Fresh vegetables as a source of multidrug resistant pathogens

Abstract

Background: Global increase of fresh vegetables consumption as a healthy food, often eaten raw, raises concerns about foodborne illnesses and the spread of antimicrobial-resistant pathogens due to contamination through various sources.

Objective: To determine the microbial quality of common vegetables in the Gaza Strip and the antimicrobial resistance of bacteria recovered from vegetables samples.

Methods: 150 vegetable samples (10 typesof vegetables; 10 samples of Cabbage, 20 Red cabbage, 20 Cucumber, 20 Tomato, 10 Parsley, 20 Eruca, 10 Green mint, 10 Lettuce, 20 Radish, and 10 Green onion)were collected from local farms and markets. Standard microbiological techniques were used to quantify (total viable count, coliform, *Staphylococcusaureus*, *Pseudomonas* and *Enterococcus*) and identify bacteria.Antimicrobial susceptibility testing (disk diffusion and microbroth dilution) was performed in accordance to clinical laboratory standards institute.

Results: Most samples (98%) were positive for coliforms, followed by *Pseudomonas* (94%), *Staphylococcus aureus*(89.3%), and *Enterococcus* (81.3%). High levels of antibiotic resistance were observed across various bacterial species. *Enterobacteriaceae* showed the highest resistance to ceftriaxone (76.2%) and the lowest to amikacin (8.2%). *Staphylococcus aureus* had the highest resistance to penicillin (92.6%) and lowest to clarithromycin/levofloxacin (22.8%). *Enterococcus* spp. displayed highest resistance to penicillin (94.4%) and lowest to norfloxacin (21.6%). Finally, *Pseudomonas* exhibited the highest resistance to ertapenem (70.9%) and the lowest to amikacin (25.4%).

Conclusion: Microbial contamination and antimicrobial resistance in vegetables pose a significant public health threat in the Gaza Strip. Improved agricultural practices, enhanced food safety, responsible antimicrobials use, and continuous monitoring are crucial to mitigate these risks.

Keywords: Vegetable contamination, Antimicrobial-resistantbacteria, Gaza Strip

Introduction

The escalating global crisis of antimicrobial resistance (AMR) poses a significant threat to public health, demanding urgent attention across various sectors (Andersson and Hughes 2014).While numerous studies have focused on the human health implications of AMR (Chandler 2019), effective mitigation strategies require a multi-pronged approach, including comprehensive surveillance to monitor microbial populations and detect resistant species that compromise food security (Mastin, Gottwald et al. 2020).

A crucial component of this approach is antimicrobial stewardship—the responsible use and management of antimicrobials to minimize the selection pressure that drives resistance development (Oliver, Cooper et al. 2021). This involves optimizing agricultural practices to reduce pathogen loads and create less favorable conditions for infection. While antimicrobial stewardship programs exist for human medicine and animal husbandry (Patel, Babady et al. 2020), the role of antimicrobials in plant agriculture, though minimal compared to other sectors (O'Neill 2016), still warrants careful consideration due to the potential for resistance development through intensive seasonal use, as evidenced by the emergence of streptomycin-resistant *Erwinia amylovora* in apple-producing regions (McEwen 2012).

The World Health Organization (WHO) and Food and Agriculture Organization (FAO) advocate for increased fruit and vegetable consumption for optimal health (santé, Organization et al. 2003). However, this increased consumption brings with it a parallel increase in the risk of exposure to foodborne pathogens, including antimicrobial-resistant bacteria (ARB) and antimicrobial-resistance genes (ARGs) (Rahman, Azad et al. 2021).

Studies have documented the presence of ARB and ARGs on fresh produce globally, such as the finding of extended-spectrum β-lactamase (ESBL)-producing pathogens on produce in Japan (Usui, Ozeki et al. 2019). Fresh produce can harbor opportunistic bacteria, previously considered non-pathogenic, which can cause serious illness in immunocompromised individuals (Al-Kharousi, Guizani et al. 2016). Contamination often occurs during various stages of production and distribution, including contact with fecal waste through practices like wastewater irrigation or the use of biosolids as fertilizer (He, Yuan et al. 2020).

The environmental release of antimicrobial residues and resistant bacteria from various human activities, including livestock and fish production, wastewater treatment, and antimicrobial manufacturing, contributes to the growing burden of antimicrobial resistance in environmental matrices (Suzuki and Hoa 2012). Agricultural practices, particularly the

application of animal manure, wastewater, or waste treatment residues, can significantly increase the abundance and mobility of antimicrobial-resistance genes in soil (Wellington, Boxall et al. 2013).

