

## Characterization of *Escherichia Coli* Associated With Vegetable Salad

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### Structured Abstract

**Background:** Raw vegetables, particularly leafy greens, are frequently linked to foodborne illness outbreaks due to potential contamination with pathogenic bacteria such as *Escherichia coli*. *E. coli* is a diverse species comprising both harmless and pathogenic strains. In 2018, a romaine lettuce outbreak in Canada underscored the severity of contamination events (Hossain et al., 2021). This study investigates the microbial safety of locally grown vegetable salads, focusing on the isolation, identification and antibiotic resistance profiling of *E. coli* strains.

**Methods:** Ten vegetable salad samples were collected from local markets in sterile containers and transported to the laboratory. Samples were cultured on MacConkey Agar, Eosin Methylene Blue Agar (EMBA), and Nutrient Agar. Presumptive *E. coli* isolates underwent Gram staining, catalase and coagulase tests. Antibiotic susceptibility test was assessed using Kirby-Bauer disk diffusion method against six antibiotics like ampicillin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), vancomycin (30 µg), kanamycin (30 µg) and tetracycline (30 µg). DNA was extracted from two selected isolates, followed by PCR amplification of the 16S rRNA gene. The amplified products were sequenced and analyzed using BLAST.

**Results:** Out of ten vegetable salad samples labelled EEC1 to EEC10, six isolates (EEC3, EEC4, EEC5, EEC6, EEC9 and EEC10) exhibited characteristics *E. coli* morphology on EMBA, with greenish metallic sheen colonies. Biochemical tests confirmed these isolates as Gram-negative rods, catalase-positive and coagulase-negative (Supriatin et al., 2021; Hossain et al., 2021). Antibiotic susceptibility results showed all six isolates were resistant to ampicillin and vancomycin, suggesting the potential presence of  $\beta$ -lactamase-producing strains (Anu Monisha et al., 2022). However, all isolates remained susceptible to ciprofloxacin, gentamicin, kanamycin and tetracycline, similar to findings by Karatay et al. (2023). PCR amplification and BLAST analysis of the 16S rRNA gene identified EEC3 and EEC6 as *E. coli* strains with 98.61% and 99.22% similarity, respectively, to strain 30859\_5#357. These findings suggest that non-MDR *E. coli* strains may be present in fresh produce and contamination could occur during handling or distribution.

**Conclusion:** This study confirms the presence of *E. coli* in locally grown vegetable salads. Although multidrug resistance was not observed, resistance to first-line antibiotics such as ampicillin warrants concern. These results emphasize the need for improved hygiene practices across the food production chain and stricter enforcement of food safety protocols to protect consumers from potential foodborne illnesses.

**Keywords:** *Escherichia coli*, vegetable salad, antibiotic resistance, 16S rRNA, food safety

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