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Assessment of Biofilm Formation and Shiga-like Toxin Genes in ESBL-producing *E. coli* Isolates Recovered from Retail Salad Vegetables in Mirzapur District, Uttar Pradesh, India

Arti Pande Achyutrao[1], Shweta Yadav[1], Kaushik Satyaprakash[2], Rajesh Kumar[1], Manish Kumar[3], Thulasiraman Parkunan[3], Mukesh Kumar Bharti[3]

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ABSTRACT

Background: The present study assessed the occurrence and biofilm forming potential of ESBL-producing *Escherichia coli* isolates recovered from raw salad vegetables collected from 32 retail vegetable shops of Mirzapur district, Uttar Pradesh, India (n=224).

Methods: Standard bacteriological culture methods using cefotaxime-supplemented EMB agar were used for initial isolation of cefotaxime-resistant *E. coli* which were subjected to phenotypic detection of ESBL production by Kirby-Bauer disc diffusion test. Subsequently, all the phenotypically positive ESBL-producing *E. coli* isolates were subjected to determine their biofilm-producing ability by Congo red agar (CRA) assay and microtiter plate assay (MPA) followed by genotypic confirmation of biofilm production by PCR assay targeted at *fim-H*, *Sfa* and *papC* genes. Additionally, the isolates were genotypically explored for a potential presence of Shiga-like toxin genes (*stx*1 and *stx*2).

Result: Altogether, 276 cefotaxime-resistant *E. coli* isolates were recovered, of which, 99.27% (274/276) were observed to be phenotypically positive for ESBL production. The brown, dry and rough (BDAR) and red, dry and rough (RDAR) colony morphotypes were observed by 70.80% (194/274) and 29.19% (80/274) of the isolates, respectively by CRA method revealing their potential to form biofilms. Biofilm formation was evident in 94.16% (258/274) of the isolates by MPA method, of which 79.45% (205/258), 17.05% (44/258) and 3.48% (9/258) were considered as weak, moderate and strong biofilm producers, respectively. The *fimH* gene was found to be the predominant genetic determinant of biofilm formation which was detected in 77% (211/274) of the tested isolates followed by *Sfa* gene (11.31%) and *papC* gene (4.01%). A significant difference ($p<0.05$) was evident between CRA and MPA methods as well as phenotypic and genotypic methods for detection of biofilm formation among the recovered isolates. In addition, significant differences ($p<0.05$) were also observed upon vegetable-wise comparison of ESBL-producing *E. coli* isolates and the presence of biofilm producing genes. The *stx*1 and *stx*2 genes were found to be present in one isolate recovered from a carrot sample and two isolates from coriander samples, respectively. . The findings of this study highlight the widespread occurrence of ESBL-producing *E. coli* from raw salad vegetables with significant biofilm-forming potential posing a public health risk. The detection of Shiga-like toxin genes in some isolates further underscores the need for stringent surveillance and improved hygiene practices to mitigate contamination risk in fresh produce.

Key words: Biofilm formation, Congo red agar assay, ESBL-producing *E. coli*, Microtitre plate assay, Raw salad vegetables, Shiga-like toxin.

INTRODUCTION

Salad vegetables are an integral component of a healthy diet because they include essential vitamins, minerals and phytonutrients. Human health is seriously threatened by the microbial burden of fruits and vegetables since a substantial percentage of plant-based meals are consumed uncooked. In recent years, exposure to antimicrobial-resistant (AMR) pathogens throughout the food chain has been increasingly reported (Pérez-Rodríguez and Mercanoglu Taban, 2019).

The bulk of the microorganisms linked to raw vegetables are gram-negative bacteria. Fresh vegetables are very susceptible to microbial contamination at multiple points throughout the production and supply chain when they come into contact with the faecal matter of animals, dirt, dust and untreated wastewater during farming (Chee Sanford *et al.,* 2009) as well as when they are handled during harvest or postharvest preparation (Jung *et al.,* 2014). Besides contributing to the spread of foodborne pathogens, the vegetables represent a vehicle for the transfer of antibiotic-resistant bacteria or AMR genes to humans (Verraes *et al.,* 2013)

[1]Department of Genetics and Plant Breeding, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.
[2]Department of Veterinary Public Health and Epidemiology, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.
[3]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary and Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.

