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Research Article

Antibiotic resistance and prevalence of *Salmonella* spp. In the vegetable wash water from the tomato market, Lafia, Nasarawa, Nigeria

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ABSTRACT

The prevalence of Salmonella spp. in vegetables and their wash water presents a considerable public health threat, particularly in regions characterised by inadequate sanitation and hygiene practices. This study aimed to evaluate the prevalence, antimicrobial resistance, and multidrug resistance (MDR) patterns of *Salmonella* spp. in vegetable wash water samples collected from the Tomato Market located on Shinge Road, Lafia, Nasarawa State, Nigeria. *Salmonella* spp. were identified in 14 out of 30 samples, indicating a prevalence rate of 46.67%. Notably, high resistance rates were observed for Ampicillin (92.86%) and Amoxicillin-Clavulanic acid (64.29%). The highest sensitivity was recorded for Ciprofloxacin and Ceftazidime (100% each), as well as for Ceftriaxone (85.71%). Various multidrug resistance (MDR) patterns were observed, with resistance to multiple drug classes being prevalent. The elevated prevalence and MDR patterns in *Salmonella* spp. from vegetable wash water underscore critical public health issues. Enhancing sanitation practices and ensuring prudent antibiotic utilisation are essential measures needed to effectively tackle these challenges.

Keywords: Multidrug resistance, Foodborne pathogens, Microbial contamination, Fresh produce safety, Public health risk

Introduction

Salmonella, a genus of rod-shaped, Gram-negative bacteria in the Enterobacteriaceae family, is a leading foodborne pathogen responsible for salmonellosis. This disease can cause gastroenteritis, systemic infections, and, in severe cases, death. The World Health Organisation (WHO) estimates that *Salmonella* causes 93.8 million cases of gastroenteritis and 155,000 deaths globally each year (Heredia & García, 2018). While traditionally linked to animal products, recent outbreaks have highlighted the contamination of fresh produce, underscoring the need to study its presence in vegetables. Between 1973 and 2011, *Salmonella* was implicated in 18% of all produce-associated outbreaks in the United States (Bennett et al., 2015), and this trend persists, with fresh produce contributing significantly to foodborne *Salmonella* outbreaks (CDC, 2021).

Vegetables may become contaminated at various points along the food supply chain, including cultivation, harvesting, processing, and distribution (Luna-Guevara et al., 2019). One critical stage is the washing process, where contaminated wash water can facilitate the spread of pathogens to uncontaminated produce (Government of British Columbia, 2012). Reusing the same water for multiple batches of vegetables heightens the risk of cross-contamination, complicating efforts to ensure food safety (Fletcher, 2015; Popa & Papa, 2021). For example, Allende et al. (2017) demonstrated that a single contaminated lettuce leaf could transfer *Salmonella* to an entire batch through wash water, emphasising the importance of water quality management during produce processing. *Salmonella*'s ability to survive in water environments further exacerbates the issue, with strains persisting for up to 54 days in sterile water (Jongman et al., 2017).

Studies from Africa, Asia, and Latin America have highlighted the widespread contamination of fresh produce and washing water with *Salmonella*. In Nigeria, Oranusi et al. (2018) detected *Salmonella* in irrigation water and vegetables, indicating contamination at the pre-harvest stage. Simi*lar* findings have been reported in Ethiopia (Tafesse et al., 2020) and India (Choudhury et al., 2019), where irrigation water and post-harvest handling practices have been identified as contributing factors to Salmonella outbreaks. These studies underscore the importance of enhancing hygiene practices in produce markets, particularly in developing countries where regulatory enforcement of food safety standards is often inadequate.

Beyond its presence in fresh produce, the antibiotic resistance of Salmonella poses a serious public health concern. A global study by Hendriksen et al. (2019) found high levels of resistance to commonly used antibiotics, such as ampicillin, chloramphenicol, and tetracycline, across over 4,000 Salmonella genomes from 42 countries. Similarly, Vital et al. (2018) reported the presence of multidrug-resistant (MDR) Salmonella in irrigation water and vegetables, with resistance to critical antibiotics such as ciprofloxacin and ceftriaxone. The increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing Salmonella further complicates treatment, making infections more challenging to manage (Tian et al., 2020). Studies from Nigeria also indicate a rising trend in antibiotic resistance among foodborne Salmonella isolates, with resistance to third-generation cephalosporins and fluoroquinolones (Eze et al., 2019). These findings align with reports from developing nations, where the unregulated use of antibiotics in agriculture and veterinary medicine has contributed to the emergence of resistant strains.

