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Antimicrobial Susceptibility Pattern of Microbes Isolated from Vegetables Served and Eaten Raw in the Wa Municipality, Ghana

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ABSTRACT

Background: Raw vegetables are an essential part of the Ghanaian diet but can pose health risks due to microbial contamination. This study aimed at determining the prevalence of pathogenic bacteria in raw vegetables and analyse their antimicrobial susceptibility patterns in the Wa Municipality, Ghana. **Methods:** A total of 43 vegetable samples were collected from farms, markets, and street vendors. Bacteria were isolated and identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. **Results:** The study isolated 69 bacterial strains, with *Salmonella* spp. (37.7%) and *Citrobacter freundii* (31.9%) being the most prevalent. High levels of antimicrobial resistance were observed, particularly in *Salmonella* spp., which showed 100% resistance to several antibiotics including ceftazidime, cefdinir, cefuroxime, and ceftriaxone. **Conclusion:** The high prevalence of pathogenic bacteria and their significant antimicrobial resistance in raw vegetables highlight a serious public health concern in the Wa Municipality. Improved food safety measures and antimicrobial stewardship are urgently needed to address this issue.

INTRODUCTION

Vegetables are crucial in human nutrition, providing essential vitamins, minerals, and dietary fiber (Slavin & Lloyd, 2012). In Ghana, vegetables are an integral part of the daily diet and are consumed in various forms, including raw, cooked, and processed. Raw vegetables have gained popularity due to their perceived health benefits and convenience (Su & Arab, 2006; Brookie *et al.*, 2018). However, the consumption of raw vegetables can pose a significant risk to public health, as they can serve as vehicles for the transmission of pathogenic microorganisms (Iwu & Okoh, 2019; Balali *et al.*, 2020; Osafo *et al.*, 2022).

Contamination of vegetables can occur at any stage of production, from cultivation to consumption. Factors such as contaminated irrigation water, the use of animal manure, and poor hygiene practices during harvesting, handling, and processing can contribute to microbial contamination (Olaimat & Holley, 2012). Pathogenic bacteria such as *Salmonella* spp., *Escherichia coli, Listeria monocytogenes,* and *Staphylococcus aureus* have been frequently associated with vegetable-related foodborne outbreaks worldwide (Srisamran *et al.*, 2022; Asfaw *et al.*, 2023)

The Upper West Region, where Wa Municipality is located, cultivates diverse vegetable crops, including okra, pepper, tomatoes, and leafy vegetables (Ministry of Agriculture, Ghana). These vegetables are sold in local markets and supplied to other regions. Although consuming these vegetables is important to diet and health, there is a gap in knowledge about their microbial quality and safety, especially those consumed raw.

Also, the emergence and spread of antimicrobial resistance (AMR) among foodborne pathogens complicate the situation further. AMR has become a global public health concern as it limits the effectiveness of antibiotics in treating infections caused by resistant bacteria(Aslam *et al.,* 2018). The indiscriminate use of antibiotics in both human medicine and animal husbandry has contributed to the development and spread of antimicrobial resistance (Yevutsey *et al.*, 2017; Ma *et al.*, 2021; Chinemerem Nwobodo *et al.*, 2022). The presence of antimicrobial-resistant bacteria in vegetables is a serious threat to public health, as it can lead to the spread of resistant genes to other bacteria and potentially to human pathogens (Poeys-Carvalho & Gonzalez, 2023; Sun *et al.*, 2024).

Given the importance of vegetables in the Ghanaian diet and the potential public health risks associated with consuming contaminated raw vegetables, this study aims to determine the prevalence of pathogenic bacteria in raw vegetables consumed in the Wa Municipality and to analyse the antimicrobial susceptibility patterns of the isolated microbes.

MATERIALS AND METHODS

Sample Collection:

A total of 43 vegetable samples were collected from various locations in the Wa Municipality, including farms (n=18), markets (n=18), and street vendors (n=7) from January 2019 to March 2019. The samples included cucumber (n=6), tomatoes (n=6), cabbage (n=6), carrot (n=6), green pepper (n=6), lettuce (n=6), and chopped vegetables (n=7). The samples were collected aseptically in sterile plastic bags and transported to the laboratory in an ice box for immediate processing.

