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Identification and Antibiotic Susceptibility of Bacterial Isolates from Ready to Eat Vegetables Salad

Sura Qusay Ali , Thanoon Yahya AL-Azzawi
Department of biology, College of education, Al-Iraqia, University, Baghdad, Iraq.

Abstract :

This study analysed the antimicrobial resistance patterns and microbiological quality of vegetables that are ready to eat (RTE). The overall bacterial counts ranged from 8.00×10^5 to 9.30×10^5 cfu/ml, above the established safety standards, and 153 bacterial isolates were obtained from 100 samples. In terms of frequency, *Escherichia coli* was the most common isolate at 49%, followed by *Salmonella enterica* at 21%, *Staphylococcus aureus* at 20%, and *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Shigella flexneri* at lower levels of 3% each. Both Gram-negative and Gram-positive bacteria showed concerning resistance trends when tested for antibiotic susceptibility using the disc diffusion technique. The percentage of resistant Klebsiella pneumoniae bacteria to Aztreonam, Ticarcillin, Tobramycin, and Trimethoprim/Sulfamethoxazole was 80%, but the percentage of resistant *Escherichia coli* bacteria to Imipenem was 24%. *S. aureus* and *E. faecalis* were the Gram-positive bacteria with the highest rates of resistance to Rifampicin (39% and 40%, respectively) and Vancomycin, respectively (VRE). Stricter “farm-to-table” safety measures are required in light of these results, which show that RTE vegetables are a reservoir for multidrug-resistant bacteria, most likely caused by careless handling and the overuse of antibiotics.

تشخيص العزلات البكتيرية من سلطة الخضراوات الجاهزة للأكل وحساسيتها للمضادات الحيوية

سرى قصي علي ، ذنون يحيى العزاوي
الجامعة العراقية/ كلية التربية - قسم علوم الحياة

مستخلص:

حللت هذه الدراسة أنماط مقاومة المضادات الحيوية والجودة الميكروبيولوجية للخضروات الجاهزة للأكل. تراوحت إجمالي أعداد البكتيريا من 8.0×10^5 إلى 9.30×10^5 وحدة تشكيل مستعمرة/ مل، وهو ما يتجاوز معايير السلامة المحددة. تم الحصول على 153 عزلة بكتيرية من 100 عينة. من حيث التكرار، كانت الإشريكية القولونية هي العزلة الأكثر شيوعًا بنسبة 49٪، تليها السالمونيلا المعوية بنسبة 21٪، والمكورات العنقودية الذهبية بنسبة 20٪، والكليبسيلا الرئوية، والإنتيروكوكوس فيكالس، والشجيلة الفلكسنيارية بمستويات أقل بنسبة 3٪ لكل منها. أظهرت كل من البكتيريا سالبة الجرام وموجبة الجرام اتجاهات مثيرة للقلق في مقاومة المضادات الحيوية عند اختبارها باستخدام تقنية انتشار الأقراص. نسبة مقاومة بكتيريا كلبسييلا نيمونيا للأزتريونام، تيكارسيلين، توبراميسين، وتريميثوبريم/ سلفاميثوكسازول كانت 80٪، ولكن نسبة مقاومة بكتيريا الإشريكية القولونية للإيميبينيم كانت 24٪. كانت بكتيريا المكورات العنقودية الذهبية والإنتيروكوكوس فيكالس من البكتيريا إيجابية الغرام ذات أعلى معدلات المقاومة لريفامبيسين (39٪ و 40٪ على التوالي) وفانكومايسين. تتطلب هذه النتائج اتخاذ تدابير سلامة أكثر صرامة من «المزرعة إلى المائدة»، والتي تظهر أن الخضروات الجاهزة للأكل هي خزان للبكتيريا المقاومة لمضادات الميكروبات المتعددة، على الأرجح بسبب التعامل غير الحذر والإفراط في استخدام المضادات الحيوية.