Corresponding Author: Mukesh Kumar Bharti, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary and Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.
Email: mukeshbharti@bhu.ac.in

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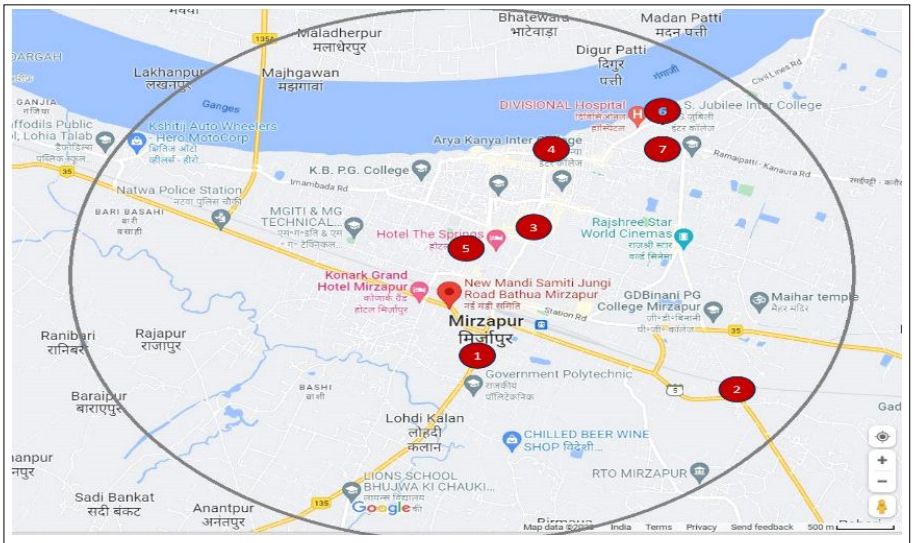
Enterobacteriaceae are opportunistic bacterias that have been associated with human illnesses (Sivakumar *et al.,* 2021). One of the most prevalent pathogens in the Enterobacteriaceae family all over the world is *Escherichia coli.* The production of Extended spectrum β-lactamase (ESBL) enzyme makes the organism resistant to all β-lactams (except carbapenems and cephamycins) and in many instances, co-resistant to other classes of antimicrobials have been observed in the same isolates making them multi-drug resistance (MDR) which are difficult to treat (Chaturvedi *et al.,* 2020; Satyaprakash *et al.,* 2024). The emergence of ESBL-producing *E. coli* in fresh vegetables is alarming, as these bacteria can be introduced into the food chain through contaminated irrigation water, soil, organic fertilizers, or cross contamination during harvesting, transportation and retailing (Said *et al.,* 2015). The worldwide dissemination of ESBL-producing *E. coli* in clinical, foods of animal and plant origin as well as environmental ecosystem is a matter of serious concern from public health point of view (Girijan and Pillai, 2023). Several studies have reported the occurrence of ESBL-producing *E. coli* in raw vegetables, emphasizing the risk of their transfer to humans *via* consumption of contaminated produce (Zurfluh *et al*., 2015; Gundappa and Gaddad, 2016).

Another crucial element in drug resistance and bacterial virulence is the formation of biofilms, the extracellular polymeric substances that allow the bacteria to proliferate and adhere to one another on living or non-living surfaces (Fazeli-Nasab *et al.,* 2022). Curli (fimbriae) and cellulose production by pathogenic bacterial strains are often considered necessary for biofilm formation that are important for the persistence and survival of bacteria in food processing environments and foods (Beshiru *et al.,* 2023). The bacteria can gain several benefits from biofilm, including mechanical resistance, physical protection and protection against chemicals, antimicrobials and disinfectants (Flemming *et al.,* 2016). Biofilm-forming ability of *E. coli* and *Salmonella* isolated from fresh fruits and vegetables have been documented (Amrutha *et al.,* 2017). Of the pathogenic *E. coli*, the verotoxic or Shiga-like toxin-producing *E. coli* (STEC) are often considered an important threat in food-borne illnesses that may lead to several health issues such as diarrhea (Majowicz *et al.,* 2014). STEC strains can survive on fresh leafy green vegetables and form biofilms. Biofilm production by STEC has been reported on various produces and abiotic surfaces that may serve as a source of cross-contamination during harvesting, storage, or transportation of produce (Carter *et al.,* 2019)