Sample Processing, Bacterial Isolation and Identification:

Each vegetable sample was weighed (25 g) and homogenized in 225 mL of sterile buffered peptone water (BPW) (Merck, Darmstadt, Germany, Cat. No. 1.07228) using a stomacher for 2 minutes. Serial dilutions of the homogenates were prepared in sterile BPW, and 0.1 mL aliquots of appropriate dilutions were plated onto various selective and differential media, including MacConkey agar (HiMedia Laboratories, Mumbai, India, Cat. No. M081), Salmonella-Shigella agar (liofilchem srl 610042), Eosin Methylene Blue agar (Oxoid Limited, Hampshire, UK, Cat. No. CM0069), and Mannitol Salt agar (HiMedia Laboratories, Mumbai, India, Cat. No. M118). The plates were incubated at 37^{o}C for 24-48 hours. Presumptive colonies were selected from each media and subcultured onto nutrient agar medium (BD Difco™, Franklin Lakes, NJ, USA, Cat. No. 213000) plates to obtain pure cultures. The isolated bacteria were identified based on their colony morphology, Gram staining, and biochemical characteristics using standard identification protocols (Parasuraman et al.,

2024). The biochemical tests included catalase, coagulase, oxidase, urease, indole, methyl red, Voges-Proskauer, citrate utilization, and triple sugar iron agar tests.

Antimicrobial Susceptibility Testing:

The antimicrobial susceptibility testing of the isolated bacteria was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar medium (Oxoid Limited, Hampshire, UK, Cat. No. CM0337) plates, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI), 2021). The antibiotics tested included Ciprofloxacin (5 µg), Norfloxacin (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Ceftriaxone (30 µg), Aztreonam (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Cefdinir (5 µg), Ofloxacin (5 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), and Cefotaxime (30 µg). The diameter of the inhibition zones was measured, and the results were interpreted as sensitive, intermediate, or resistant based on the CLSI breakpoints (Clinical and Laboratory Standards Institute (CLSI), 2021).

Data Analysis:

The data analysis was performed using SPSS version 24 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated to determine the prevalence of bacterial isolates, expressed as frequencies and percentages of the total number of isolates. For antimicrobial susceptibility analysis, the zones of inhibition were measured and categorized as sensitive, intermediate, or resistant according to CLSI breakpoints. Results were presented in tabular and graphical formats showing the distribution of bacterial isolates across different vegetable types and their antimicrobial susceptibility patterns.

RESULTS

Prevalence of Bacterial Isolates:

A total of 69 bacterial isolates were obtained from the 43 vegetable samples (Table 1). The most prevalent bacteria were *Salmonella* spp. (37.7%) and the least prevalent were *Enterobacter* spp. (1.4%), and *Bacillus* spp. (1.4%). Details of the prevalence is presented in Table 1.

Table 1: The distribution of organisms isolated from each vegetable and the total number of bacteria in each sample type.

Samples	Organism							
	Bacillus	***Citrobacter diversus***	***Citrobacter freundii***	***Enterobacter* species**	***Proteus Vulgaris***	***Yersinia***	***Salmonella* species**	***Staphylococcus* species**
Cabbage	0	0	3	0	1	0	4	0
Carrot	0	2	3	0	0	0	5	0
Tomatoes	1	2	3	0	0	0	4	0
Cucumber	0	3	4	0	0	0	3	0
Green pepper	0	1	2	0	0	1	3	1
Lettuce	0	0	3	1	1	1	4	1
Chopped vegetables	0	3	4	0	1	0	3	1
Total	**1 (1.4%)**	**11 (15.9%)**	**22 (31.9%)**	**1 (1.4%)**	**3 (4.3%)**	**2 (3%)**	**26 (37.7%)**	**3 (4.3%)**

The sampling points and bacterial species isolated from vegetables in the Wa Municipality is described in Figure 1 below. The isolated bacterial species include *Salmonella* spp., *Citrobacter freundii, Citrobacter diversus, Staphylococcus* species, *Proteus vulgaris, Yersinia* spp., *Enterobacter* spp., and *Bacillus* spp.

