chicken shawarma (seven isolates), chicken sandwich (three isolates) and mayonnaise (two isolates). *bla*$_{CTX-M}$ was

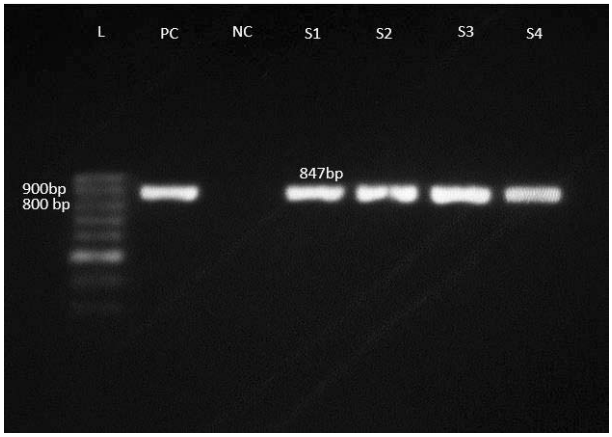

Fig. 1. Detection of *bla*$_{TEM}$ gene (847 bp) by PCR

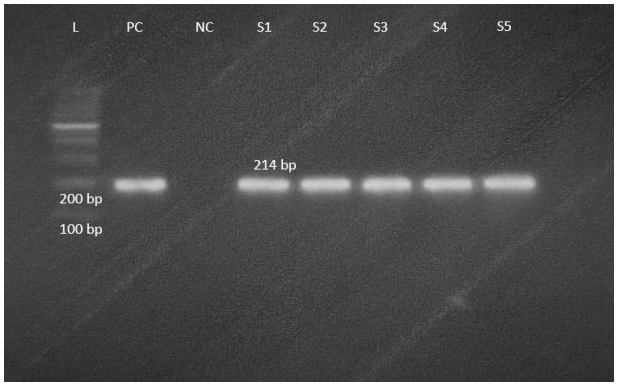

Fig. 2. Detection of *bla*$_{SHV}$ gene (214 bp) by PCR

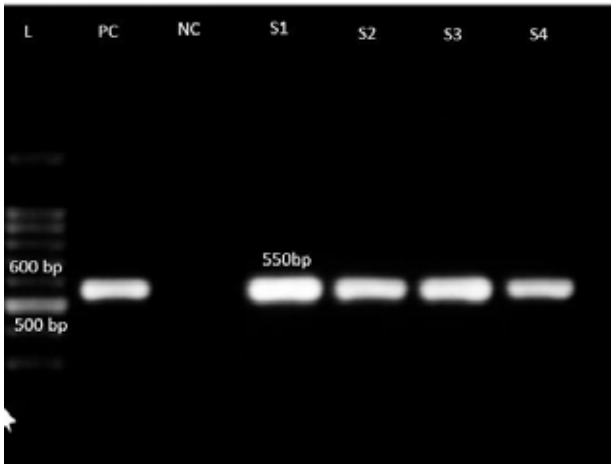

Fig. 3. Detection of *bla*$_{CTX-M}$ gene (550 bp) by PCR

L-Molecular ladder (100bp); PC- Positive control; NC- Negative control; Lane S1-S5 - Sample

Table 4. *Occurrence of ESBL-producing genes among E. coli* isolates

Gene	Total number of ESBL phenotypic isolates	No. of isolates harbouring the gene	Per cent of positive isolates (%)	Cochran's Q value	p-value
bla$_{TEM}$	23	12	52.17^{a}	8.167	0.017*
bla$_{SHV}$	23	4	17.39^{b}		
bla$_{CTX-M}$	23	9	39.13^{ab}		

Values bearing the different superscript within the column differ significantly while bearing the same superscript within the column do not differ significantly *significant at 0.05

identified in shawarma (five isolates) and sandwich (four isolates) isolates, but was not detected in mayonnaise. Gene bla_{SHV} occurred at a lower frequency, being observed in one shawarma isolate, two sandwich isolates and one mayonnaise isolate (Table 5). Overall, the distribution of ESBL genes did not differ significantly among the three food categories, indicating comparable ESBL gene occurrence across sample types despite minor product-specific variation in individual gene detection patterns.

Evidence from food and agricultural settings supports this pattern of shared gene pools across matrices. Fernandez-Gomez *et al.* (2022) reported that 83 per cent of food-derived isolates carried bla_{TEM}, whereas bla_{CTX-M} was present in only 33 per cent, with gene distribution varying between food and clinical sources but not clearly segregated within foods. Igbinosa *et al*. (2023) likewise found $bla_{CTX-M-1}$ and bla_{TEM} among *E. coli* from matrices such as RTE salads, lettuce and tomatoes, without a consistent dominance linked to any single food type. Gundran *et al*. (2019) observed comparable frequencies of bla_{CTX-M}, bla_{TEM} and bla_{SHV} in cloacal and boot swab samples from broiler farms, suggesting exposure to common ESBL reservoirs.

Taken together, these observations support the present finding that ESBL gene carriage in shawarma, sandwich and mayonnaise was likely driven by shared contamination pathways rather than intrinsic matrix differences.