Literature is scarce particularly from a developing country like India on the occurrence of ESBL-producing *E. coli* in fresh salad vegetables, which have the potential to form biofilms and may harbour Shiga-like toxin genes. Hence, the present study was undertaken to isolate ESBL-producing *E. coli* from raw salad vegetables sold in the retail vegetable markets of Mirzapur district, Uttar Pradesh, India. The recovered isolates were further subjected to evaluate their biofilm-forming potential by phenotypic and genotypic methods and a possible presence of Shiga-like toxin genes. The findings of this study will provide insights into the potential risks associated with the consumption of raw salad vegetables and contribute to the development of improved food safety strategies to mitigate microbial contamination in fresh produce.

MATERIALS AND METHODS

The Present study was carried out at the Faculty of Veterinary and Animal Sciences, RGSC, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh from March 2023 to October 2023. A total of 224 fresh vegetable



(1. Bathua, 2. Muhkuchwa, 3. Ghanta ghar, 4. Ganesh ganj, 5. Mukeri bazaar, 6. Kachehri road, 7. Ramaipatti).

Fig 1: Sampling area across seven vegetable market areas of Mirzapur district.

samples comprising of tomatoes, cucumbers, carrots, green chillies, radishes, coriander leaves and beetroots were collected from 32 different local retail vegetable shops spread across 7 different markets of Mirzapur district, which are located within a 5 km radius around the district headquarter (Fig 1, Table 1). The samples were collected in sterile sample collection bags and transported to the laboratory in a cold chain (4±1°C) and processed within 6 hours.

The collected samples were rinsed with sterile distilled water. The contents were then homogenized with sterile PBS (pH 7.2±0.2) and 1 mL of the suspension was enriched in 9 mL of McConkey broth and incubated overnight at 37°C in a bacteriological incubator. A loopful of the enriched culture was streaked on a sterile EMB agar plate supplemented with cefotaxime (4 µg/mL) and incubated for 24 hrs at 37°C. At least three colonies showing a greenish metallic sheen with a dark purple background were randomly selected from different streaking lines of each plate and processed for Gram staining and biochemical tests (Catalase, Oxidase, IMViC, TSI and Nitrate test) for the phenotypic identification of *Escherichia coli*. All the cefotaxime-resistant *E. coli* isolates were screened for phenotypic ESBL production by combination disc method (CDM) using sterile Muller Hinton Agar (MHA) and the results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2019).

Qualitative detection of biofilm production in ESBL-producing *E. coli* isolates was determined by streaking a loopful of the culture on Congo red agar (CRA). The media was formulated with BHI broth (37 g/L), Congo red (0.8 g/L), sucrose (36 g/L) and agar powder (30 g/L). Inoculated plates were incubated at 37°C for 24 hrs. The plates were observed for the formation of different morphotypes as RDAR (red, dry and rough) indicating expression of curli fimbriae and cellulose, PDAR (pink, dry and rough) indicating expression of cellulose but not fimbriae, BDAR (brown, dry and rough) indicating expression of fimbriae but not cellulose and SAW (smooth and white) indicating expression of neither cellulose nor fimbriae as described by (Nesse *et al.,* 2020).

The Microtiter Plate Assay (MPA) was performed as per (Stepanović *et al.,* 2007) with slight modifications. In brief, 180 µl of BHI broth containing 1% sucrose was dispensed into each well of a 96-well polystyrene microtiter plate (Corning, USA). Subsequently, 20 µl of freshly prepared bacterial suspensions of each of the isolates were added to the respective wells, while the last well served as a negative control containing only 200 µl of BHI broth with 1% sucrose. The inoculated plate was covered with a lid and incubated aerobically at 37°C for 24 hours. The optical density (OD) of each well was measured at 570 nm using an ELISA reader (TECAN Infinite, Switzerland) The OD values OD_C, 2 X OD_C and 4 X OD_C were taken into consideration during the analysis. Isolates with OD value falling within the range of ≤ OD_C, between OD_C and 2 X OD_C, between 2 X OD_C and 4 X OD_C and ≥ 4 X OD_C were classified as non-biofilm producers, weak biofilm producers, moderate biofilm producers and strong biofilm producers, respectively (Stepanović *et al.,* 2007).

