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Prevalence and Antimicrobial Resistance of *Escherichia coli* in Ready-to-Eat Vegetables and Salads in Mashhad

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ABSTRACT

Introduction: The rising global consumption of ready-to-eat (RTE) vegetables has highlighted concerns about their microbiological safety, particularly regarding contamination with antibiotic-resistant *Escherichia coli* (*E. coli*). This study aimed to isolate *E. coli* from green vegetables and salads sold in Mashhad, Iran, and assess their antimicrobial resistance profiles.

Methods: A total of 120 RTE vegetable samples were analyzed using microbiological procedures outlined in the Iranian National Standard ISIRI 2946. Confirmatory identification of *E. coli* was performed via culture-based methods and indole testing. Antibiotic susceptibility was evaluated using the Kirby-Bauer disk diffusion method, following CLSI guidelines.

Results: Out of 120 samples, 40 (33.33%) tested positive for *E. coli*. The highest susceptibility rates were to nalidixic acid (57.5%) and chloramphenicol (55%). However, significant resistance was observed against cefazolin (67.5%), cefixime (62.5%), and ciprofloxacin (62.5%). Intermediate resistance to colistin (47.5%) raises concern due to its role as a last-resort antibiotic. Multidrug resistance (MDR) was prevalent, with 80% of isolates resistant to at least two antibiotics.

Conclusion: The detection of multidrug-resistant *Escherichia coli* in one-third of RTE vegetable and salad samples from Mashhad highlights a significant public health concern. These findings underscore the urgent need for enhanced hygiene practices, regular microbial surveillance, and antibiotic resistance monitoring to ensure the safety of RTE produce and prevent potential transmission of resistant strains through the food chain.

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Introduction

The consumption of fresh and ready-to-eat (RTE) vegetables has increased globally due to heightened awareness of their nutritional benefits and their role in preventing chronic diseases such as cardiovascular disorders and cancer [1]. However, these food products can also serve as vehicles for foodborne pathogens, particularly when consumed raw or with minimal processing [2]. Among the major bacterial contaminants of concern is *E. coli*, which serves not only as a fecal indicator organism but also includes pathogenic strains that can cause serious gastrointestinal illness [3]. E. coli contamination in vegetables may result from multiple sources, including the use of untreated water for irrigation, organic fertilizers such as manure, contaminated harvesting equipment, or poor hygiene during post-harvest handling and packaging [4]. Particularly, the presence of antibiotic-resistant *E. coli* in food items is a growing public health concern. The misuse and overuse of antibiotics in agriculture and clinical settings have contributed to the emergence and dissemination of antimicrobial-resistant bacteria in the environment, which may be transmitted to humans through the food chain [5].

The detection and antimicrobial resistance profiling of *E. coli* in fresh produce are thus crucial for assessing the microbial safety and public health risks associated with these food items. Standardized microbiological methods, such as those outlined in the Iranian National Standard ISIRI 2946, provide guidance for the isolation and enumeration of *E. coli* in food samples. Additionally, the Kirby-Bauer disk diffusion method remains a widely accepted technique for determining antimicrobial susceptibility patterns.

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This study aimed to isolate and identify *E. coli* strains from ready-to-eat green vegetables and salads marketed in local outlets, assess their antimicrobial resistance patterns using the Kirby-Bauer method, and contribute to the growing body of evidence on the microbiological safety of fresh produce in Iran.

Materials and Methods

Sample Collection and Bacterial Isolation

A total of 120 ready-to-eat green vegetable and salad samples were collected and submitted to the Huanna Zist Binaloud Laboratory (Mashhad, Iran) for microbiological analysis. Isolation of *E. coli* strains was carried out according to the Iranian National Standard No. 2946 [6]. For each sample, 10 grams of the vegetable or salad material were added to 90 mL of Ringer's solution (Merck, Germany) as a diluent. Subsequently, 10 mL of it were transferred into a test tube containing 10 mL of double-strength Lauryl Sulfate Broth (LSB) (Ibersco, Iran) equipped with a Durham tube. The tubes were incubated at 37°C for 24 hours; if no turbidity or gas formation was observed, incubation was extended to 48 hours.

