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

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Abstract :

This study investigated the microbiological quality and antimicrobial resistance profile of ready to eat (RTE) vegetables. Total bacterial counts varied from 8.00×10^5 to 9.30×10^5 cfu/ml which is over the safety criteria and 153 bacterial isolates were isolated from 100 samples. The most prevalent isolate was *Escherichia coli* (49%) followed by *Salmonella enterica (*21%), *Staphylococcus aureus* (20%) and *Klebsiella pneumoniae, Enterococcus faecalis* and *Shigella flexneri* (3% each). Antibiotic susceptibility testing of Gram-negative and Gram-positive bacteria by the disc diffusion method showed worrying patterns of resistance. *K pneumoniae* bacteria resistant to Aztreonam, Ticarcillin, Tobramycin and Trimethoprim/Sulfamethoxazole were 80% and *E coli* bacteria resistant to Imipenem were 24%. *S. aureus* was the most resistant to Rifampicin (39%) and *Enterococcus faecalis* was the most resistant to vancomycin (40%). The findings indicate that ready-to-eat vegetables are a source of multidrug-resistant bacteria, perhaps because of poor handling and indiscriminate use of antibiotics, and hence require improved ''farm-to-table'' safety protocols.

تشخيص العزلات البكتيرية من سلطة الخضراوات الجاهزة للأكل وحساسيتها للمضادات الحيوية

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مستخلص:

هدفت هذه الدراسة إلى تقييم الجودة الميكروبيولوجية ومقاومة المضادات الحيوية في الخضراوات الجاهزة للأكل. تراوحت أعداد البكتيريا الكلية بين 8.00×10^5 و 9.30×10^5 مستعمرة/ مل، وهو ما يتجاوز معايير السلامة، وتم عزل 153 عزلة بكتيرية من 100 عينة. كانت الإشريكية القولونية أكثر العزلات شيوعًا (49٪)، تليها السالمونيلا المعوية (21٪)، والمكورات العنقودية الذهبية (20٪)، والكلبسيلة الرئوية، والمكورات المعوية البرازية، والشيغيلا الفلكسنرية (3٪ لكل منها). أظهر اختبار حساسية المضادات الحيوية للبكتيريا سالبة الغرام وموجبة الغرام باستخدام طريقة انتشار القرص أنماطًا مقلقة للمقاومة. بلغت نسبة بكتيريا الكلبسيلة الرئوية المقاومة للأزتريونام، والتيكارسيلين، والتوبرايسين، والتريميثوبريم/ سلفاميثوكسازول 80٪، بينما بلغت نسبة بكتيريا الإشريكية القولونية المقاومة للإيميبينيم 24٪. كانت بكتيريا المكورات العنقودية الذهبية الأكثر مقاومة للريفامبيسين (39٪)، بينما كانت بكتيريا المكورات المعوية البرازية الأكثر مقاومة للفانكومايسين (40٪). تشير هذه النتائج إلى أن الخضراوات الجاهزة للأكل تُعد مصدرًا للبكتيريا المقاومة للأدوية المتعددة، ربما بسبب سوء التعامل معها والاستخدام العشوائي للمضادات الحيوية، وبالتالي فهي تتطلب بروتوكولات سلامة مُحسّنة من المزرعة إلى المائدة.