The presence of biofilm-forming genes in the recovered ESBL-producing *E. coli* isolates was determined by multiplex polymerase chain reaction (m-PCR) assay targeted at *fimH*, *Sfa* and *papC* gene.

The possible presence of Shiga-like toxin producing genes in the recovered isolates was explored by PCR assay targeted at the *stx1* and *stx2* genes as per Fagan *et al.* (1999) and Paton and Paton (1998), respectively.

The association between phenotypic (CRA and MPA) and genotypic method (PCR) for detection of biofilm formation among the ESBL-producing *E. coli* isolates and between fresh salad vegetable categories and the presence of biofilm forming genes were compared by Pearson chi-square (when n>3) and Fisher's exact test (when n<3) using IBM SPSS software (Version 23, IBM, Armonk, NY, USA). The level of significance was determined at a 95% confidence interval, where p<0.05 was considered significantly different and p>0.05 was non-significantly different.

RESULTS AND DISCUSSION

As per the available literature, this is the first-ever study on ESBL-producing *E. coli* from salad vegetables collected from retail shops in Mirzapur district, Uttar Pradesh, India. The initial screening of raw salad vegetables in our study revealed the presence of cefotaxime-resistant *E. coli* in 41.96% (94/224) of the samples from which a total of 276

Table 1: Area-wise occurrence of Cefotaxime-resistant/ESBL-producing *E. coli* among the raw salad vegetable samples.

Market area	No. of shops	Total number of samples collected	No. of samples positive for the presence of Cefotaxime-resistant *E. coli*	No. of samples positive for the presence of ESBL-producing *E. coli*
Bathua	3	21	8 (38.09%)	8 (38.09%)
Muhkuchwa	3	21	5 (24.0%)	5 (24.0%)
Ghanta ghar	11	77	25 (32.46%)	25 (32.46%)
Ganesh ganj	2	14	8 (57.14%)	8 (57.14%)
Mukeri bazaar	4	28	18 (64.28%)	18 (64.28%)
Kachehri road	7	49	21 (42.85%)	21 (42.85%)
Ramaipatti	2	14	7 (50.0%)	7 (50.0%)
Total	32	224	94 (41.96%)	94 (41.96%)

cefotaxime-resistant *E. coli* isolates were recovered and subjected to phenotypic detection of ESBL production by CDM (Table 1). A similar result was reported by Shakerian *et al.* (2016), who found *E. coli* in 49.6% of salad vegetable samples, suggesting that contamination level in raw vegetables may be consistently high across different geographical regions due to improper hygiene and handling practices. However, a significantly higher isolation rate of *E. coli* (86%) was documented in ready-to-eat salad vegetables collected from an area where crops were irrigated with untreated sewage water (Castro-rosas *et al.,* 2012). This highlights the critical role of irrigation water quality in bacterial contamination, supporting the hypothesis that poor agricultural practices exacerbate microbial risks in fresh produce. Studies from India have reported a wide range of *E. coli* occurrence in raw vegetables, varying from 12.6% to 66.0% (Saksena *et al.,* 2020), along with the occurrence of other pathogens like *Salmonella* spp. (38.14%) and *Shigella* spp. (25.25%) (Gundappa and Gaddad, 2016). These variations can be attributed to differences in sampling locations, agricultural practices and post-harvest methods. In our study, vegetable-wise comparison indicated that radish had the highest contamination rate (62.50%), followed by coriander (53.12%), carrot (46.87%), green chilies and beetroot (43.75% each), tomato (21.87%) and cucumber (18.75%) (Table 2). The higher contamination rate in root vegetables such as radish, carrot, beet root, as well as coriander could be due to their direct contact with soil, which is often contaminated with animal excreta, irrigation water (pond, canal or river) or untreated sewage. This finding aligns with previous studies where *E. coli* contamination was found in 15% of tomatoes and 20% of carrot samples (Mritunjay and Kumar, 2017). In contrast, a much lower occurrence of *E. coli* was observed in fresh vegetables in Aizawl, India, where only 4.88% of tomatoes, potatoes and cabbage samples were tested positive. Similarly other studies (Fiedler *et al.*, 2017) reported minimal contamination rates in tomatoes (0.08%) and leafy vegetables (0.79%-1.30%). These discrepancies might stem from variations in environmental conditions, food safety regulations and differences in hygienic agricultural practices. Additionally, (Tambekar and Mundhada, 2006) highlighted the link between salad vegetables and bacterial contamination, emphasizing that these vegetables are frequently consumed raw with minimal washing without any heat treatment, thereby increasing the risk of food-borne infections. Our study reinforces these concerns, indicating that raw vegetables serve as a significant reservoir of *E.coli*, necessitating improved hygiene measures during cultivation, handling and distribution to mitigate potential health risks.