Introduction

Individuals' dietary practices are shaped by societal lifestyles, and it appears that contemporary life is increasingly frenetic, resulting in diminished leisure time; hence, convenience foods are increasingly favoured. Ready-to-consume products necessitate no further preparation prior to consumption and are available in a diverse array, including fresh-cut fruits, salads, vegetables, prepared meat and poultry, smoked or salted seafood, smoked or salted meat, and dairy products (Gonçalves et al., 2023; Smigic, et al., 2023). The presence of both fruits and vegetables in the market may enhance their consumption among the general populace, thereby aiding in the attainment of the World Health Organization's recommendation of a daily intake of 400 grams of fruits and vegetables per capita (Raffo et al., 2021). Globally, salad vegetables are regarded as a significant source of nutrients, notably as providers of anti-cancer chemicals for the skin (Ramteke et al., 2016). Recent research have demonstrated that the consumption of salad vegetables can avert heart diseases and skin cancers (Coulibaly-Kalpy et al., 2017). Salad vegetables are mostly consumed for their nutritional components and their flavour when paired with other foods (Choudhury et al., 2011; Alimi et al., 2016). Salads serve as sources of vitamins, minerals, proteins, and other dietary elements necessary for the optimal functioning of the human body (Amoah, 2014). Ready-to-eat (RTE) vegetables salads are items that have undergone minimal processing (Łepecka et al., 2022; (Ababio and Lovatt, 2014). including sorting, chopping, washing/disinfection, rinsing, centrifugation, packaging in air or a modified atmosphere, refrigeration, and transportation. These salads are then consumed in a short period of time [Mir et al., 2018; Finger et al., 2023]. It has been stated by Ulger et al. [Ülger et al., 2018]. Multiple factors contribute to the elevated risk of microbial contamination linked to vegetable salads. This encompasses environmental conditions (hygiene) in which vegetable salads are prepared, the washing of vegetables with contaminated water, farming, post-harvest processing, and transportation (Taban and Halkman, 2011). Additional elements utilised in the preparation of the vegetable salad may potentially serve as a source of contamination (Gantois et al., 2009). Consequently, from cultivation to harvesting, preparation, and consumption, the likelihood of these vegetable salads encountering harmful microbes is significantly elevated. According to Mensah et al. (2002), pre-prepared foods such as vegetable salads provide a significant risk of food poisoning due to the presence of entropathogens. The presence of pathogens RTE vegetables poses a greater public health risk than in raw meat products, as RTE vegetables typically do not undergo further treatments to eradicate these bacteria; additionally, (Osaili et al., 2011) they may harbour native microflora, including pathogenic bacteria from the raw materials used in their preparation (Francis and Thomas, 1999). The predominant bacteria found in RTE vegetables include *Salmonella, Listeria monocytogenes, Campylobacter jejuni*, *S aureus*, *Bacillus cereus, and Clostridium perfringens* (Christison and Lindsay , 2007; Yang et al., 2016). Salmonella is a gram-negative bacillus belonging to the Enterobacteriaceae family and is a significant foodborne disease affecting humans. Furthermore, it is regarded as a significant contributor to mortality and economic detriment globally. Each year, there are 93.8 million instances of gastroenteritis caused by *Salmonella* varieties, resulting in 155,000 fatalities globally. It is estimated that 80.3 million are attributable to food-borne sources.Fourteen To date, more than 2,500 Salmonella serotypes have been recognised, with approximately fifty percent being *Salmonella enterica* Serovar *Typhimurium*. The primary transmission routes of *Salmonella* species are chicken meat RTE vegetables, dairy products, fruits, and vegetables (Keithlin et al., Keithlin). The World Health Organization estimates that 550 million individuals get salmonella poisoning year, with around 220 million of those victims being children younger than five years old. One of the four main infectious agents that can cause diarrhoea on a global scale is Salmonella (who, 2021). Feglo and Sakyi (2012) documented varying concentrations of *S aureus*, Bacillus species, *K pneumoniae*, and *E coli* in several RTE vegetables. Salmonella, *Shigella*, *E. coli*, *Clostridium*, *Staphylococcus*, *Campylobacter*, and *Vibrio* are prevalent bacteria responsible for foodborne illnesses (Amoah, 2014). Bruce et al. (2005) indicated that diarrhoeal disorders are the primary reasons for hospital visits. Research has revealed that vegetables prepared by food vendors, particularly street food vendors, are significantly contaminated with faecal matter and harmful microorganisms (Amoah et al., 2006). Additionally, various associated risk practices in food handling have been identified by Henseler (2005). Significant efforts have been directed towards the surveillance of antimicrobial resistance (AMR) in bacteria from humans and food-producing animals in developed nations in recent years (World Health Organization and the European Centre for Disease Prevention and Control 2022); however, insufficient focus has been placed on other One Health contexts, such as the environment and food, especially in low- and middle-income countries (Ikhimiukor et al. 2022). The existence of antimicrobial-resistant bacteria in fruits and vegetables poses a significant food safety issue, as these items are frequently ingested raw or with minimum processing. Detecting antimicrobial-resistant bacteria in fruits and vegetables would elucidate the risk of local populations' exposure to AMR via food and aid in formulating suitable strategies to guarantee food safety throughout the value chain of fresh produce from cultivation to consumption. Although certain studies have identified antimicrobial-resistant pathogens in retail samples across various regions over the word (Adesetan et al. 2013, Oyedele et al. 2020, Ajiboye and Emmanuel 2021, Ugwu et al. 2022), there is a paucity of information regarding antimicrobial-resistant pathogens in fruits and vegetables. This study aims to identify antimicrobial-resistant pathogens in RTE vegetables in Baghdad, Iraq.