Introduction

The way society lives influences the eating habits of people and it seems that today life is getting faster and faster leaving less free time; for this reason convenience foods are more and more popular. Ready-to-eat items are available in a wide variety of fresh-cut fruits, salads and vegetables, prepared beef and poultry, smoked or salted seafood, smoked or salted meat and dairy products and do not need any further processing before they are eaten (Gonçalves et al., 2023; Smigic, et al., 2023). The availability of fruits and vegetables in the market may promote the consumption by the general population, thereby helping to reach the World Health Organization recommendation of a daily diet of 400 grams of fruits and vegetables for each capita (Raffo et al., 2021). Salad veggies are an important source of nutrients and are considered an important source of anti-cancer compounds for skin (Ramteke et al., 2016) worldwide. Recently, it has been shown that salad greens consumption helps prevent heart illnesses and skin malignancies (Coulibaly-Kalpy et al., 2017). Salad veggies are mostly used for its nutritional components and its flavour when used along with other dishes (Choudhury et al., 2011; Alimi et al., 2016). Salads are providers of vitamins, minerals, proteins and other nutritional nutrients important for the normal functioning of the human body (Amoah, 2014). Ready-to-eat (RTE) vegetables salads are products that have been little processed (Łepecka et al., 2022; (Ababio and Lovatt, 2014). as sorting, cutting, washing /disinfection, rinsing, centrifugation, packaging in air or modified atmosphere, refrigeration and transportation. These salads are then consumed in a short time [Mir et al., 2018; Finger et al., 2023]. It was said by Ülger et al. [Ülger et al., 2018]. The risk of microbial contamination is higher for vegetable salads for a number of reasons. This includes the environmental conditions (hygiene) in which vegetable salads are produced, washing of vegetables with polluted water, cultivation, processing and transportation after harvest (Taban & Halkman, 2011). Other ingredients used in the production of the vegetable salad may be possibly a source of contamination (Gantois et al., 2009). So, from growth to harvest, preparation and consumption, the risk of these vegetable salads being exposed to dangerous microorganisms is much higher. As stated by Mensah et al. (2002), Pre-prepared foods such as vegetable salads have a great danger of food poisoning, as they contain entropathogens. The health risk to the public from pathogens in RTE vegetables is higher than in raw meat products since RTE vegetables are not subject to further treatment to eliminate these bacteria and may also contain native microflora including pathogenic bacteria from the raw materials used in their preparation (Osaili et al., 2011; Francis and Thomas, 1999). The most common RTE veggies are mainly contaminated with the following bacteria, including 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 of the Enterobacteriaceae family and is a major food-borne illness of humans. Furthermore, it is regarded as a leading cause of mortality and economic loss internationally. Each year, 93.8 million cases of gastroenteritis and 155,000 deaths are caused by Salmonella strains over the world. Of those, 80.3 million are related to foodborne sources.Fourteen To far, more than 2,500 Salmonella serotypes have been recognised, around 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 people around the world become sick from salmonella poisoning annually, and about 220 million of the victims are children younger than 5 years old. Salmonella is one of the four major infectious pathogens that can cause diarrhoea worldwide (who, 2021). Feglo and Sakyi (2012) reported different levels of S aureus, Bacillus spp., K pneumoniae and E coli in many RTE veggies. Salmonella, Shigella, E. coli, Clostridium, Staphylococcus, Campylobacter and Vibrio are common bacteria that cause foodborne diseases (Amoah, 2014). According to Bruce et al. (2005) the major causes of hospital attendance are diarrhoeal illnesses. Research has shown that vegetables produced by food sellers especially street food vendors are highly polluted with faecal matter and dangerous micro-organisms (Amoah et al, 2006). Henseler (2005) has also highlighted numerous risk practices related with food handling. In recent years, extensive efforts have been made for the detection of antimicrobial resistance (AMR) in bacteria from humans and food-producing animals in affluent countries (World Health Organization and the European Centre for Disease Prevention and Control 2022); But other One Health settings like environment and food have not been as well attended to, especially in low- and middle-income countries (Ikhimiukor et al. 2022). The occurrence of antibiotic resistant bacteria on fruits and vegetables is a major food safety concern as these foods are often consumed raw or with minimal processing. The presence of antimicrobial resistant bacteria in fruits and vegetables would elucidate the risk of local populations' exposure to AMR through food and would guide the development of appropriate solutions to ensure food safety along the value chain of fresh produce from farm to table.

While some studies have reported antimicrobial resistant pathogens in retail samples in different parts of the word (Adesetan et al. 2013, Oyedele et al. 2020, Ajiboye and Emmanuel 2021, Ugwu et al. 2022), there is limited information on antimicrobial resistant pathogens in fruits and vegetables. The present investigation seeks to discover the antibiotic resistance organisms in RTE veggies in Baghdad, Iraq.

Methodology

Sample collection and processing

Between November and January 2026, 100 samples of packaged RTE vegetable salad were collected from 15 different restaurants in Baghdad. These were leaves; carrots; a mixture of leaves and carrots; and a mixture of leaves, carrots and maize. All samples were randomly collected before their best before date, transported to the laboratory in their original packing within a maximum time of 1 hour and immediately analysed following their arrival.