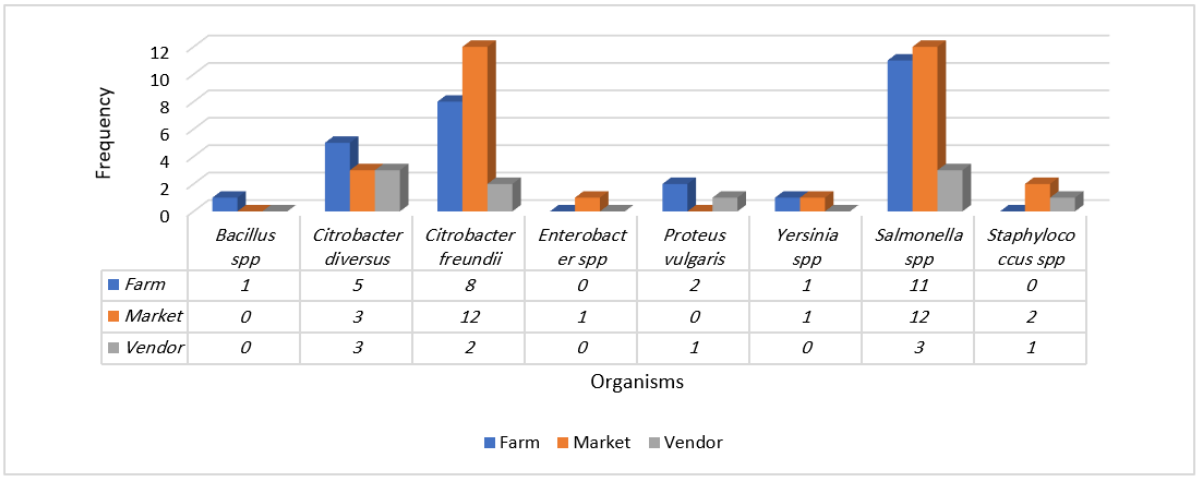

Fig. 1: Vegetable Sampling Locations and Corresponding Isolated Bacterial Species.

The antimicrobial susceptibility testing revealed varying levels of resistance among the isolated bacteria. *Salmonella* spp. showed sensitivity to Ciprofloxacin (76.9%), Ofloxacin (76.9%), Gentamicin (76.9%), Amikacin (76.9%), and Norfloxacin (76.9%). However, *Salmonella* spp. exhibited high resistance to Ceftazidime (100%), Cefdinir (100%), Cefixime (75%), Cefuroxime (100%), Nitrofurantoin (100%), Nalidixic acid (100%), Ceftriaxone (100%), Cefotaxime (100%), and Aztreonam (100%). Details of the susceptibility and resistance pattern are shown in Figure 2a and 2b, respectively.

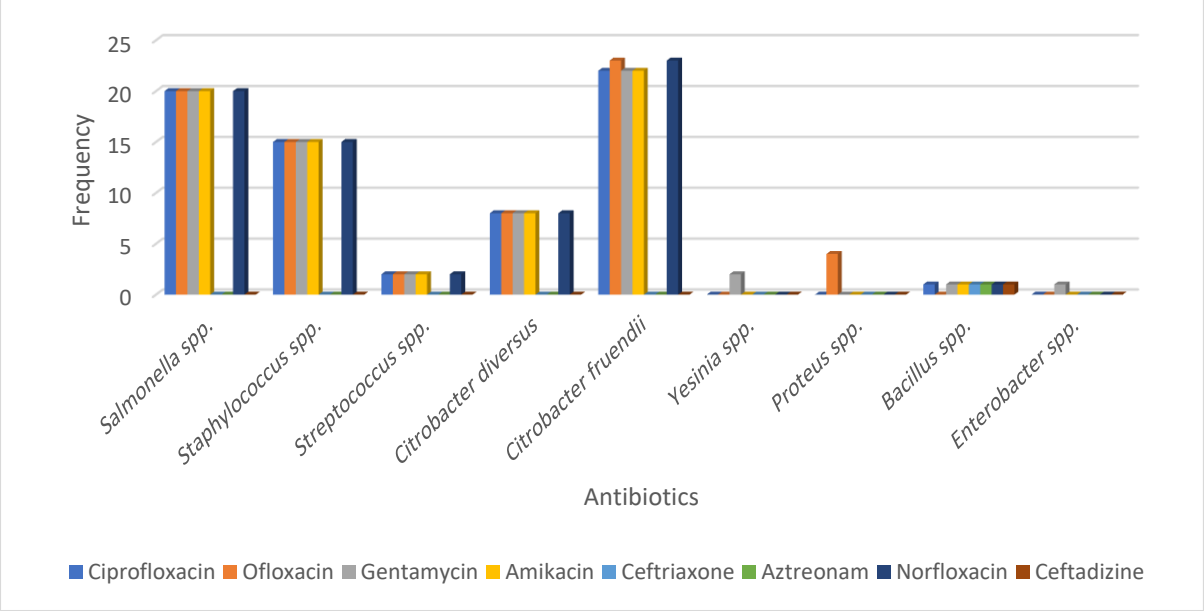

Fig. 2a: Susceptibility profile of organisms isolated.

