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Research Article

Antimicrobial Resistant Profile And ESBL Production in *Enterobacteriaceae* Isolated from Fresh Vegetables Sold in Markets in Benghazi City, Libya

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ABSTRACT

Fresh vegetables are widely consumed because of their high nutritional value, however, they may serve as vehicles for the transmission of pathogenic and antimicrobial-resistant bacteria. This study aimed to isolate and identify members of the *Enterobacteriaceae* from fresh vegetables sold in markets in Benghazi, Libya, and to determine their antimicrobial resistance profiles as well as the presence of Extended-Spectrum Beta-Lactamase (ESBL)-producing isolates. A total of 31 raw vegetable samples were collected from six different markets in April 2025. Samples were processed conventionally, with antimicrobial susceptibility determined via CLSI-compliant Kirby-Bauer disc diffusion on Mueller-Hinton agar. Phenotypic detection of ESBL production was performed using the synergy test with a β-lactam antibiotics. Microbial growth was detected in 26 out of 31 samples (83.9%). The most predominant are *E.coli* (22.58%), followed by *Shigella* spp. (19.35%), *Proteus* spp., *Klebsiella* spp. (16.13%), *Salmonella* spp. (12.90%), *Enterobacter* spp. (6.45%), and *Serratia* spp. (6.45%) each. Antimicrobial susceptibility testing revealed high sensitivity to imipenem, amikacin, and meropenem (100%), followed by gentamicin (96.8%) and ciprofloxacin (93.5%). In contrast, high resistance was observed to amoxicillin/clavulanic acid (87%), while moderate resistance was detected to ampicillin (38.7%) and cefotaxime and ceftriaxone (32.3%). ESBL production was confirmed in 6 isolates (19.3%).This study found that commonly consumed vegetables are contaminated with antibiotic-resistant bacteria. The notable presence of multidrug-resistant bacteria on vegetables requires immediate, effective contamination control strategies from local health authorities.

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1. Introduction

Fresh vegetables are an essential component of a healthy diet and are widely consumed around world due to their nutritional value and health benefits. Additionally, fruits and vegetables are regarded as nutrient-dense foods, offering significant nutritional value [1,2] . As a result, organizations such the World Health Organization (WHO) have advised people to eat a substantial portion every day not only for the prevention of chronic diseases such as cardiovascular disease, cancer, diabetes, and obesity, but also for the prevention and alleviation of various micronutrient deficiencies [3]. The consumption of fruits and vegetables is rising due to their correlation with the protection of specific cancer types, ophthalmic illnesses, and cardiovascular conditions [4]. However, the majority of unprocessed food, such as fresh fruits, roots, greens, and sprouts, still serves as a pathway to bacterial illnesses, including those that are resistant to the most common antibiotics [5]. Microbiological safety of vegetables and fruits is a big challenging in the food chain of modern world. These food materials get in contact with unwanted bacteria at one stage- in farming, during packing, transportation and during sale. Among the sources of contamination, both in and contact with polluted soil remain the most important, just to mention unsafe irrigation water, untreated manure and poorly treated bio solids. The poor sanitation during post-harvest handling, transport, processing and storage further compounds it. The microbial load of the produce also grows when the food is improperly handled either by the retailers, or by the consumers omitting the washing process or cooking raw foods together with cooked foods [6,7,8]. Although vegetables are popular due to their widespread acceptability and convenience of use, they are vulnerable to high levels of microbial contamination and cross-contamination, which could result in outbreaks of food-borne pathogens because they do not undergo a terminal heating step before consumption. The possibility of widespread contamination is increased by these problems, which are further exacerbated by poor handling procedures and lax food safety laws in these settings. All vegetables sold in public markets, supermarkets, and roadside stands are susceptible to contamination, which can cause serious health problems for customers and contribute to the rise in food-borne illnesses associated with vegetables in many nations [9].

It has been found that on fresh produce, both antimicrobial-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) can be detected. In Japan, as an example, the researchers have found long-spectrum b-lactamase (ESBL)-creating strains in leafy vegetables [10]. Similarly, *Escherichia coli* isolates from fresh vegetables in Czech republic have been found to contain ESBL-encoding plasmids [11]. Antimicrobial-resistant strains of *Salmonella* and *E. coli* have also been found on fresh fruit in other countries , such as the US, Canada, Australia, and Finland [12]. Furthermore, fresh produce contains opportunistic bacteria that were once thought to be non-pathogenic but can seriously infect an immune-compromised host. For instance, in various retail market contexts, opportunistic bacteria including *Klebsiella* spp. and *Enterobacter* spp. have been detected on vegetables like tomatoes, capsicum, and cabbage [13,14]. In immunocompromised people, consuming vegetables tainted with *Klebsiella* spp. can result in acute bronchopneumonia and lobar pneumonia [15].

Fresh vegetables and fruits are frequently contaminated in the stage of agricultural cultivation, especially in those cases when water used to irrigate the crops is feces-contaminated. Raw manure is sprinkled. The contamination of water with feces can bring in resistome into the food chain, e.g., $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} (food safety) [16]. These ARB can readily inhabit the human bowel, following consumption, and take advantage of horizontal gene transfer to pass the resistance genes to indigenous or pathogenic microbiota [17,18].