The increased consumption of fresh vegetables, coupled with more frequently documented outbreaks linked to their consumption since the early 1990s, underscores the growing public health concern (Falomir, Gozalbo et al. 2010). These outbreaks are often linked to bacterial contamination, especially from the Enterobacteriaceae family (Tyler and Triplett 2008). The popularity of pre-packaged and prepared "four-range" vegetables further complicates the issue, presenting a concentrated source of potential contamination.

Fresh produce contamination is a major cause of foodborne outbreaks, as highlighted by the estimated 131 outbreaks in the United States between 1996 and 2010 (Newell, Koopmans et al. 2010). The distribution and prevalence of antimicrobial resistance in both commensal and pathogenic bacterial populations are influenced by numerous factors, including exposure to antimicrobials used in human medicine and industrial animal production (McEwen 2012).

The widespread availability of antimicrobials has dramatically improved human and animal health, but their use has inadvertently driven the evolution of AMR (Verraes, Van Boxstael et al. 2013). This global issue affects both human and animal health, with the potential for resistant microorganisms and ARGs to spread through the food chain (Stockwell and Duffy 2012). A major concern is the rise of Gram-negative bacteria (GNB) resistant to β-lactam antimicrobials, particularly through the production of β-lactamases, including extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and carbapenemases production represents a critical pathway to carbapenem resistance, rendering treatment of multidrug-resistant (MDR) bacterial infections extremely challenging(Chelaghma, Loucif et al. 2022).

This study aims to evaluate the microbial quality and antimicrobial resistance patterns of bacteria isolated from locally available vegetables.

Methods and material

Study design and settings

This study employed a descriptive cross-sectional design to investigate microbial contamination and antimicrobial resistance (AMR) in fresh vegetables from the Gaza Strip.The study was conducted at Communicable Diseases Surveillance Center (CDSC) of the Islamic University of Gaza in Gaza.

Sampling and sample collection

Random sampling procedure was adopted to collect samples of vegetables (cabbage, red cabbage, cucumber, tomato, parsley, eruca, green mint, lettuce, radish, and green onion; n=150), 75samples from markets and 75samples from the field—fromthe five governorates of Gaza Strip (North, Gaza, Midzone, Khan Younis, and Rafah). All of the vegetable samples were collected aseptically using a sterile zipper bag. Within two hours of sample collection, an iceboxmaintained at 6-8 °C was used to transport samples to the microbiology laboratory of the CDSC.

Samples processing

For the analysis, 25 g of each vegetable sample was weighed and transferred to 225 ml of sterile normal salinethen blended (1:10 dilution).Serial dilutions (10^{-2}-10^{-8}) were conducted, and aliquots of 100 ul were then inoculated onto the respective media then incubated.

Microbiological indicators determination

For the enumeration of (Total viable count, Coliform, *Pseudomonas*, *S. aureus*, and *Enterococcus*,) 0.1 ml aliquot was drawn and dispensed into a sterile Petri dish (Nutrient Agar, M-endo Agar, Cetrimide Agar, Mannitol Salt Agar, and Bile Esculin Agar)and incubated at 37 °C for 24 hours.On MSA, *S. aureus* appear golden-yellow colonies, it changes the color of the medium to yellow, and the colonies as circular, smooth, and shiny. While on BEA, *Enterococcus* form small, dark brown to black colonies, but on m-endo agar, the *Enterobacteriaceae* appear as red to pink with metallic sheen especially (E. coli). *Pseudomonas* typically greenish, can also appear blue-green or even yellow-green. and incubated at 37 °C for 24 hours. On MSA, S. aureus appears as golden-yellow colonies. The

number of typical colonies was counted and was expressed as colony-forming units (CFU/g). Thefollowing equation was used to calculate the total colony number:

$$\boldsymbol{Total\ count} = no\ of\ colonies \times 1/volume\ plated \times 1/dilution$$

Identification of bacterial isolates

After the incubation period, positive cultures were subcultured onto MacConkey agar plates and Blood agars to identify isolates that were used in the antimicrobial susceptibility testing. Isolates were identified based on colony color and morphology in addition to specific biochemical reactions and the final identification using the API 20E (biomerieux, France) test system for Gram negative isolates. Gram staining, catalase, DNase, coagulase, hemolytic activity on Blood agar, specific antimicrobial susceptibilities, and Api staph and srep systems were used to identify Gram-positive isolates.