Methodology

Sample collection and processing

One hundred samples of packaged RTE vegetables salads from 15 separate restaurants in Baghdad were collected between November and January of 2026. They comprised leaves, carrots, a mixture of leaves and carrots, and a combination of leaves, carrots, and corn. All samples were randomly acquired prior to their best-before date, transported to the laboratory in their original packaging within a maximum duration of 1 hour, and analysed immediately upon arrival.

Characterization and Identification of bacterial isolates

A sterile blade was employed to excise the epicarp and mesocarp of each specimen in a sterile tray following the disinfection of each specimen's surface. One gram of each sample was measured into a sterile stomacher bag (Seward, UK) and homogenised using a stomacher (Stomacher 80 Biomaster, Seward, UK) using 9 ml of sterile water for 2 minutes. The homogenate was serially diluted with sterile water and pour plated onto nutrient agar (NA) (HiMedia, India), MacConkey agar (MCA) (HiMedia, India), Mannitol Salt Agar - MSA (HiMedia, India), Chromogenic Media and Salmonella–Shigella agar (SSA) (HiMedia, India) in duplicate. All isolated bacteria were identified utilising conventional methods, including colonial characteristics, Gram staining, and biochemical reactions such as carbohydrate fermentation, coagulase test, indole test, hydrogen sulphide production, urease production, and citrate utilisation, as stipulated in Bergey's Manual of Determinative Bacteriology (Bergey and Holt 1994).

Antibiotic susceptibility test

In order to determine which antibiotics were most effective against various groups of pathogenic bacteria, we used the disc diffusion method as described in reference (CLSI, 2014). Here's what we did: after 18 to 24 hours, we transferred the colonies of the growing bacterial isolates to nutrient haemocyte medium using a standard culture conveyor. This was done in a test tube that also contained 5 ml of physiological salt solution. To get a cell count of 1.5-1 x 10^8 cells/ml, the bacterial suspension's density was adjusted using a McFarland turbidity scale 0.5 tube. We pressed a cotton swab against the inside of the tube to extract any excess inoculum after dipping it in the bacterial suspension. Then, we inoculated the Muller-Hinton Agar (MHA) plate by wiping the swab across the middle surface in multiple directions to achieve uniform growth. After that, the dishes were allowed to dry at room temperature for around ten to fifteen minutes. Using sterile forceps, the antibiotic tablets were carefully placed on top of the inoculation (MHA) and pressed down so that they would adhere firmly; there should be at least 25 mm of space between the centers of the tablets. The plates were kept in an airy, inverted incubator at 37 ° C for 24 hours. After that, the diameter of the inhibition zone around each disc was measured in millimetres. This measurement was then compared to the standard rates for the inhibition zone diameter for the antibiotics listed in (CLSI, 2014), which determine whether the bacteria are resistant or sensitive to those antibiotics.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 16.0.0, SPSS Inc., Chicago. The Univariate technique includes regression analysis and analysis of variance for a singular dependent variable affected by one or more factors and/or variables. The variables of the factor classified the population into categories. The null hypotheses on the impact of different variables on the means of separate groupings of a single dependent variable using the General Linear Model method. We utilised the Bivariate Correlations approach to calculate the coefficient of Pearson's correlation and its significance levels. Correlations evaluate the association among variables or rank values (Released, 2007).