"As He *et al*. have shown, livestock waste is a major source of antibiotic resistance genes that can enter the agricultural environment, spread

through irrigation or soil, and possibly contaminate vegetables" [19].

Food contamination poses a major environmental health challenge, impacting human well-being by affecting various food sources, including vegetables. Bacterial contamination of vegetables is particularly concerning, as consuming tainted produce can lead to severe health issues. Such contamination can occur through exposure to polluted water, contaminated soil, or poor handling practices during harvesting, transportation, and storage [20]. This study aimed to evaluate the presence of *Enterobacteriaceae* in raw vegetables sold in Benghazi markets and to identify the most prevalent species. It also sought to examine their antibiotic susceptibility patterns and detect extended-spectrum beta-lactamase (ESBL) producers.

2. Methodology

2.1. Sampling, Bacterial Culture, Antimicrobial Susceptibility Profile

This cross-sectional study was conducted in April 2025 with 31 samples of raw vegetables (lettuce, tomatoes, cucumbers, radishes, watercress, and coriander). were collected from 6 different markets in Benghazi city (Alberka, Bouatni, Old laithy, Sedi Khalifa, Alsabry, and Bo Snaib) to evaluate the most predominant strain of bacteria. The samples were processed as per standard microbiological methods. The identification of bacteria was performed by biochemical tests, antimicrobial susceptibility test (AST) was done according to Clinical and Laboratory Standard Institute (CLSI) Guidelines , M100 ,35th edition (2025). Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method on Mueller Hinton agar. The presence of ESBL enzymes was confirmed by synergy of cefotaxime, ceftazidime and ceftriaxone with amoxicillin/clavulanic acid.

2.2 Statistical Analysis

Data were analysed using IBM SPSS version 26. Descriptive statistic were used to classify the data in mean and stander variation . fisher's Exact test was performed.

3. Results and Discussion

3.1. Isolation and Identification of Bacteria

During the study, 26 out of 31 vegetable samples showed microbial growth, representing 83.9% of the total samples . This high percentage indicates a considerable level of bacterial contamination among examined vegetables it also indicates inadequate hygienic practices during cultivation, harvesting, transportation , or handling of vegetables prior to consumption. . The results are in accordance with similar works, thus in Spain (2021) Pintor-Cora et al.(21) detected *Enterobacteriaceae* counts in (82.9%) of vegetable samples. also in Poland (2022) Kowalska et al. (22) the isolation of *Enterobacteriaceae* in this report was (84%). Moreover, 5 samples harboured two different strains of *Enterobacteriaceae* (16.1%), demonstrating the possibility of mixed contamination within a single sample .

The identification of the isolates have shown that 6 different gram negative bacterial species were isolated (Table1). The predominant and the highest percentage were *E.coli* accounting (22.58%) followed by *Shigella* spp. (19.35%) , *Proteus* spp, *klebsiella* spp (16.13%) each . In addition, *salmonella* spp. (12.90%). Whereas *Enterobacter* spp and *Serratia* spp each constituted (6.45%) (Figure1).

Overall, these finding highlight the diversity of bacterial contamination present in fresh vegetables and emphasize the potential public health risk associated with their consumption.

3.2 Antimicrobial Sensitivity Test

Antimicrobial susceptibility testing was conducted on all 31 bacterial isolates to determine their resistance profiles. The results showed that all isolates were highly sensitive to Imipenem, Amikacin, and Meropenem, with a Sensitivity rate of 100%. Furthermore, a marked sensitivity was observed to Gentamicin (96.8%) and Ciprofloxacin (93.5%) in addition to Levofloxacin.

Trimethoprim/Sulfamethoxazole (89.3%).

In contrast, the isolates exhibited a high resistance rate to several β- lactam antibiotics, particularly amoxicillin/clavulanic acid, with a resistance rate of (87%). Moderate resistance was recorded to Ampicillin (38.7%) and to both Cefotaxime and Ceftriaxone (32.3%). However, a lower resistance rate was observed for Ceftazidime (16.1%) (Figure 2). Overall, these findings indicate the high persistence of resistance to commonly used β-lactams, while maintaining susceptibility to Carbapenems, Aminoglycosides and Fluoroquinolones. . Consuming fresh fruits and vegetables, which possess MDR bacteria on it, can cause issues in the intestinal tract and infections that are hard to treat. A recent study showed that children with MDR bacteria had less microbial diversity, a changed intestinal microbiota, and an intestinal bacteria that was full of AMR genes. Consequently, the intestinal microbiota serves as a reservoir of resistance genes. [23].

Table 1. Occurrence of Enterobacterales in vegetables.