Antimicrobial susceptibility testing

The test was performed using the Kirby-Bauer disk diffusion method, following CLSI guidelines. A standardized inoculum of each bacterial isolate was spread on Mueller-Hinton agar plates, and antimicrobial disks were placed on the agar. After incubation, the zones of inhibition were measured, and the results were interpreted as susceptible, intermediate, or resistant based on CLSI criteria. For colistin and vancomycin, microbroth dilution was used due to the irregularity of inhibition zones making measurement unreliable by the disk diffusion method.

Data analysis

Statistical Package for Social Science (SPSS) software was used to compile, tabulate, and analyze the data obtained. Pie charts, tables, and histograms were used to present the results. Each isolate's Multiple Antimicrobial Resistance Index (MAR index) was calculated.

Results

Microbiological Analysis

Among the 150 samples that were collected, 147 (98%) werepositive for coliforms, 141 (94%) for *Pseudomonas*, 136 (90.6%) for *S. aureus*, and 125 (83.3%) for *Enterococcus* were the lowest positive result.Almost all(149; 99.3%) samples showed positive growth on the TVC as shown in table (1).

Table (1): The percentage of positive microbiological indicatorsin the study samples (N=150).

Microorganism	Positive		Negative		Min	Max	Mean colonies for positive
	N	%	N	%			
Coliform	145	98	3	2	$1X10^3$	$1 X 10^9$	$2.3 X 10^8$
Pseudomonas	141	94	9	6	$2X10^3$	$1 X 10^9$	$2.4 X 10^8$
S. aureus	136	90.6	16	10.7	$3X10^2$	$1 X 10^9$	$5.5 X 10^7$
Enterococcus	125	83.3%	28	18.7%	$2X10^1$	$1 X 10^9$	$1.7 X 10^7$

Five genera of the family *Enterobacteriaceae* were identified in this research from the vegetable samples. The highest frequency was for *E. coli* 51 (34.5%), followed by *K. pneumoniae* 43 (29.1%), *Enterobacter* spp. 22 (14.9%), *Citrobacter* spp. 19 (12.8%) and *Proteus* spp. 12 (8.1%) out of 147 positive samples.

The distribution of microbiological indicators according to sample source

No statistically significant differences between sample sources and the indicators (P>0.05). Showed Table (2).

Table (2): Comparison between sample sources according to microorganism.

Microorganism		Sample sources				Statistical test	
		Market		Farm		$\chi 2$	P-value
		N	%	N	%		
Coliform	Positive	76	50.	71	47.3	3.144	0.76
	Negative	0	0	3	2		

S. aureus	Positive	69	46	67	43	0.343	0.558
	Negative	6	4.6	8	6		
Pseudomonas spp.	Positive	72	48	69	46	0.148	0.702
	Negative	4	2.6	5	3.3		
Enterococcus spp.	Positive	66	44	59	37.3	3.079	0.079
	Negative	9	6.6	16	12		

Distribution of microbiological indicators according to sample type

There was no statistically significance difference between the two microbial indicators (Coliform, and *Pseudomonas*), and the sample type from which samples were collected (P value >0.05). However, the results showed statistically significant differences in the sample type with the *Enterococcus* and *S. aureus* among study samples (P<0.05). See table (3).

Table (3): The distribution of microbiological indicators according to sample type.

Sample type	N of samples	Percentage positive				
		TVC	Coliform	*Pseudomonas*	*S. aureus*	*Enterococcus*
Cabbage	10	100	100	100	100	80
Red cabbage	20	100	100	95	85	65
Cucumber	20	100	95	90	90	95
Tomato	20	100	90	90	65	85
Parsley	10	100	100	100	90	100
Eruca	20	100	100	100	100	95
Green mint	10	100	100	100	100	100
Lettuce	10	100	100	100	100	80
Radish	20	100	100	90	100	80
Green onion	10	96	100	90	70	20

Distribution of positive microbiological indicators according to governorates

There was no statistically significance difference between the three microbiological indicators (*Enterococcus*, *S. aureus*, and Coliforms), and the region from which samples were collected (P value >0.05). However, the results showed statistically significant differences in the sample collection region and *Pseudomonas* among study samples (P<0.05). See Table (4).