Characterization and Identification of bacterial isolates

The surface of each specimen was disinfected and epicarp and mesocarp of each specimen were excised in a sterile tray using a sterile blade. One gram of each sample was weighed into a sterile stomacher bag (Seward, UK) and homogenised using a stomacher (Stomacher 80 Biomaster, Seward, UK) with 9 ml of sterile water for 2 minutes. The homogenate was serially diluted with sterile water and poured plated in triplicate 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). All isolated bacteria were identified by conventional methods such as colonial characteristics, Gram staining and biochemical reactions including carbohydrate fermentation, coagulase test, indole test, hydrogen sulphide production, urease production and citrate utilisation as described in Bergey's Manual of Determinative Bacteriology (Bergey and Holt 1994).

Antibiotic susceptibility test

The disc diffusion approach (CLSI, 2014) was used to determine the best drugs against the different pathogenic bacterial species. What we did was shifting the colonies of the growing bacterial isolates after 18 to 24 h to nourishing haemocyte media with a regular culture conveyor. This was done in a test tube containing 5 ml of physiological salt solution. The bacterial suspension was standardised to the McFarland 0.5 tube to get a concentration of 1.5-1 x 10^8 cells/ml. The bacterial suspension was transferred to a cotton swab and excess inoculum was removed by tapping the swab against the side of the tube. Then, the swab was streaked in numerous directions on the center surface of Muller-Hinton Agar (MHA) plate to get homogenous growth. Then the dishes were allowed to air dry for around ten to fifteen minutes. Antibiotic tablets were carefully laid on top of the inoculation (MHA) with sterile forceps and pressed down such that they would attach tightly, with at least 25 mm of space between the centers of the tablets. The plates were incubated for 24 hours at 37 ° C in an airy, inverted incubator. The diameter of the zone of inhibition around each disc was then measured in millimeters. Then, this measurement was compared with standard rates for inhibition zone diameter for drugs stated in (CLSI, 2014) which determine if the bacteria are resistant or sensitive to these antibiotics.

Statistical analysis

Statistical analysis was done by SPSS (Statistical Package for Social Sciences) version 16.0.0, SPSS Inc., Chicago. The Univariate technique combines regression analysis and analysis of variance for a single dependent variable modified by one or more factors and/or variables. The factor's variables divided the population into categories. The null hypothesis on the effect of distinct variables on the means of separate groupings of a single dependent variable using General Linear Model technique. The Bivariate Correlations method was used to calculate Pearson's correlation coefficient and its significance levels. Correlations quantify the degree of relationship between variables or rank values (Released, 2007).

Results

Microbiological pattern isolated from ready to eat vegetables

The results showed that a total of 153 isolates were recovered from 100 samples during the sampling trips. The total bacterial count obtained in the present study was 8.00×10^5 to 9.30×10^5 cfu/ml. The identified bacterial isolates from the sample were: *E coli* (n=75) 49%; *S enterica* (n=32) 21%; *S aureus* (n=31) 20%; *K pneumoniae* (n=5) 3%; *E faecalis* (n=5) 3%; and *S flexneri* (n=5) 3%. The results showed high levels of coliforms and enteric pathogens in the studied environment. The distribution of microorganisms throughout the investigation is presented at Figure (1) and Table 1.

Table 1: Shows the isolated bacteria with their frequency and percentage

Isolated bacteria	Number of occurrences	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%**