Samples showing turbidity and gas production were considered presumptive positive and were transferred to EC Broth (Ibersco, Iran). These tubes were incubated at 44°C for 24 to 48 hours. Tubes exhibiting turbidity and gas production were then inoculated into Peptone Water without indole (Ibersco, Iran), used as a confirmatory medium, and incubated at 44°C for an additional 48 hours.

After incubation, Kovac's reagent was added to the tubes. The appearance of a pink ring indicated a positive indole reaction, confirming the presence of *E. coli*. For further confirmation, a loopful of the EC Broth from positive tubes was streaked onto Nutrient Agar (Ibersco, Iran) and incubated for colony isolation and subsequent analysis.

Antimicrobial Susceptibility Testing

The antibiotic resistance profiles of the isolated *E. coli* strains were determined using the Kirby-Bauer disk diffusion method, following the guidelines provided by the Clinical and Laboratory Standards Institute [7]. Fresh bacterial suspensions equivalent to 0.5 McFarland standard were prepared and uniformly spread on Mueller-Hinton agar plates. Antibiotic-impregnated disks were applied to the surface, and plates were incubated at 37°C for 18–24 hours.

The antibiotic susceptibility of the *E. coli* isolates was assessed using the following antibiotics and their respective disk concentrations: Nalidixic acid (30 µg) and Ciprofloxacin (5 µg), Amoxicillin (25 µg), Cefixime (5 µg), Cefazolin (30 µg), Chloramphenicol (30 µg), Colistin (10 µg), and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg).

After incubation, the diameters of the inhibition zones were measured in millimeters and interpreted as resistant, intermediate, or sensitive based on CLSI breakpoints.

Results

Prevalence of *E. coli* Isolates:

Of the 120 green vegetable and salad samples analyzed, 40 (33.33%; 95% CI: 25.53–42.17) tested positive for the presence of *E. coli*. (Figure 1).

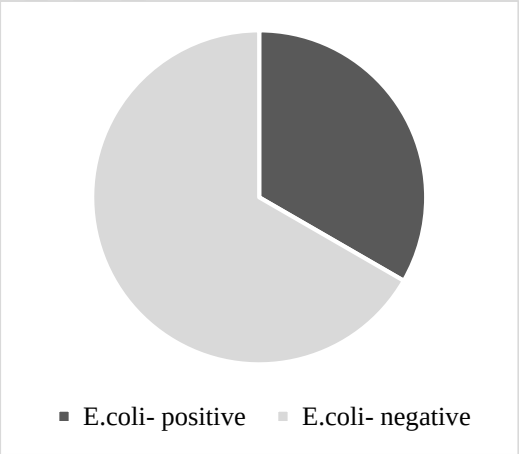

Figure 1. Proportion of E. coli in 120 samples

Antimicrobial Resistance Profiles of *E. coli* Isolates:

Antibiogram analysis revealed that the highest susceptibility among the *E. coli* isolates was to nalidixic acid (57.5%; 95% CI: 42.20–71.49) and chloramphenicol (55.0%; 95% CI: 39.83–69.29). The highest resistance rate was observed against cefazolin (67.5%; 95% CI: 52.02–79.92), followed by cefixime (62.5%; 95% CI: 47.03–75.78) and ciprofloxacin (62.5%; 95% CI: 47.03–75.78) (Table 1). Multiple antibiotic resistance was detected in 32 isolates (80.0%; 95% CI: 65.24–89.50), as detailed in Table 2. (Figure 2)

In relation to colistin susceptibility, previous studies by Jayol et al. (2017) and Olaitan et al.

(2014) proposed a non-standard yet widely cited correlation between inhibition zone diameter and MIC values in *E. coli*. Isolates with inhibition zones ≥ 13 mm generally correspond to MIC values ≤ 2 µg/mL and are considered susceptible according to EUCAST criteria. Zones of 10–12 mm typically indicate MIC values of 2–4 µg/mL, suggesting possible resistance, while zones ≤ 9 mm are often associated with MIC values ≥ 4 µg/mL and are therefore interpreted as resistant [8, 9].