In this study, 99.27% (274/276) of the recovered cefotaxime-resistant *E. coli* isolates were observed to be phenotypically positive for ESBL production. This means that, all the samples that were contaminated with cefotaxime-resistant *E. coli* (41.96%) also harboured ESBL-producing *E. coli*, as the same isolates were evaluated for ESBL production (Table 1). Our results contrast sharply with those of Pintor-Cora *et al.* (2021), where only 11.96% (14/117) and 2.85% (7/47) of vegetable samples were positive for ESBL-producing *E. coli*. Similarly, a study from Nigeria reported a lower occurrence, with 24.4% vegetables, 20% ready-to-eat salad vegetables and 36.6% vegetables obtained from vendors and open markets, were found to harbour ESBL-producing *E. coli*. These discrepancies may be due to differences in agricultural and food handling practices, the level of antimicrobial use in farming and regional variations in microbial contamination sources. The high occurrence of ESBL phenotypes in our study may be attributed to the potential transfer of resistance genes from environmental and commensal microbiota (Zurfluh *et al.,* 2015). Additionally, the use of a selective medium (cefotaxime-supplemented EMB agar) and the strategy of picking multiple colonies from each sample likely contributed to a higher detection rate in our study. This is further supported by the findings of Satyaprakash *et al.* (2024), who reported a similarly high occurrence of phenotypic ESBL production (91.29%) among the cefotaxime-resistant *E. coli* isolates (n=310) recovered from environmental sources of eastern parts of Uttar Pradesh (Mirzapur, Prayagraj, Varanasi, Jaunpur and Sonbhadra districts), India. In one more study Jaya *et al*. (2025) potentiate the use of Antimicrobial peptide on production performance, egg quality and serum biochemical parameters of laying hens. Another similar kind of study done in Indonesia by Wibawati *et al*. (2025), on occurrence of Antibiotic-resistant *Proteus mirabilis* and *Proteus*

Table 2: Vegetable wise occurrence of Cefotaxime-resistant/ESBL-producing *E. coli*.

Type of the vegetables	Total no. of samples collected	No. of samples positive for the presence of Cefotaxime-resistant *E. coli*	No. of samples positive for the presence of ESBL-producing *E. coli*
Radish	32	21 (62.50%)	21 (62.50%)
Coriander	32	17 (53.12%)	17 (53.12%)
Carrot	32	15 (46.87%)	15 (46.87%)
Green chilli	32	14 (43.75%)	14 (43.75%)
Beet root	32	14 (43.75%)	14 (43.75%)
Tomato	32	7 (21.87%)	7 (21.87%)
Cucumber	32	6 (18.75%)	6 (18.75%)
Total	224	94 (41.96%)	94 (41.96%)

vulgaris in African Catfish (*Clarias* sp.) Since *E. coli* is a well-recognized faecal indicator organism, its detection in raw salad vegetables strongly suggests unhygienic conditions during cultivation, harvesting, transportation and retailing-whether due to accidental contamination or anthropogenic activities. The presence of such high levels of ESBL-producing *E. coli* in fresh produce underscores the urgent need for stricter food safety measures and better sanitation practices to minimize the risk of AMR bacterial transmission through food chain.