Despite these global challenges, there is a lack of data on the antibiotic resistance profiles of Salmonella isolates from vegetable wash water, particularly in Lafia, Nasarawa State. Most studies in Nigeria have focused on poultry, irrigation water, and processed foods, leaving a critical gap in understanding the role of market-based water sources in bacterial contamination (Eze et al., 2019). Addressing this gap is crucial to developing effective control measures and ensuring the safety of vegetables consumed in the region.

This study aims to determine the prevalence and antibiotic resistance patterns of Salmonella species isolated from vegetable wash water in the tomato market of Lafia, Nasarawa State. Understanding these resistance trends offers valuable insights into associated risks and guides intervention strategies.

Materials and Methods

Study Location

The Tomato Market on Shinge Road in Lafia, Nasarawa State, is a bustling hub renowned for trading in fresh produce, particularly tomatoes. Located centrally, it attracts vendors, farmers, and buyers from nearby areas. The market features organised stalls where vendors display a variety of vegetables and fruits. The Market is crucial to the study due to its central role in the local food supply chain. As a significant marketplace for fresh produce, including vegetables, it represents a critical point of potential contamination or pathogen spread, such as *Salmonella*.

Sample Collection

Samples were collected using a modified technique, as described by Nwinyi and Nduchukwuka (2016), from 15 randomly selected vendors selling vegetables at the Tomato Market on Shinge Road, Lafia, Nasarawa State. A total of 30 samples were obtained using sterile containers and aseptic techniques directly from these vendors. Each sample was labelled with details including date, time, and specific location. Samples will then be transported in coolers to maintain integrity during transit to the Microbiology laboratory at the Department of Science Laboratory Technology, Isa Mustapha Agwai 1 Polytechnic, Lafia, Nasarawa State, for bacterial analysis and antibiogram profiling.

Bacteriological Analysis and Identification of Salmonella Isolates

Bacteriological analysis was conducted using standard methods for examining water and wastewater (American Public Health Association [APHA], 2017). Samples were thoroughly mixed to ensure homogeneity. An aliquot of 25 g or 25 mL of each sample was transferred aseptically into 225 mL of Buffered Peptone Water (BPW) and incubated at 37°C for 18–24 hours to allow for pre-enrichment of stressed or low-level bacterial cells. After enrichment, ten-fold serial dilutions were prepared as necessary, and 0.1 mL aliquots were plated onto *Salmonella*-*Shigella* Agar (SSA) using the pour plating method. The plates were incubated at 37°C for 24-48 hours, and well-isolated colonies were selected (Ashefo & Habibu, 2024). Small, round, transparent colonies, with or without black centres due to hydrogen sulfide (H_2S) production, were subcultured onto freshly prepared SSA plates for purity checks and biochemical characterisation (Atlas, 2013). Smears of bacterial isolates were heat-fixed on clean, grease-free slides and subjected to Gram staining. The slides were stained with crystal violet, followed by Lugol's iodine and 95% alcohol for decolourisation, before being counterstained with safranin. After air-drying, the slides were examined under an oil immersion objective lens, where Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared pink (Verawaty et al., 2020).

Presumptive *Salmonella* colonies, identified based on their morphology on *Salmonella*-*Shigella* Agar and Gram-staining as Gram-negative rods, were further analysed using the KB003 H125™ Enterobacteriaceae Identification Kit. Pure colonies were suspended in sterile normal saline and adjusted to 0.5 McFarland turbidity. The bacterial suspension was inoculated into the wells of the kit, which contains dehydrated biochemical substrates for various metabolic tests. After sealing, the kit was incubated at 37ºC for 24 hours. Following incubation, reagents were added to specific wells, and the results were interpreted according to the manufacturer's instructions for identifying *Salmonella* species.