Results

Microbiological pattern isolated from ready to eat vegetables

The results indicated that from the 100 samples, a total of 153 isolates were obtained over the sampling trips. The overall bacterial count recorded in this investigation varied from 8.00×10^5 to 9.30×10^5 cfu/ml. The bacterial isolates detected from the samples were: *Escherichia coli* (n=75) 50%; *Salmonella enterica* (n=32) 21%; *Staphylococcus aureus* (n=75) 20%; *Klebsiella pneumoniae* (n=75) 3%; *Enterococcus faecalis* (n=75) 3%; and *Shigella flexneri* (n=75) 3%. Figure (1), Table 1 illustrates the prevalence of bacteria throughout the study.

Table 1: Illustrates the isolated bacteria with its frequency and percentage

Isolated bacteria	Number of occurrence	Frequency of occurrence
Escherichia coli	75	49%
Salmonella enterica	32	21%
Staphylococcus aureus	31	20%
Klebsiella pneumoniae	5	3%
Enterococcus faecalis	5	3%
Shigella flexneri	5	3%
Total number	**153**	**100%**

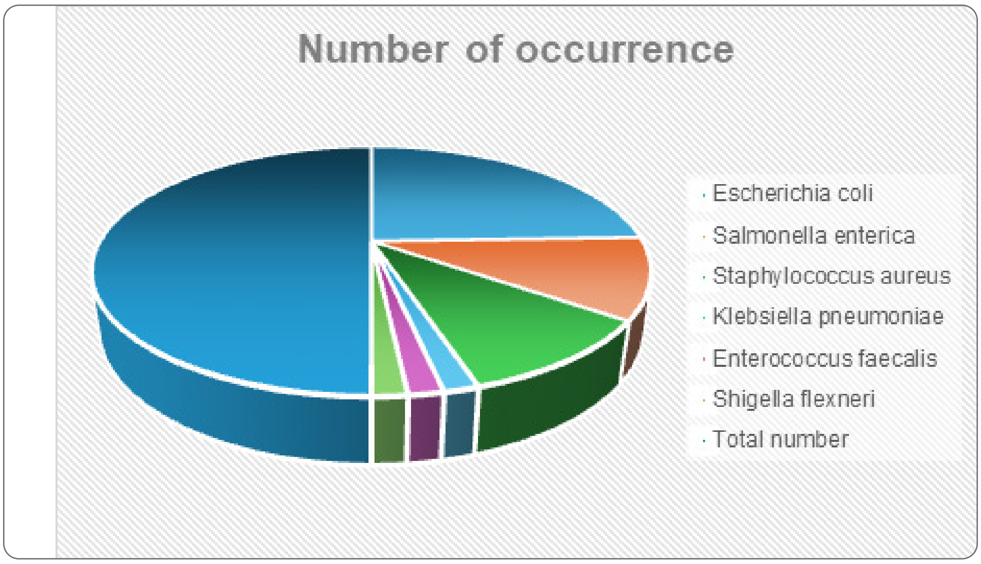

Figure (1): chart of isolated bacteria from ready to eat vegetables.

In this investigation, 153 bacterial strains were identified and characterised. 49% of the total isolates were classified as *E coli* (n=75), constituting nearly half of the studied microbial community. Subsequently, *Salmonella enterica* constituted 21% (n=32) of the isolates. According to these findings, the studied environment had an elevated level of coliforms and enteric pathogens.

Antimicrobial susceptibility test

This is the test for antibiotic susceptibility. This model is used in the random use of antibiotics, and this is a different usage in use depending on the dependence on sensitivity testing (Rivera , 2003). Multiple antibiotic resistance forms in the context, and it is medically risky. This model is used in the random use of antibiotics. In order to determine antibiotic sensitivity, the hard disc diffusion method was applied to MHA. There are 14 antibiotics from a variety of antibiotics that are effective against Gram-negative bacteria, while 17 drugs are effective against Gram-positive bacteria. Bacteria were examined (Pollack, 2012), and the findings were analysed as indicated in (CLSI, 2014). As illustrated in the tables (2,3).