Table (4): Relation between positive microbiological indicators according to governorates

Governorate	N of samples	Percentage positive				
		TVC	Coliforms	*Pseudomonas*	*S. aureus*	*Enterococcus*
Gaza	30	100	93	83	93	73
North	30	100	96	90	83	83
Khan Younes	30	100	100	100	93	83
Rafah	30	100	100	96	83	76
Midzone	30	96	100	100	93	90

Passed and failed samples according to FDA microbial limits in fresh vegetables

The collected vegetables were evaluated to determine their compliance with safety limits based on FDA permissible standards for bacteria in vegetables. The acceptable TVC for fresh vegetables is less than 10^6 CFU/g, total number of coliform in fresh vegetables of 10^2 (CFU) per gram, that less than 10^3 CFU/g of food should be considered the acceptable amount of *S. aureus* in ready-to-eat food, and fresh vegetable counts of *Enterococcus* must not exceed 10^3 CFU/g. The results were categorized into 'passed' and 'failed' based on bacterial counts and contamination levels. Table (5) illustrate the classification of the samples according to various microbiological indicators.

Table (5): Passed and failed samples according to FDA microbial limits in fresh vegetables.

Type of samples	N	Total viable count		Coliform		*Pseudomonas*		*S. aureus*		*Enterococcus*	
		Passed	Failed	Passed	Failed	Passed	Failed	Passed	Failed	Passed	Failed

Cabbage	**10**	3	7	2	8	8	2	7	3	9	1
Red cabbage	**20**	6	14	4	16	7	13	8	12	7	13
Cucumber	**20**	9	11	4	16	11	9	9	11	7	13
Tomato	**20**	7	13	4	16	13	7	13	7	11	9
Parsley	**10**	10	0	2	8	2	8	3	7	5	5
Eruca	**20**	3	17	3	17	2	18	2	18	3	17
Green mint	**10**	2	8	0	10	4	6	0	10	6	4
Lettuce	**10**	0	10	0	10	4	6	1	9	2	8
Radish	**20**	9	11	8	12	14	6	10	10	11	9
Green onion	**10**	6	4	4	6	8	2	7	3	10	0

Antimicrobial Susceptibility of *Enterobacteriaceae*

*Enterobacteriaceae*isolates (N=147), were tested for their susceptibilities to 16 different antimicrobial agents, 92.6% (N=136) were classified as s multi drug resistance with a MAR index ($\geq$ 0.2).The results showed thatthe highest resistance was to chloramphenicol (76.2%), and the lowest resistance was to amikacin (8.2%). See Figure (1)

Antimicrobial Susceptibility of*Pseudomonas*

*Pseudomonas*isolates (N=141) were tested for their susceptibilities to 13 antimicrobial agents and the results showed that thehighest resistance was to ertapenem (70.9%), and the lowest resistance was to amikacin (25.4%). Among the 141 isolates of *Pseudomonas,* 91.5% (N=129)were classified as MDR with a MAR index ($\geq$ 0.2).(See Figure 1).
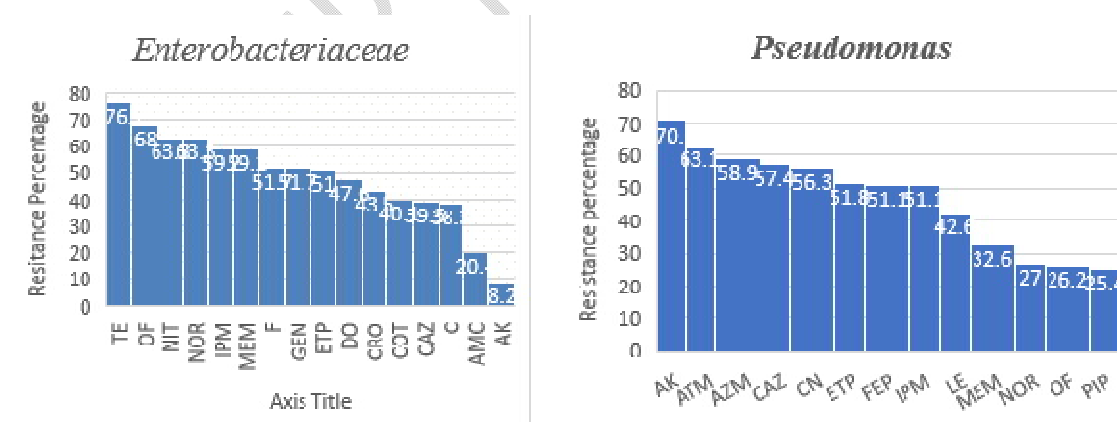