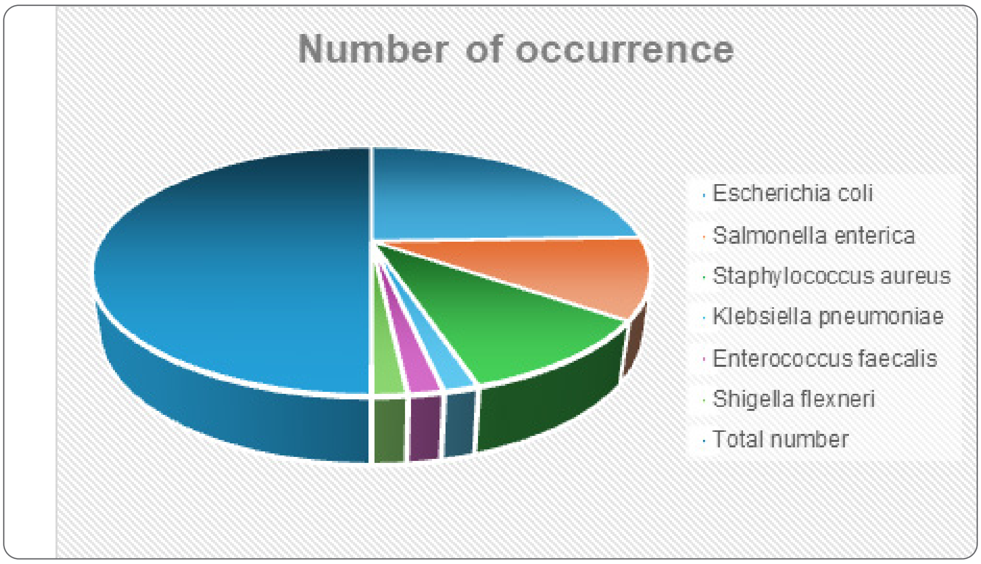

Figure (1): chart of isolated bacteria from ready to eat vegetables.

Antimicrobial susceptibility test

This is the test for antibiotic sensitivity. This model is employed in the random use of antibiotics. This is a varied usage in use dependent upon the dependency on the sensitivity testing (Rivera, 2003). Multiple antibiotic resistance occurs in a medical environment and is dangerous. The model is used for random use of antibiotics. Antibiotic sensitivity was determined by the hard disc diffusion technique in MHA. 14 medicines from different classes of antibiotics were active against gram negative bacteria and 17 medications were active against gram positive bacteria. Bacteria were studied (Pollack, 2012) and the results were interpreted as described in (CLSI, 2014). As shown in (tables 2, 3).

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

Antibiotics \ Bacterial isolates	*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 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