Antibiotic Susceptibility Testing

Fourteen confirmed Salmonella spp isolates were picked, and isolates were grown in nutrient broth for 18–24 hours, diluted in physiological saline, and adjusted to match the 0.5 McFarland standard (Clinical and Laboratory Standards Institute [CLSI], 2017). Antibiotic susceptibility was determined using the disc diffusion method (Olovo et al., 2019). Antibiotics tested included Ampicillin (AMP: 10 µg), Amoxicillin Clavulanic acid (AMT: 30 µg), Cefotaxime (CTX: 30 µg), Ceftazidime (CAZ: 30 µg), Ceftriaxone (CRO: 30 µg), Ciprofloxacin (CIP: 5 µg), Cotrimoxazole (SXT: 25 µg), Gentamicin (CN: 10 µg), and Streptomycin (S: 10 µg). Mueller Hinton Agar (MHA) plates were inoculated with the test cultures, dried, and antibiotic discs were placed using sterile forceps. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimetres, including disc diameter (CLSI, 2017).

Classification of Antibiotic Resistance

Antibiotic resistance in the isolates were classify into: multidrug resistance (MDR: non susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non susceptible to all antimicrobial listed) (Magiorakos *et al.*, 2012).

Results and Discussion

The prevalence of *Salmonella* spp. in vegetable wash water from the Tomato market in Lafia, Nasarawa State, was notably high, with 14 out of 30 samples (46.47%) testing positive. This finding aligns with previous studies, such as Nkang et al. (2018), who reported a prevalence of 42%, and Okonko et al. (2019), who observed a prevalence of 48% in wash water from Nigerian street markets. Similarly, a study in Jordan found *Salmonella* contamination in 32.2% of irrigation water samples, indicating significant contamination risks associated with irrigation water (Burjaq & Abu-Romman, 2020). These consistent findings across regions highlight the substantial contamination risks associated with vegetable wash water in open market environments, emphasising the urgent need for improved sanitation practices (Quansah & Chen, 2021).

The antibiotic susceptibility patterns of the *Salmonella* isolates (Table 1) revealed high resistance to Ampicillin (92.86%) and Amoxicillin-Clavulanic acid (64.29%), indicating reduced effectiveness of these commonly used antibiotics. This finding is consistent with those of Iroha et al. (2020), who reported similar resistance levels in *Salmonella* isolates from food samples in Nigeria. Such high resistance rates are concerning, as these antibiotics are often first-line treatments for *Salmonella* infections (Hosseini et al., 2024). Moderate resistance was observed to Cotrimoxazole (21.43%) and Gentamicin (50%), suggesting these antibiotics retain partial effectiveness.

In contrast, the isolates exhibited 100% sensitivity to Ciprofloxacin and Ceftazidime and

85.71% sensitivity to Ceftriaxone. These findings are in agreement with Ashefo and Habibu (2024), who reported high sensitivity to Ciprofloxacin (95%) and Ceftriaxone (90%) in clinical *Klebsiella pneumonia* isolates. The continued effectiveness of these antibiotics is promising; however, careful management is required to prevent the emergence of resistance (Quansah & Chen, 2021).

Table 2 outlines the multidrug resistance (MDR) profiles observed among the *Salmonella* isolates. All resistance phenotypes fell into the MDR category, with common patterns including resistance to Ampicillin, Streptomycin, and Gentamicin (AMP-STP-CN) and a more extensive pattern involving Ampicillin, Amoxicillin-Clavulanic acid, Streptomycin, and Gentamicin (AMP-AMT-STP-CN). Similar MDR profiles were reported by Ochei et al. (2017) in *Salmonella* isolates from food sources in Nigeria.

The diversity of MDR phenotypes, including combinations of beta-lactams and other antibiotic classes, aligns with findings from Abebe et al. (2022), who documented a wide range of MDR patterns in *Salmonella* spp. from environmental samples in Ethiopia. These resistance profiles underscore the complexity of managing Salmonella infections and the challenges presented by multidrug-resistant (MDR) strains in both clinical and environmental settings.