Figure (1): Antimicrobial Susceptibility of Gram-negativeisolates; left*Enterobacteriaceae*, Right, *Pseudomonas*

Antimicrobial Susceptibility of *S. aureus*

S. aureus isolates had a greater degree of multidrug resistance than other bacteria tested in this study.Isolates of *S. aureus* 100% (N=136) were classified as s multi drug resistance with a MAR index ($\geq$ 0.2).They were tested against 19 different antimicrobial agents. The results showed that the highest resistance was to penicillin (92.6%),and the lowest resistance were to clarithromycin (22.8%) and levofloxacin (22.8%). See Figure (2).

Antimicrobial Susceptibility of *Enterococcus*spp.

Among the122 isolates of *Enterococcus* spp., 92% (N=117) were classified as MDR resistance with a MAR index ($\geq$ 0.2). the isolates for their susceptibilities against14 different antimicrobial agents and the results showed thatthe highest resistance was to penicillin (94.4%), and the lowest resistance was to norfloxacin (21.6%). Shown Figure (2).

Figure (2): Antimicrobial Susceptibility of Gram-positiveisolates; left*S. aureus*, Right, *Pseudomonas*
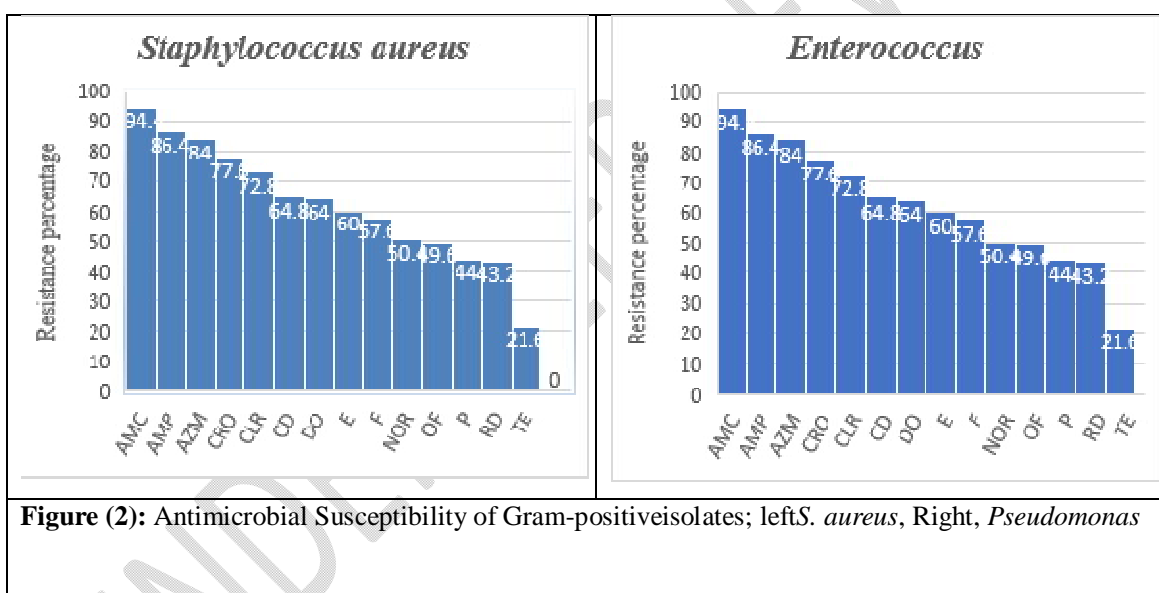
Discussion

This study reports a high prevalence of microbial contamination in raw vegetables from the Gaza Strip, highlighting a significant public health concern. The findings underscore the need for improved food safety practices throughout the entire agricultural and distribution chain. The high percentage of coliforms (98%) detected aligns with previous research in the US, indicating widespread fecal contamination likely stemming from factors such as proximity to animal areas, contaminated irrigation water, inadequate hygiene, and sanitation practices (Steinka, Barone et al. 2017).