Antimicrobial Resistance Index (AMR Index)

The Antimicrobial Resistance (AMR) index for each isolate was calculated using the formula:

$$\text{AMR Index} = \frac{\text{Number of antibiotics to which the isolate was resistant}}{\text{Total number of antibiotics tested}}$$

In this study, resistance was tested against 8 antibiotics; therefore, the AMR index for an isolate resistant to n antibiotics was calculated as:

$$AMR\ Index = \frac{n}{8}$$

Isolates were classified as multidrug-resistant (MDR) if they showed resistance to three or more classes of antibiotics.

Multiple Antibiotic Resistance Patterns

Of the 40 *E. coli* isolates, 32 (80.0%; 95% CI: 65.24–89.50) were multidrug-resistant (MDR), showing resistance to at least one antimicrobial agent in three or more classes.

The most frequent MDR profile was resistance to five antibiotics — observed in 10 isolates (25.0%). This profile typically included cefazolin, cefixime, ciprofloxacin, amoxicillin, and either sulfamethoxazole-trimethoprim or chloramphenicol.

The resistance pattern with the highest number of resistant antibiotics was resistance to seven agents (cefazolin, cefixime, ciprofloxacin, amoxicillin, chloramphenicol, sulfamethoxazole-trimethoprim, and nalidixic acid), detected in 3 isolates (7.5%). This pattern yielded the highest AMR index of 1.0, indicating complete resistance to all tested antibiotics.

The second-highest AMR index (0.86) corresponded to resistance to six antibiotics, observed in 1 isolate (2.5%).

MDR profiles involving four antibiotics were recorded in 5 isolates (12.5%), and those with three antibiotics in 11 isolates (27.5%). Two isolates (5.0%) showed resistance to only two antibiotics and were not classified as MDR.

The AMR index across MDR isolates ranged from 0.43 to 1.0, with a mean of 0.71, reflecting a substantial resistance burden among *E. coli* strains isolated from fresh vegetables and salads in this study (Table 2).

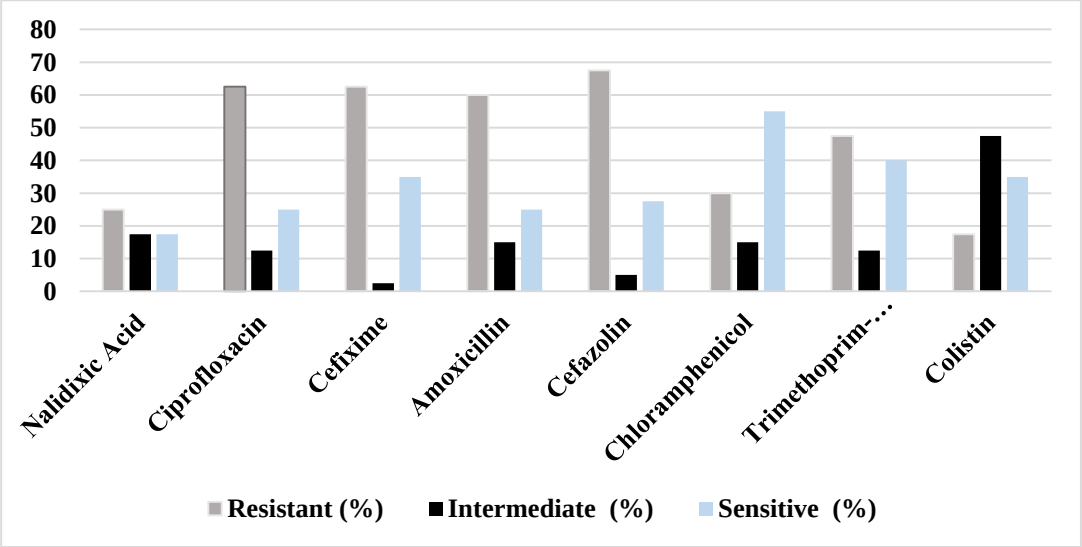

Figure 2. Comparison of the resistance patterns of *E. coli* isolates in the analyzed samples against eight selected antibiotics.