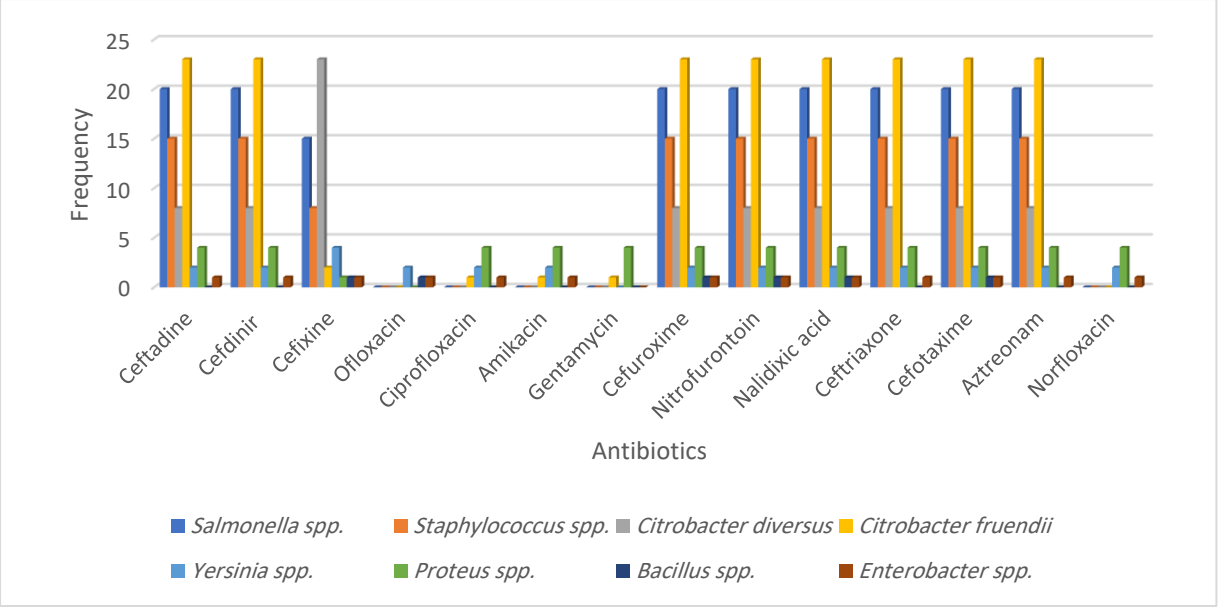

Fig. 2b: Resistance profile of organisms isolated.

DISCUSSION

This study reports a significant prevalence of pathogenic bacteria in vegetables served and consumed raw in the Wa Municipality of Ghana. The high occurrence of *Salmonella* spp. (37.7%) and *Citrobacter freundii* (31.9%) is particularly alarming, given their potential to cause foodborne illnesses in humans. These findings are consistent with earlier studies, which highlight the persistent challenge of microbial contamination in fresh produce (Verraes *et al.*, 2013; Kumar *et al.*, 2023).

Salmonella spp., a leading cause of foodborne infections globally, poses a substantial risk to public health when present in raw vegetables. Recent outbreaks linked to contaminated produce underscore the importance of vigilance in vegetable safety. The high prevalence observed in this study suggests a potential risk, especially for vulnerable populations such as children, the elderly, and immunocompromised individuals (Kirk *et al.*, 2015). The presence of *Salmonella* spp. in these samples may be attributed to various factors, including contaminated irrigation water, improper use of manure, or poor hygiene practices during handling and processing (Alegbeleye & Sant'Ana, 2023; Ma *et al.*, 2024).

Citrobacter freundii, while less commonly associated with foodborne outbreaks, is an opportunistic pathogen capable of causing various infections, including urinary tract and respiratory infections (Liu *et al.*, 2018). Its high prevalence in the vegetable samples indicates poor hygiene practices throughout the production chain. Recent studies have shown that *C. freundii* can acquire and transfer antimicrobial resistance genes, potentially contributing to the spread of resistance in the environment (Majumder *et al.*, 2020).