Table (2) Antimicrobial susceptibility test of G-negative resistant bacterial isolates isolated from ready to eat vegetables

Bacterial isolates / Antibiotics	***Escherichia coli***		*Salmonella enterica*		***Klebsiella pneumoniae***		*Shigella flexneri*	
	№	٪	№	٪	№	٪	№	٪
Amikacin	5	7%	4	13%	2	40%	0	0%
Aztreonam	12	16%	1	3%	4	80%	2	40%
Cefepime	3	4%	2	6%	1	20%	3	60%
Ceftazidime	1	1%	3	9%	1	20%	4	80%
Ciprofloxacine	10	13%	0	0%	2	40%	0	0%
Gentamicin	0	0%	8	25%	2	40%	1	20%
Imipenem	18	24%	1	3%	1	20%	0	0%
Meropenem	1	1%	0	0%	2	40%	1	20%
Minocycline	1	1%	9	28%	0	0%	0	0%
Piperacillin	14	19%	1	3%	2	40%	0	0%
Piperacillin/Tazobactam	4	5%	0	0%	0	0%	1	20%
Ticarcillin	10	13%	3	9%	4	80%	3	60%
Tobramycin	0	0%	4	13%	4	80%	1	20%
Trimethoprime/ Sulfamethoxazole	17	23%	13	41%	4	80%	0	0%
Total Tested Samples	**75**		**32**		**5**		**5**	

Table (3) Antimicrobial susceptibility test of G-positive resistant bacterial isolates isolated from ready to eat vegetables

Antibiotics	*Staphylococcus aureus* NO.	(%)	*Enterococcus faecalis* NO.	(%)
Benzylpenicillin	5	16%	1	20%
Oxzacillin	5	16%	0	0
Gentamicin	2	6%	1	20%
Tobramycin	3	10%	0	0
Levofloxacin	3	10%	1	20%
Moxifloxacin	3	10%	0	0
Erythromycin	7	23%	0	0
Clindamycin	3	10%	0	0
Rifampicin	12	39%	0	0
Teicoplanin	2	6%	0	0
Fucidic Acid	5	16%	0	0
Vancomycin	1	3%	2	40%
Nitrofuranation	1	3%	0	0
Tetracycline	8	26%	0	0
Tigecycline	4	13%	1	20%
Linezole	2	6%	0	0
Trimethoprime/ Sulfamethoxazole	3	10%	0	0
Total Tested samples	**31**		**5**	