Table 1. Number and percentage of susceptible, intermediate, and resistant isolates to 8 antibiotics

Antibiotic	Abbreviation	Resistant(%)	Intermediate(%)	Sensetive(%)
Nalidixic Acid	NA	10(25)	7(17.5)	23(57.5)
Ciprofloxacin	CP	25(62.5)	5(12.5)	10(25)
Cefixime	CFM	25(62.5)	1(2.5)	14(35)
Amoxicillin	AMC	24(60)	6(15)	10(25)
Cefazolin	CF	27(67.5)	2(5)	11(27.5)
Chloramphenicol	C	12(30)	6(15)	22(55)
Trimethoprim-sulfamethoxazole	SXT	19(47.5)	5(12.5)	16(40)
Colistin	CL	7(17.5)	19(47.5)	14(35)

Table 2. Number and percentage of isolates resistant to more than one antibiotic.

Number of antibiotic	Antibiotics	Number of isolates	Sum(%)
2	CP, CF	2	2(5)
3	CP, CFM, SXT	3	11(27.5)
	CFM,CF,SXT	1	
	NA,CP,CFM	1	
	CP, CFM,CF	1	
	AMC,CF,C	1	
	CL, AMC,SXT	2	
	AMC,CF,SXT	1	
	NA,CFM,CF	1	
4	CP,CFM,AMC,CF	2	5(12.5)
	CFM,AMC,CF,C	1	
	CP,CFM,AMC,CF	1	
	CL,CP,AMC,CF	1	
	CP,AMC,CF,C,SXT	1	
5	CP,CFM,AMC,CF,SXT	2	10(25)
	CP,CFM,AMC,CF,C	2	
	NA,CFM,AMC ,CF,SXT	1	
	NA,CFM,AMC,C,SXT	1	
	CL,CP,AMC,C,SXT	1	
	CL,NA,AMC,CF,SXT	1	
	NA,CP,CFM,AMC,CF	1	
6	NA,CP,CFM,AMC,CF,SXT	1	1(2.5)
7	CL,CP,CFM,AMC,CF,C,SXT	1	3(7.5)
	NA,CP,CFM,AMC,CF,C,SXT	2	

Discussion

The presence of *E. coli* in RTE green vegetables and salads represents a growing public health concern, particularly when these products are consumed raw and without further processing. While recent studies in northern Iran report low or undetectable levels of *E. coli* in RTE vegetables and salads [10], emerging evidence indicates that such contamination may still be present in other regions, including Mashhad, especially with the emergence of multidrug-resistant (MDR) strains [11,12].

In this study, analysis of RTE vegetable and salad samples from Mashhad revealed the presence of *E. coli* at a considerable level. The antimicrobial susceptibility profile highlighted a mix of sensitivity to some agents and pronounced resistance to several commonly used antibiotics, with a notably high proportion of isolates classified as multidrug-resistant (MDR). Azimirad et al. analyzed 92 RTE leafy green samples in Tehran and reported an *E. coli* prevalence of 23.2%, which is lower than the rate observed in our study. This variation may reflect differences in regional hygiene standards, environmental conditions, or postharvest handling practices [13]. Similarly, Soltan Dallal et al. evaluated 65 vegetable and salad samples in Tehran, finding that 71% of salads exceeded acceptable microbiological thresholds, including contamination with *E. coli*. Although the precise prevalence was not specified, their results further emphasize the widespread microbial contamination of RTE produce in Iran [14].

This study found the presence of *E. coli* in RTE vegetables sold in Mashhad and characterized their antimicrobial resistance (AMR) patterns. Our findings are consistent with national and

international studies demonstrating high resistance rates in *E. coli* isolates, particularly against β-lactam antibiotics such as ampicillin and cefoxitin, as well as the detection of key resistance genes including *blaTEM* and *qnr* variants [15, 16, 17]. Similar resistance trends have been reported across Iran, with significant prevalence of ESBL-producing strains in RTE lettuce and salads [15].