The isolation of other bacteria such as *Citrobacter diversus*, *Staphylococcus* spp., *Proteus vulgaris*, *Yersinia* spp., *Enterobacter* spp., and *Bacillus spp.*, albeit in lower frequencies, further emphasizes the diverse microbial ecosystem present on raw vegetables. Each of these organisms has the potential to cause infections, particularly in individuals with compromised immune systems (Tack *et al.*, 2020). Their presence emphasizes the need for comprehensive food safety measures from farm to fork.

The antimicrobial susceptibility testing revealed alarming levels of resistance among the isolated bacteria, particularly in *Salmonella* spp. and *C. freundii*. The high resistance rates to multiple antibiotics, including third-generation cephalosporins, fluoroquinolones, and aminoglycosides reflect a growing global concern about antimicrobial resistance (AMR) in the food chain (Fernández-Trapote *et al.,* 2024). This multidrug resistance pattern is particularly worrisome as it limits treatment options for potential infections.

The observed resistance to ciprofloxacin and other fluoroquinolones in some isolates is concerning, as these antibiotics are often used as first-line treatments for severe Salmonella infections (Khanal *et al.,* 2017; Chen *et al.,* 2020). The high resistance rates to cephalosporins (ceftazidime, cefotaxime, ceftriaxone) observed in this study align with recent reports of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, a trend increasingly documented worldwide (Ye *et al.,* 2017).

The presence of antimicrobial-resistant bacteria in vegetables presents a multifaceted risk to public health. Beyond the immediate threat of difficult-to-treat infections, these resistant organisms can serve as reservoirs for resistance genes, potentially transferring them to other pathogenic bacteria (Lammie & Hughes, 2016). Recent studies have demonstrated the potential for horizontal gene transfer of resistance determinants in the plant production environment, highlighting vegetables as vectors for the spread of AMR (Araújo *et al.,* 2017) .

Contamination of vegetables with pathogenic and antimicrobial-resistant bacteria can occur at multiple points along the production chain. Recent research has highlighted the role of irrigation water quality in introducing pathogens to crops (Alegbeleye & Sant'Ana, 2023). In the context of Wa Municipality, where vegetable farming is a significant economic activity, ensuring the safety of irrigation water sources is crucial. The use of untreated wastewater for irrigation-a practice observed in some developing countries-can introduce not only pathogens but also antibiotic residues and resistance genes into the vegetable production system (Phan *et al.,* 2024).

Post-harvest handling and processing also play critical roles in vegetable safety. Cross-contamination during transportation, storage, and market display can introduce or amplify microbial contamination (Ssemanda *et al.,* 2018). The high prevalence of pathogens observed in this study suggests that current practices may be inadequate in preventing contamination or growth of these organisms. Addressing these challenges requires a multifaceted approach that encompasses improved hygiene practices and stricter regulations across all stages of food production.

Conclusion and Recommendations:

Conclusion:

This study revealed a high prevalence of pathogenic bacteria, particularly *Salmonella* spp. and *Citrobacter freundii*, in raw vegetables from the Wa Municipality in Ghana. The high levels of antimicrobial resistance observed among these isolates, especially to clinically important antibiotics, emphasizes a significant public health risk. These findings highlight the urgent need for improved food safety measures and antimicrobial stewardship in the region's food chain sector.

Recommendations:

Based on the findings of this study, we recommend the following;

1. The ministry of Agriculture should establish and enforce comprehensive hygiene protocols throughout the vegetable production chain, from farm to market. This should include proper handling techniques, regular sanitation of equipment and storage facilities, and personal hygiene training for all workers involved in vegetable

cultivation, harvesting, and distribution.

2. Strategies to ensure the use of safe irrigation water should be developed and implemented. This may involve treating water sources, promoting alternative irrigation methods, or implementing regular water quality testing programs to minimize the risk of introducing pathogens to crops.
3. Antimicrobial stewardship programs should be developed and implemented. These should focus on reducing unnecessary antibiotic use in agriculture, promoting alternative pest management strategies, and educating farmers and consumers about the risks of antimicrobial resistance in the food chain.

DECLARATIONS:

Ethical Approval: Ethical clearance for this study was obtained from the ethical and protocol review committee of the Science Laboratory Technology Department of Dr. Hilla Limann Technical University, Ghana, with reference number EPRC/SLT/0020.

Authors' Contributions: EUO, REA and EPKP participated in the conceptualization and the design of the research protocol as well as participated in the laboratory work. AWI, MD and ANU collected and processed samples as well as wrote the first and final draft of the manuscript. All authors read and approved the final draft of the manuscript.

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