The high prevalence of *Salmonella* spp. and the diversity of resistance patterns observed in this study emphasise the importance of stringent hygiene practices and robust antibiotic stewardship. These measures are crucial for reducing contamination risks, ensuring effective treatment options, and mitigating the public health impact of *Salmonella* infections. Ongoing research and international collaboration remain critical in addressing the challenges posed by antibiotic resistance in *Salmonella* spp. globally.

Table 1. Antimicrobial Resistance and Sensitivity Profile of *Salmonella* spp. No. of Isolates =14

Antibiotic	% Resistance	% Sensitivity
AMP	92.86 (13)	7.14 (1)
AMT	64.29 (9)	35.71(5)
STP	42.86(6)	57.14(8)
CIP	0(0)	100(14)
SXT	21.43 (3)	71.43(11)
CN	50.00 (7)	50.00(7)
CAZ	0 (0)	100(14)
CRO	14.29 (2)	85.71(12)
CTX	7.14(2)	92.86(13)

***: Values in parentheses are the number of isolates**
Key: Ampicillin (AMP: 10 µg), Amoxicillin-Clavulanic acid (AMT: 30 µg), Cefotaxime (CTX: 30 µg), Ceftazidime (CAZ: 30 µg), Ceftriaxone (CRO: 30 µg), Ciprofloxacin (CIP: 5 173 µg), Co-trimoxazole (SXT: 25 µg), Gentamicin (CN: 10 µg) and Streptomycin (STP: 10 µg)

Table 2: Classes of Antimicrobial Resistance in *Salmonella* spp.

Antibiotic Resistance Phenotype (ARP)	Number (%) Isolates (n=14)	Classification
AMP-STP-CN	3 (21.43)	MDR
AMT-CN-CAZ-CRO	1 (7.14)	MDR
AMP-STP-CN-CRO	2 (14.29)	MDR
AMP-AMT-SXT-CN	3 (21.43)	MDR
AMP-AMT-STP-CN	3 (21.43)	MDR
AMP-AMT-SXT-CRO-CTX	1 (7.14)	MDR
AMP-AMT-STP-CN-CAZ	1 (7.14)	MDR

Key: MDR= multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories).
No. = Number, % = Percentage, AMP = Ampicillin (Beta-lactams),
AMT = AmoxicillinClavulanic acid (Beta-lactams), CTX = Cefotaxime (Beta-lactams),
CAZ = Ceftazidime (Betalactams), CRO = Ceftriaxone (Beta-lactams), FOX = Cefoxitin (Beta181 lactams),
CIP = Ciprofloxacin (Fluoroquinolones), SXT = Co-trimoxazole (Folate pathway inhibitors),
CN = Gentamicin (Aminoglycosides), STP = Streptomycin (Aminoglycosides)

Conclusion

This study highlights a significant prevalence of *Salmonella* spp. (46.47%) in vegetable wash water from the Tomato market in Lafia, Nasarawa State, underscores the contamination risks in market environments. The high resistance rates to commonly used antibiotics such as Ampicillin (92.86%) and Amoxicillin-Clavulanic acid (64.29%) reveal alarming challenges in managing *Salmonella* infections and suggest the need for alternative treatment options. However, the retained sensitivity to Ciprofloxacin, Ceftazidime (100%), and Ceftriaxone (85.71%) offers a glimmer of hope for effective treatment. The identification of multidrug-resistant (MDR) phenotypes further complicates treatment strategies, reflecting the growing burden of antimicrobial resistance (AMR). These findings underscore the need for urgent interventions, including improved sanitation practices in food markets, enhanced public health monitoring, and robust antimicrobial stewardship, to mitigate the spread of MDR *Salmonella*. To address these challenges effectively, ongoing research and international collaboration are essential. Surveillance efforts such as monitoring antibiotic resistance profiles and enforcing food safety standards are critical to protecting public health and reducing the risks associated with *Salmonella*-contaminated fresh produce.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: The authors declare that this study does not involve experiments with human or animal subjects, and therefore, ethics committee approval is not required.

Data availability: Data will be made available at the request of the author(s).

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