All 274 ESBL-producing *E. coli* isolates were subjected to phenotypic detection of biofilm formation by CRA and MPA methods. The CRA method revealed BDAR and RDAR morphotypes in 70.80% (194/274) and 29.19% (80/274) of the isolates, respectively, while PDAR and SAW morphotypes were not exhibited by any of the tested isolates, suggesting that all isolates had the potential to form biofilms. By MPA, biofilm production was evident in 94.16% (258/274) of the isolates, with 79.45% (205/258) being weak, 17.05% (44/258) moderate and 3.48% (9/258) strong biofilm producers. A significant difference ($p<0.05$) was observed between CRA and MPA methods for detecting biofilm formation in ESBL-producing *E. coli* isolates (Table 3). These results align with a study on 130 ESBL-producing *E. coli* isolates obtained from clinical samples, where the majority were also weak biofilm producers followed by moderate and strong biofilm producers as exhibited by MPA (Kim *et al.,* 2018). Similarly, Abdulhaq *et al.* (2020) reported that the detection of biofilm formation in *Pseudomonas aeruginosa* increased from 44.23% using CRA method to 94.23% when tested by MPA, highlighting the greater sensitivity of the microtiter plate method. Like wise, Amrutha *et al.* (2017) found that, among 35 *E. coli* isolates from fruit and vegetable samples, only 22.2% exhibited biofilm formation by the CRA method, while all were positive by MPA, with varying degrees of biofilm production. These findings reinforce that MPA is more reliable and quantitative method for biofilm detection, whereas CRA, despite being rapid and simple screening method, has lower sensitivity, specificity and accuracy (Amrutha *et al.,* 2017). The interpretation of CRA results varies across studies, which may explain the differences in positivity rates. Many studies consider the red colonies on CRA plates as negative for biofilm formation and black colonies as positive (Harika *et al.,* 2020). However, our study followed the interpretation criteria of Nesse *et al.* (2020), which recognizes the RDAR morphotypes as indicative of curly fimbriae and cellulose expression, while, BDAR results from fimbriae expression without cellulose- both confirming biofilm forming potential. Differences in CRA interpretation across studies could explain the lower positivity rate reported in some research compared to our findings.

Genotypic analysis revealed that one or more biofilm forming genes (*fim*H, *Sfa* and *papC*) were detected in 92.33% (253/274) of phenotypically positive ESBL-producing *E. coli* isolates using multiplex PCR assay. The *fim*H gene was predominant (77%, 211/274), followed by *Sfa* (11.31%, 31/274) and *papC* (4.01%, 11/274) (Table 4a). These findings are consistent with a study in Northwest Iran, where *fim*H was identified as the major genetic determinant of biofilm formation (Tajbakhsh *et al.* 2016). However, other studies have reported varying degree of detection of genes such as Davari Abad *et al.* (2019), who found *sfa* gene at a higher frequency among uropathogenic *E. coli*. Similar study was done by Lade *et al*. (2022), in India on Milk sample as coexpression of Methicillin-resistant *S. aureus* and ESBL Producing *E. coli* in Mastitic Milk of Buffaloes. The observed significant difference

Table 3: Statistical significances between CRA and MPA methods for biofilm formation in ESBL-producing *E. coli* isolates.

	CRA		MPA			χ^2 Value	p value
	BDAR	RDAR	Weak	Moderate	Strong		
Positive result	194	80	205	44	9	514.145	0.00
Negative result	80	194	69	230	265		

Table 4a: Statistical significance between CRA and PCR methods for biofilm formation in ESBL-producing *E. coli* isolates.

	CRA		PCR			χ^2 value	p value
	BDAR	RDAR	*fim*H	*Sfa*	*papC*		
Positive result	194	80	211	31	11	522.756	0.00
Negative result	80	194	63	243	263		
Total	274	274	274	274	274		

Table 4b: Statistical significance between MPA and PCR methods for biofilm formation in ESBL-producing *E. coli* isolates.

	MPA			PCR			χ^2 value	p value
	Weak	Moderate	Strong	*fim*H	*Sfa*	*papC*		
Positive result	203	44	9	211	31	11	784.458	0.00
Negative result	71	230	265	63	243	263		
Total	274	274		274	274	274		

Table 5: Statistical significance between biofilm producing genes and vegetable-wise occurrence of ESBL-producing *E. coli* isolates.