Globally, AMR in *E. coli* from RTE vegetables shows significant regional variation. In Lebanon, 60% of *E. coli* isolates from salads were multidrug resistant, with inadequate washing practices contributing to microbial persistence [18]. In Côte d'Ivoire, alarming resistance rates of 100% to cefuroxime and 87.5% to ampicillin and cefoxitin were recorded [15]. In contrast, resistance rates in European lettuce farms were relatively low (ampicillin: 7%; no resistance to gentamicin or ciprofloxacin), likely reflecting better agricultural practices and antibiotic stewardship [19].

From a genomic standpoint, resistance genes such as *blaTEM*, *blaCTX-M*, *tetA*, *sulI*, and aminoglycoside resistance genes like *aadA* and *aac(3)-IV* have been frequently reported in *E. coli* isolates from vegetables across various countries, including Ethiopia, Germany, and Bangladesh [20, 21, 22]. Many of these genes are plasmid-borne and capable of horizontal transfer, raising concerns about their potential transmission to human gut microbiota following consumption [23].

Moreover, global analyses of over 94,000 *E. coli* genomes have shown that nearly 50% carry antibiotic resistance genes (ARGs), with multidrug-resistance profiles being especially common in low- and middle-income countries due to suboptimal food safety systems and widespread antibiotic misuse [24]. Notably, high-risk clones such as *E. coli* ST131 are emerging worldwide and contribute to the persistence and dissemination of resistance traits, particularly to fluoroquinolones and third-generation cephalosporins [25].

While the overall prevalence of *E. coli* in RTE vegetables in Mashhad appears moderate, the detection of MDR isolates with resistance to multiple antibiotics including β-lactams, aminoglycosides, and fluoroquinolones highlights a significant public health risk. This is especially critical given the growing global trend of carbapenem and colistin resistance, even though such resistance was low or absent in our isolates [26].

Hygiene practices play a central role in determining contamination levels. Studies from Kenya and Cameroon show that while vendors may be aware of protective practices (e.g., glove use), improper food handling and insufficient washing contribute to contamination [27, 28]. Vinegar washing has been shown to significantly reduce *E. coli* loads compared to water-only washing [29], yet some studies demonstrate that even repeated washing may not completely eliminate bacterial contamination [30].

In Iran, although no large outbreaks of foodborne *E. coli* linked to salads have been documented, high rates of antibiotic resistance in isolates from animals and food sources suggest a potential for cross-contamination and foodborne transmission of MDR strains [12, 31].

From a policy perspective, the increasing prevalence of MDR *E. coli* in food products has led to stricter food safety regulations and monitoring systems in many countries. Integration of food safety policies with antimicrobial stewardship and improved sanitation infrastructure (WASH) is critical to mitigating the risks associated with resistant foodborne pathogens [32, 33].

Conclusion

The detection of *E. coli*—particularly multidrug-resistant strains—in RTE vegetables and salads from Mashhad underscores the intersection of food safety and antimicrobial resistance as a pressing public health challenge. Regional variation in contamination rates, both within Iran and internationally, highlights the role of agricultural practices, hygiene standards, and postharvest handling in shaping microbial risks. The persistence of resistance genes in foodborne *E. coli* and their potential for horizontal transfer further elevates the threat of disseminating resistance to the human gut microbiota. Strengthening food safety surveillance, enforcing hygienic handling practices, and aligning national strategies with global antimicrobial stewardship initiatives are essential steps to reduce the burden of resistant *E. coli* in the food chain and safeguard consumer health.

Declaration

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Conflicts of Interests
None declared by Authors.

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Code of Ethics
The research did not involve human participants or animal subjects, and thus did not require approval from an ethics committee. All procedures were conducted in accordance with relevant guidelines and regulations for microbiological studies of food samples.

Authors' Contribution
Author 1 conceptualized the study, designed the methodology, and supervised the research process.
Author 2 conducted sample collection, performed laboratory experiments, and contributed to data analysis.
Author 3 analyzed the data, wrote the initial manuscript draft, and handled literature review and referencing.
All authors reviewed and approved the final version of the manuscript.

Artificial Intelligence (AI)
Perplexity AI was utilized to search for relevant articles during manuscript preparation, while ChatGPT was employed to enhance the language and improve readability.

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