Type of vegetables	Contamination rate	Identified bacteria
Lettuce	13%	*Salmonella* spp., *klebsiella* spp. & *Serratia* spp.
Tomatoes	13%	*Enterobacter* spp., *E.coli. Salmonella* spp.. *Shigella* spp. & *Klebsiella* spp.
Cucumbers	16%	*E.coli, Salmonella* spp., *Shigella* spp. & *Klebsiella* spp.
Coriander	16%	*Salmonella* spp., *Shigella* spp., *Proteus* spp.& *Enterobacter* spp.
Watercress	16%	*E.coli*, *Proteus* spp., *Salmonella* spp. & *Klebsiella* spp.
Radishes	19.3%	*E.coli* , *Proteus* spp. &*Shigella* spp

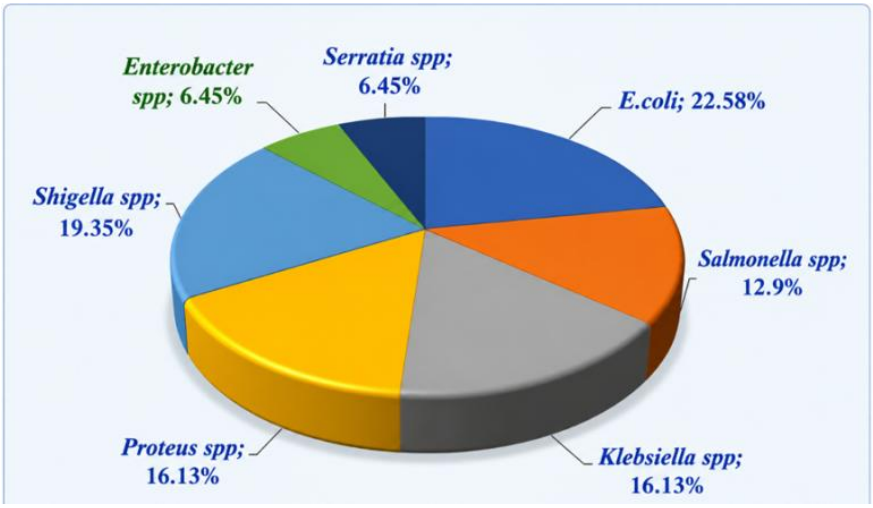


Figure 1. percentage distribution of different bacterial species isolated from vegetable samples

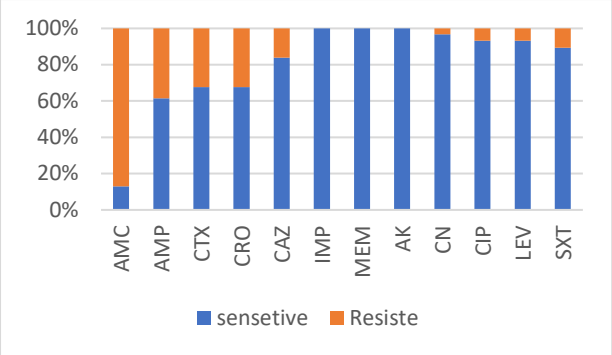


Figure 2. Antibiotic Susceptibility Pattern of *Enterobacteriaceae*

3.3 Phenotypic Detection of Extended Spectrum Beta Lactamases

The results shows the distribution of ESBL-producing isolates among the total samples tested. Out of 31 isolates of gram-negative bacteria, 6 (19.4%) were confirmed as ESBL producers, while 25 isolates (80.6%) were non-ESBL producers. *Salmonella* spp (n= 2) 33.3%, *E.coli*, *Proteus* spp., *Shigella* and *Enterobacter* each showed one isolates (16.6%) that were ESBL producers ($p > 0.05$) (Table2).

Table 2. Prevalence of ESBLs production among Enterobacteriaceae

ESBL PRODUCER	PERCENTAGE (%)
*Salmonella*s Pp	(n=2) 33.3%
Enterobacter	(n=1) 16.7%
Shigella	(n=1) 16.7%
Proteus	(n=1) 16.7%
E.coli	(n=1) 16.7%

Our results corroborate the finding of Amra et al. (2024) in Algeria[24] , where ESBL-producing bacteria were isolated at rate of (19.4%). similarly, we aligned the study of Pintor-cora et al (2021)[21] , which also reported ESBL-producing bacteria were isolated at rate of (17.8%). In contrast, a study conducted in Amsterdam, Netherlands (2014), Reuland et al. reported much lower isolation rate of ESBL-producing microbes at just (6%) [25].

The study had some limitations that should be considered. The sample size were relatively small (n=31), mainly due to limited resource and cost constraints, which the number of samples that could be collected and analysed. This may reduce the statistical power of the study and limit the generalizability of the finding. Therefore, further studies with large sample size are recommended to confirm and expand these findings.

4. Conclusions

As a result of our findings, it's clear that the contamination of vegetables with microbes was significantly high, necessitating urgent action across all stages of production. In addition, our study highlights the occurrence of ESBLs *Enterobacterales* in fresh vegetables, typically ingested raw, thus becoming a public health risk if these vegetables are insufficiently washed or undercooked. Therefore, the consumer needs to be aware about the health risks of fruits and vegetables by maintaining good hygiene at their homes.

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Conflict of interest

The authors declare that there is no conflict of interest.

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