Discussion

This study's analysis of the microbiological profile of ready-to-eat (RTE) vegetables indicates substantial contamination by enteric pathogens and varied antibiotic resistance patterns. A total of 153 isolates were obtained from 100 samples, highlighting a significant food safety issue, as these products are generally ingested without additional heat treatment. The investigation documented a substantial bacterial load, varying from $8.00\times1010^5$to 9.30×10^5cfu/ml. This over the permissible microbiological thresholds for raw vegetables in most regulatory standards, indicating possible concerns regarding irrigation water quality or post-harvest management. A serious concern is the preeminent prevalence of *E coli*, which comprised 49% (n=75) of the whole microbial population. This discovery closely parallels recent results from Al-Najaf, Iraq (Ridha, 2026), which documented a 53.3% frequency of *E. coli* in lettuce. The elevated prevalence of this bacteria is a conclusive evidence of recent faecal contamination. In contrast, analogous research in European markets generally indicate *E. coli* prevalence rates under 10%, underscoring a notable discrepancy in sanitary regulations. The identification of obligatory pathogens namely *S enterica* (21%, n=32) and *Shigella flexneri* (3%, n=5) is notably concerning. This result that 21% for *Salmonella* falls within the high-risk spectrum established in research from the Philippines (24.7%) (Vital et al., 2014) and Ethiopia (15%) (Bekele, 2022). International guidelines, such as those established by the WHO and FDA, uphold a “zero tolerance” approach in fresh produce; their presence indicates a direct danger of Salmonellosis and Shigellosis epidemics. Although *Salmonella* is frequently associated with tainted irrigation or manure, the detection of Shigella present in commercially produced food in developed areas indicates direct human-to-food contamination or the infiltration of untreated sewage. their presence in ready-to-eat vegetables presents a direct danger of foodborne outbreaks, including Salmonellosis and Shigellosis. Additionally, *S aureus* was recognised as the third most common isolate at 20% (n=31). This frequency far exceeds the 11% documented in comparable street-vended vegetable studies (Bekele, 2022). The notable occurrence of *S. aureus* in this study, given its prevalence on human skin and in nasal cavities, suggests that contamination likely arises during the “market phase” due to unsanitary handling by vendors or handlers. Similar to research conducted in other developing urban centers, these results classify the samples under investigation as “High Risk.” The contamination is thought to be the consequence of a multi-stage failure in the food safety chain, beginning with the use of polluted environmental water and ending with inadequate hygiene practices during the final distribution of the produce.The susceptibility testing, according to CLSI (2014) and Pollack (2012) criteria, demonstrated varying levels of resistance among the 14 antibiotics evaluated for Gram-negative bacteria (Table 2). *E coli*, the main isolate, shown significant resistance rates, specifically 24% to Imipenem and 23% to Trimethoprim/Sulfamethoxazole. The 24% resistance to Imipenem is markedly greater than the 9.25% resistance observed in recent urban garden research (Pramanik et al., 2025). In contrast, the total susceptibility (0% resistance) to Gentamicin and Tobramycin identified in this investigation aligns with data from (Fatoba, 2022), where E. coli from agricultural soil and litter exhibited minimal resistance levels (3.1%) to Gentamicin and Imipenem (Fatoba et al., 2022). This indicates that although your isolates are developing carbapenem resistance, aminoglycosides continue to be an effective treatment option for these particular organisms. Isolates of *S enterica* shown significant resistance to Trimethoprim/Sulfamethoxazole (41%) and Minocycline (28%). This supports the claim that fresh food is a significant source of “multiple antibiotic resistance forms” (Rivera, 2003). Multidrug-resistant (MDR) patterns are being increasingly documented in non-typhoidal *Salmonella* worldwide; for example, a 2017 study in Morocco revealed that 39.6% of *Salmonella* food isolates exhibited resistance to at least one antibiotic, with particularly high resistance noted for sulfonamides and trimethoprim (Amajoud et al., 2017). The elevated prevalence of multidrug-resistant *Salmonella* in this study samples underscores the potential for treatment failure in foodborne illnesses. Salmonellosis cases derived from these vegetable sources. *K pneumoniae* demonstrated a low incidence of 3%, although its resistance profile was notably severe. The isolate exhibited 80% resistance to Aztreonam, Ticarcillin, Tobramycin, and Trimethoprim/Sulfamethoxazole. This significant resistance threshold corresponds with recent research in Saudi Arabia, indicating that *Klebsiella* species isolated from leafy vegetables exhibited a substantial prevalence of resistance genes, with almost 20% identified as carbapenemase producers (Elsafi et al., 2024). The elevated resistance to Tobramycin and Aztreonam observed in this study is alarming, as it resembles clinical-grade resistance patterns commonly associated with infections acquired in hospitals rather than standard environmental strains.The antibiotic susceptibility profiles for Gram-positive isolates, as presented in Table 3, indicate significant weaknesses in existing treatment protocols. These findings underscore the importance of RTE vegetables as substantial reservoirs for opportunistic infections and clinically important antibiotic resistance.This study revealed that *S aureus* had the greatest resistance to Rifampicin (39%), followed by Tetracycline (26%) and Erythromycin (23%). The enhanced resistance rates align with studies by (Rahimi et al., 2024), where *S. aureus* isolates from fresh produce exhibited significant resistance to Erythromycin (>30%) and Tetracycline (>25%). Nonetheless, a significant observation in your study is that resistance to Vancomycin, frequently regarded as a "last-resort drug," remained low at 3%. This corresponds with the global trend indicating that Vancomycin-resistant *S. aureus* (VRSA) is infrequent in environmental samples relative to clinical environments, yet the detection of any VRSA in RTE vegetables serves as a notable public health indication. A significant treatment issue highlighted in this study is the 40% resistance to Vancomycin detected in Enterococcus faecalis. The incidence of Vancomycin-resistant Enterococci (VRE) in food products is exceedingly high. A study by (Santana et al., 2023) indicated that vancomycin-resistant enterococci (VRE) were present in around 12% of vegetable samples. Your 40% result indicates a significant environmental contamination cycle, perhaps associated with the utilisation of avoparcin-related growth boosters in livestock or the inclusion of hospital effluent in irrigation water. Vancomycin-resistant Enterococcus (VRE) is a known "superbug," and its presence on common vegetables presents a significant danger of horizontal gene transfer to other intestinal pathogens.The data in Tables 2 and 3 demonstrate that RTE vegetables are not simply passive carriers of bacteria but serve as active reservoirs for multidrug-resistant (MDR) strains. The existence of resistance to powerful antibiotics such as Imipenem (in Gram-negative bacteria) and Vancomycin (in Gram-positive bacteria) corroborates Rivera's (2003) claim that the indiscriminate use of antibiotics in agriculture and human medicine is a principal catalyst for resistance. These findings require enhanced sanitation protocols and improved regulatory supervision of irrigation water and soil quality to reduce the danger of multidrug-resistant foodborne illness outbreaks.

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