The high prevalence of *Pseudomonas* (94%) is potentially linked to the humid environment of greenhouses commonly used in Gaza's agriculture, creating favorable conditions for this bacterium's growth. This contrasts with lower prevalence reported in studies from Brazil(de Melo, da Mata Gomes et al. 2019), highlighting the influence of environmental factors like climate and soil quality on microbial populations.

The high detection rate of *S. aureus* (89.3%) across all ten vegetable types emphasizes the risk of food poisoning through poor hand hygiene and contamination during post-harvest handling. This is notably higher than the prevalence observed in China (Jia, Qin et al. 2024),possibly reflecting differences in vegetable types, sample sizes, local climate, and detection methods. The lower prevalence of *Enterococcus* (81.3%) compared to studies in Georgia (McGowan, Jackson et al. 2006), may be due to the difference in sample types, with our study focusing solely on vegetables while the Georgia study included meat and fruit. The presence of *Enterococcus* in vegetables, however, underscores the potential for contamination via improper manure or wastewater usage.

Significant differences in contamination levels were observed between farm-harvested and market vegetables, particularly for *E. coli* (Kapeleka, Sauli et al. 2020). This indicates that contamination is exacerbated during post-harvest handling and distribution. The lack of statistically significant differences in *Enterobacteriaceae* among different vegetable types (P > 0.05) likely reflects the shared irrigation water source, contrasting with some studies showing higher contamination in specific vegetables like cucumbers (Alam, Bristi et al. 2013), or cabbage (Chinakwe, Nwogwugwu et al. 2022).However, statistically significant differences (P < 0.05) were observed in *S. aureus* contamination across different vegetable types, with leafy greens showing the highest levels. This aligns with observations in other studies (Wu, Huang et al. 2018), suggesting that the complex structure of leafy vegetables provides increased surface area for microbial growth. Similarly, statistically significant differences were observed for *Enterococcus* spp. (P < 0.05), with cucumber and Eruca showing the highest levels, highlighting the potential for environmental contamination during cultivation and handling.

Interestingly, there was no significant relationship between the prevalence of *Pseudomonas* and vegetable type, possibly influenced by the ubiquitous nature of this bacterium in the humid Gaza environment and contrasting with findings in other studies which reported high prevalence in lettuce and chard (Estepa, Rojo-Bezares et al. 2015), with

low levels in onion due to its layered structure and bioactive compounds(Sharma, Mahato et al. 2018).

Geographic variations in microbial contamination were less pronounced, potentially due to similar irrigation practices and transportation methods across Gaza governorates. However, *Pseudomonas* showed a geographic relationship, with Gaza governorate having the lowest levels and Khan Younis and Midzone the highest, suggesting differences in humidity levels and fertilizer composition may influence bacterial prevalence.

The antimicrobial resistance profiles of isolates revealed a high prevalence of multi-drug resistance (MDR), particularly among *Enterobacteriaceae* (92.6%), *S. aureus* (100%), and *Enterococcus* spp. (92%). This aligns with global trends showing escalating antimicrobial resistance in foodborne pathogens (Stein, Brinks et al. 2024), emphasizing the need for interventions to minimize antimicrobial usage and prevent the spread of resistance genes (Mandumpala 2023).

 Specific resistance patterns varied considerably among bacterial species and antimicrobial classes, frequently differing from results in other studies. These variations may be attributed to factors such as geographic location, antimicrobial usage patterns, sample size, detection methods, and the diverse mechanisms by which bacteria acquire and develop resistance (Li and Schneider-Futschik 2023). The high levels of resistance observed, particularly to commonly used antimicrobials, represent a significant challenge to public health, highlighting the urgent need for improved surveillance and implementation of comprehensive control strategies(Vítězová, Kohoutová et al. 2020).

In conclusion,this study found high levels of potentially pathogenic bacteriain vegetable samples collected from local farms and market indicating poor agricultural practices, handling, and storage. The findings emphasize the risks of eating raw vegetables and the need for improved government oversight of the entire vegetable supply chain. It is important identify critical food safety gaps in the Gaza Strip's vegetable production and distribution chain. The high prevalence of microbial contamination and antimicrobial resistance necessitates immediate actions focusing on improved sanitation, hygiene, and post-harvest handling practices. Further research should investigate the specific mechanisms driving antimicrobial resistance and explore the effectiveness of targeted interventions to minimize public health risks associated with consuming raw vegetables in this region.

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