Distribution of ESBL-encoding genes within E. coli isolates

Genotypic profiling of the 23 ESBL-producing *E. coli* isolates in the present study showed that bla_{TEM} and bla_{CTX-M} predominated. The most frequent pattern observed within isolates was the double-gene combination bla_{TEM} + bla_{CTX-M} (39.1 per cent), followed by bla_{TEM} alone (17.4 per cent) and bla_{TEM} + bla_{SHV} (13.0 per cent). The remaining profiles – bla_{CTX-M} + bla_{SHV}, triple-gene carriage and single-gene bla_{CTX-M} or bla_{SHV} – each accounted for 8.7 per cent of isolates, indicating that most strains carried more than one ESBL determinant (Fig. 4).

Comparable co-occurrence of ESBL genes has been reported in food and animal matrices. Akarsh *et al.* (2019) detected bla_{TEM}, bla_{CTX-M} and bla_{SHV} in 56.66, 40.00

Table 5. Occurrence of ESBL-encoding genes across sample types

Gene	No. of ESBL-producing isolates harbouring the gene			Total gene occurrence	Chi-square value	p-value
	Chicken shawarma	Chicken sandwich	Mayonnaise			
bla_{TEM}	7	3	2	12	1.120	0.571^{ns}
bla_{CTX-M}	5	4	0	9	4.353	0.113^{ns}
bla_{SHV}	1	2	1	4	1.160	0.560^{ns}
Total	13	9	3	25		

ns: nonsignificant

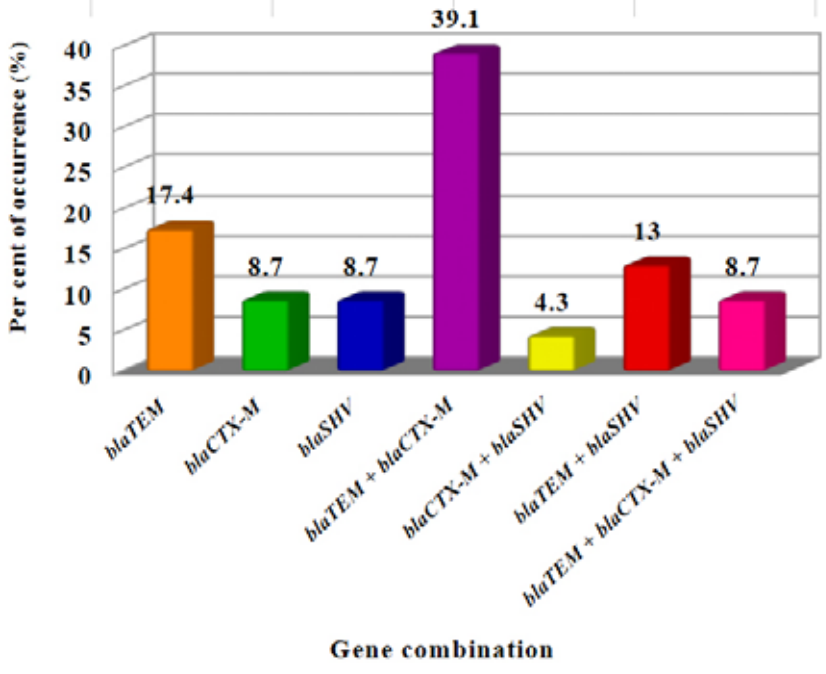

Fig. 4. Distribution of ESBL-producing genes among ESBL-producing isolates

and 26.66 per cent of raw-milk *E. coli*, with bla_{TEM} + bla_{CTX-M} the most common combination. Kalaiselvi *et al.* (2018) observed that the majority of ESBL-producing *E. coli* from chicken meat carried multiple ESBL genes, and 62.5 per cent harboured all three. Similarly, Gundran *et al.* (2019) reported bla_{CTX-M} + bla_{TEM} as the predominant genotype among broiler isolates.

The prominence of bla_{TEM} + bla_{CTX-M} and the detection of triple-gene carriers in the present work are therefore consistent with poultry-associated ESBL reservoirs and suggested plasmid-mediated accumulation of multiple ESBL determinants in *E. coli* contaminating chicken-based RTE products. Such multi-gene carriage widens the β-lactam hydrolytic spectrum, enhances the likelihood of co-selection under antimicrobial and biocide pressure and favours persistence along processing and retail environments, underscoring the need for strengthened molecular surveillance and control measures throughout the RTE food chain.

Conclusion

The present study demonstrated substantial contamination of chicken-based RTE foods with *Escherichia* spp., with significantly higher occurrence in shawarma compared to sandwiches and mayonnaise, indicating matrix-dependent contamination risk. Approximately one-third of isolates were ESBL producers, highlighting the presence of β-lactam resistance within retail RTE environments. bla_{TEM} predominated, followed by bla_{CTX-M} and bla_{SHV}, with frequent multi-gene carriage suggesting plasmid-mediated dissemination. Collectively, these findings identify poultry-based RTE foods as potential community reservoirs of ESBL-producing *Escherichia* spp. Strengthened retail hygiene, segregation of raw and cooked ingredients, and routine molecular surveillance are essential to mitigate consumer exposure and limit the spread of antimicrobial resistance within the food chain.

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Conflict of interest

The authors declare that they have no conflict of interest.

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