The present investigation on microbiological profile analysis of RTE veggies revealed that there was significant contamination of enteric pathogens with varying antibiotic resistance profiles. A total of 153 isolates were obtained from the 100 tested samples which is a severe danger to food safety as such products are generally consumed raw and without further heat treatment. The research revealed high bacterial load of 8.00×10^5 to 9.30×10^5 cfu/ml . This is above the microbiological limits permissible in raw vegetables in majority of the regulatory requirements and poses possible issues of irrigation water quality or post -harvest management. *E coli* (49%, n=75) was the most dominant species and represents a challenge to the whole microbial community. This is comparable to the earlier finding from Al-Najaf, Iraq (Ridha, 2026) who reported that *E coli* was 53.3% in the lettuce. This increase in the numbers of these bacteria is a good indicator of recent faecal contamination. The same research in the European markets shows a prevalence of *E coli* under 10%, and consequently a considerable gap in the sanitary laws with respect to this. Importantly, obligate pathogens were also discovered including *S. enterica* (21%, n=32) and Shigella flexneri (3%, n=5). The 21% detection of *Salmonella* was within the high-risk range as defined by studies from the Philippines (24.7%) (Vital et al., 2014) and Ethiopia (15%) (Bekele, 2022). The international norms especially those set by WHO and FDA defend the "zero tolerance" in fresh food. The existence of the pathogens implies imminent danger of Salmonellosis and Shigellosis epidemics. While *Salmonella* is commonly linked to contaminated irrigation or manure, the presence of Shigella in commercially prepared food in developed places indicates either direct human-to-food contamination or the intrusion of untreated sewage. Their existence in ready-to-eat vegetables constitutes a direct threat for such outbreaks as Salmonellosis and Shigellosis. *S aureus* was also identified as the third most common isolate at 20% (n=31). This frequency is much higher than the 11% reported in similar street vended vegetable studies (Bekele, 2022). The high incidence of *S aureus* in this study is not surprising as this bacterium is commonly found on the skin and nasal cavities of humans. It is therefore likely that contamination occurs during the "market phase" through unclean handling by vendors or handlers. The findings categorise the samples under review as "High Risk," similar to earlier study undertaken in other growing urban centers. The disease is believed to be the result of a succession of failures in the food safety chain, from the use of contaminated ambient water, to poor hygiene practices in the final distribution of the fruit. The susceptibility test of 14 antibiotics for gram negative bacteria revealed varied degrees of resistance based on the standards of CLSI (2014) and Pollack (2012) (Table 2). *E coli* was the most prevalent isolate with high rates of resistance 24% to Imipenem and 23% to Trimethoprim/Sulfamethoxazole. The resistance to Imipenem was 24% which is substantially higher than 9.25% resistance in previous research in urban gardens (Pramanik et al., 2025). On the other hand, the overall sensitivity (0% resistance) to Gentamicin and Tobramycin seen in this study corroborates with the data reported by (Fatoba, 2022) where *E coli* isolated from agricultural soil and litter showed low resistance levels (3.1%) to Gentamicin and Imipenem (Fatoba et al., 2022). This suggests that while the study isolates are gaining resistance to carbapenems, aminoglycosides are still an effective treatment for these organisms. The *S enterica* isolates shown substantial resistance to Trimethoprim/Sulfamethoxazole (41%) and Minocycline (28%). This confirms the statement that fresh food is a major source of "multiple antibiotic resistance forms" (Rivera, 2003). The pattern of non-typhoidal *Salmonella* has been progressively described as multidrug-resistant (MDR) worldwide. For instance, in a research conducted in Morocco in 2017, 39.6% of food isolates of *Salmonella* were resistant to at least one antibiotic with high resistance to sulfonamides and trimethoprim (Amajoud et al., 2017). The significant prevalence of multidrug-resistant *Salmonella* in the samples of our investigation indicates the possibility for treatment failure in foodborne diseases. *Salmonella* illnesses linked to these vegetable sources. *K pneumoniae* Low incidence (3%) but very significant resistance profile. The isolate was resistant to Aztreonam, Ticarcillin, Tobramycin and Trimethoprim/Sulfamethoxazole by 80% . This high level of resistance is consistent with earlier studies in Saudi Arabia, showing significant presence of resistance genes in *Klebsiella spp.* isolated from leafy vegetables, where over 20% of the isolates were carbapenemase producers (Elsafi et al., 2024). The high levels of Tobramycin and Aztreonam resistance shown in this study are disturbing as they mirror the resistance patterns observed in clinical grade organisms generally linked with hospital acquired infections rather than ambient strains. Major limitations of current therapeutic regimens are explored. These data highlight the role of RTE vegetables as a significant cause of opportunistic infection and clinically relevant antibiotic resistance. In this study greatest resistance of gram positive was shown as in *S aureus* to Rifampicin (39%) followed by Tetracycline (26%) and Erythromycin (23%). The high resistance rates are consistent with findings of (Rahimi et al., 2024) where *S aureus* strains isolated from fresh vegetables exhibited high resistance to Erythromycin (>30%) and Tetracycline (>25%). However, this study includes an interesting finding that the resistance to Vancomycin (considered as a "last resort drug") was low (3%). This is consistent with the global trend Vancomycin-resistant *S aureus* (VRSA) is less common in environmental samples than in clinical samples. The occurrence of VRSA in RTE vegetables is a serious public health problem. The present study highlights the problem of resistance to Vancomycin in *E faecalis* which was shown to be 40% a serious difficulty in therapy. The occurrence of vancomycin-resistant enterococci (VRE) in food products is quite widespread . VRE were detected in about 12% of the vegetable samples (Santana et al., 2023). The study result 40% vancomycin resistance of *E faecalis* suggests a substantial environmental contamination cycle possibly linked to the use of avoparcin related growth boosters in animals, or addition of hospital effluent in irrigation water. A recognised "superbug," VRE, if present on commonly eaten vegetables, represents a serious threat for horizontal gene transfer to other gut pathogens. The data in Tables 2 and 3 indicate that RTE veggies are not just passive carriers of germs but active reservoirs of multidrug-resistant (MDR) strains. The detection of resistance to potent antibiotics such as Imipenem (Gram-negative bacteria) and Vancomycin (Gram-positive bacteria) supports the statement of Rivera (2003) that indiscriminate use of antibiotics in agriculture and human medicine is one of the major determinants of resistance. These findings call for improved sanitation methods and better regulation of irrigation water and soil quality to limit the risk of multidrug-resistant foodborne illness outbreaks.

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