Types of vegetables	ESBL-producing *E. coli* (%)	ESBL-producing *E. coli* containing *fim*H gene	χ^2 value	p value
Statistical significance between *fimH* gene and vegetable-wise occurrence of ESBL-producing *E. coli* isolates				
Radish	62.50	37		
Coriander	53.12	46		
Carrot	46.87	29		
Green chilli	43.75	35	13.332	0.034
Beet root	43.75	33		
Tomato	21.87	17		
Cucumber	18.75	14		
Statistical significance between *Sfa* gene and vegetable-wise occurrence of ESBL-producing *E. coli* isolates				
Radish	62.50	12		
Coriander	53.12	1		
Carrot	46.87	5		
Green chilli	43.75	6	12.061	0.049
Beet root	43.75	1		
Tomato	21.87	3		
Cucumber	18.75	3		
Statistical significance between *papC* gene and vegetable-wise occurrence of ESBL-producing *E. coli* isolates				
Radish	62.50	0		
Coriander	53.12	0		
Carrot	46.87	4		
Green chilli	43.75	3	10.992	0.046
Beet root	43.75	1		
Tomato	21.87	0		
Cucumber	18.75	3		

($p<0.05$) between phenotypic (CRA and MPA methods) and genotypic (PCR) methods for detection of biofilm detection (Table 4a and 4b) further highlights the complex regulatory mechanisms of biofilm formation, which are influenced by both genetic and environmental factors. Moreover, a significant difference ($p<0.05$) was observed between biofilm forming genes and the vegetable-wise occurrence of ESBL-producing *E. coli* isolates (Table 5), suggesting that contamination sources and environmental conditions play a crucial role in biofilm formation. The co-occurrence of ESBL production and biofilm formation is particularly concerning, as biofilms enhance bacterial persistence in the environment and contribute to antimicrobial resistance by limiting antimicrobial penetration (Dutt *et al.,* 2022). This could explain why biofilm-forming bacteria pose a significant risk.

Out of the 274 ESBL-producing *E. coli* isolates, Shiga-like toxin genes were detected in only three isolates (one *stx1* gene in an isolate recovered from a carrot sample and two *stx2* genes in two isolates recovered from coriander samples) by PCR assay. All the three isolates were also biofilm producers, with *fimH* as the major genetic determinant. Similar findings were reported by Verma *et al.* (2018), who identified four Shiga toxin-producing (STEC) *E. coli* isolates from 73 *E. coli* isolates recovered from fruits and vegetables. Adator *et al.* (2018) demonstrated that STEC could survive within dry-surface biofilms and transfer to fresh lettuce, emphasizing the role of biofilms in pathogen persistence. In another study, STEC was detected in 6% of carrot samples, 12% of lettuce, 3% of cucumbers and 8% of leafy vegetables with two lettuce STEC isolates found to be positive for the O157 serogroup (Kholdi *et al.,* 2021). These findings suggest that biofilm formation may provide a survival advantage for STEC, increasing the risk of contamination in fresh produce. Raw vegetables are important components of the human diet, yet they are vulnerable to microbial contamination. Several outbreaks of STEC infections have been linked to contaminated leafy greens and salad vegetables (Wendel *et al.,* 2009). The presence of ESBL-producing *E. coli* with biofilm-forming ability, along with STEC genes in fresh produce, underscores the urgent need for improved hygiene practices during cultivation, harvesting, transportation and retailing to mitigate public health risks.

CONCLUSION

The exhibition of a higher magnitude of ESBL-producing *E. coli* in fresh ready-to-eat salad vegetables consumed raw possesses a significant yet uncertain public health risk. The biofilm producing potential of the recovered isolates, as observed through both phenotypic and genotypic methods, further complicates antimicrobial treatment with the probable presence of additional drug-resistant and virulence genes. Preventing human exposure to resistant bacteria through fresh produce remains a challenge due

to the complex interplay of food safety and security concerns. This underscores the need for a holistic approach encompassing pre-harvest and post-harvest processing, as well as carefull distribution through the retail channels. To mitigate these risks, enhanced hygiene practices, stringent regulatory measures and increased oversight throughout the supply chain are imperative. Furthermore, effective communication, behavioural initiatives and awareness programmes targeted at stakeholders, farmers, producers, retailers and consumers are essential. A coordinated effort integrating scientific research, policy interventions and public awareness will be key to reducing the threat of AMR pathogens in fresh produce and safeguarding public health.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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