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Assessment of Microbial Contamination in Pre-Packaged Vegetable Salads in Umuahia Metropolis

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Abstract

The rising consumption of ready-to-eat (RTE) vegetable salads offers nutritional benefits but raises public health concerns due to minimal processing, which may allow microbial contamination. This study evaluated the microbiological quality of five pre-packaged vegetable salad samples from various retail outlets in Umuahia Metropolis, Abia State, Nigeria, including a laboratory-prepared control. Samples were analysed using Nutrient Agar, Potato Dextrose Agar, MacConkey Agar, and Eosin Methylene Blue Agar to determine total viable counts (TVC) and identify microbial contaminants. TVC ranged from 3.8×10^2 CFU/g in the control to 2.16×10^3 CFU/g in market samples, indicating varying contamination levels. Bacterial isolates included *Proteus*, *Pseudomonas*, *Staphylococcus*, *Escherichia coli*, and *Bacillus* species, while fungal isolates include microorganisms were yeasts, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Geotrichum* spp. Antibiotic susceptibility tests revealed resistance to several commonly used antibiotics among the bacterial isolates, which included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enterica*. These results indicate that RTE salads sold in Umuahia may pose health risks due to microbial contamination and antibiotic-resistant bacteria. The study highlights the need for stricter hygiene during preparation, effective cold-chain storage, and routine microbial assessments to safeguard public health.

Keywords: Pre-packaged salads, Bacterial and fungal contamination, Food safety, Umuahia

Introduction

The consumption of ready-to-eat (RTE) salads has increased significantly worldwide due to growing awareness of their nutritional value, convenience, and health-promoting properties [1,2]. These salads, often composed of raw vegetables such as lettuce, cabbage, carrots, and cucumbers, are typically consumed without further cooking, making them especially vulnerable to microbial contamination [3,4]. Unlike cooked foods, RTE salads bypass thermal inactivation of pathogens, and as such, any microorganisms introduced during cultivation, harvesting, processing, packaging, or distribution may remain viable until consumption [5,6].

In developing countries, like Nigeria, the risk associated with microbial contamination in RTE salads is particularly high. Numerous studies have reported the presence of pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Pseudomonas* spp. in salad samples from various sources [3,7]. These pathogens are commonly associated with foodborne illnesses and can cause severe gastrointestinal disorders, especially in vulnerable populations such as children, the elderly, and immune-compromised individuals [8–10]. Contamination is often attributed to poor hygiene practices during handling, the use of contaminated water for washing vegetables, exposure to unclean packaging materials, and improper storage conditions in open markets or food outlets [11,12].

In addition to bacterial pathogens, fungal contamination of RTE salads poses another significant concern. Fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Geotrichum* have been frequently isolated from fresh produce and are capable of producing harmful mycotoxins under favourable conditions [13]. These mycotoxins, even at low concentrations, are known to cause immunosuppression, liver damage, and, in some cases, carcinogenic effects [14].

The situation is not isolated to Nigeria. Similar challenges have been reported in other parts of sub-Saharan Africa. For example, in Tamale, Ghana, RTE vegetable salads sold by street vendors showed high prevalence of *E. coli*, *Salmonella*, and other enteric bacteria, highlighting systemic lapses in food safety regulation and awareness [15]. These findings collectively underscore a broader public health concern across the region, particularly where there is poor enforcement of hygiene standards in food preparation and retail environments [16].

Furthermore, the emergence of antimicrobial resistance (AMR) among foodborne pathogens exacerbates the threat posed by contaminated RTE salads. Pathogens such as *E. coli* and *Staphylococcus aureus* are increasingly exhibiting resistance to commonly used antibiotics, limiting therapeutic options and complicating the management of foodborne diseases [17,18]. Monitoring the antimicrobial susceptibility profiles of isolates from food sources is thus essential in evaluating public health risks and informing food safety policies [19].

Despite increasing research in other parts of Nigeria, there is a scarcity of published data on the microbiological safety of RTE salads sold within Umuahia Metropolis, Abia State. To the best of our knowledge, no recent study has systematically evaluated both the microbial profile and antibiotic resistance patterns of contaminants in pre-packaged salads in this region. Most available studies have focused on other urban centers such as Lagos, Port Harcourt, and Enugu, leaving a gap in surveillance data for south-eastern Nigeria. Given the rising urban population and growing demand for convenience foods in Umuahia, such an assessment is both timely and necessary.

The present study was therefore conducted to assess the microbiological quality of pre-packaged vegetable salads available within Umuahia Metropolis, Abia State, Nigeria. Specifically, the study aims to determine the total viable counts (TVC) of microbial contaminants in salad samples from various retail locations; identify and characterise bacterial and fungal species present; and evaluate the antibiotic susceptibility patterns of the bacterial isolates. The results of this study are intended to provide valuable insights into the safety status of RTE salads sold in Umuahia, contribute to the broader food safety discourse in Nigeria, and support the implementation of improved hygiene and monitoring practices across the food supply chain.

Materials and Methods

Study Area

This study was conducted within Umuahia Metropolis, Abia State, Nigeria. Umuahia is located at approximately latitude 5.5320 °N and longitude 7.4860 °E and is characterized by multiple markets and informal food vending zones. The increasing consumption of ready-to-eat (RTE) salads in the area necessitates microbiological surveillance of such food items.

Chemicals and Reagents

The chemicals and reagents used in this study included 95% ethanol for plant extraction, distilled water, Mueller-Hinton agar, nutrient broth, sterile Petri dishes, filter paper discs, sterile cotton swabs, and standard antibiotic discs for antimicrobial susceptibility testing, along with all necessary laboratory glassware and inoculation equipment were purchased from a certified Pharmacy shop (Grace and Mercy) Umuahia, Abia State, Nigeria and were of analytical grade. All reagents and media were of analytical grade, purchased from a certified pharmaceutical outlet (Grace and Mercy Pharmacy, Umuahia), and prepared according to manufacturer specifications and ISO 11133:2014 guidelines for microbiological media preparation [1].

Sample Collection

Salad samples (5) were analyzed: Sample A – Laboratory-prepared sterile control. Sample B1 – Collected from Isi-Gate Market, Sample B2 – From Ahiaeke Market and Samples B3 & B4 – Obtained from two roadside/street vendors. Samples were aseptically collected in sterile, leak-proof containers, labelled, and immediately transported in an ice-cooled insulated box to the Microbiology Laboratory at Michael Okpara University of Agriculture, Umudike, for analysis within one hour, following WHO protocols for food microbiology sampling [2].

Microbiological Analysis

Total Viable Count (TVC)

A 10 g portion of each sample was aseptically transferred into 90 mL of sterile 0.1% peptone water and homogenized using a stomacher. Serial dilutions were performed up to 10^{-5}, and 1 mL aliquots from appropriate dilutions were plated using the pour plate technique on the following media: Nutrient Agar (NA): for total aerobic heterotrophic bacteria, MacConkey Agar (MAC): for enteric Gram-negative bacteria, EMB Agar: for coliforms (e.g., *E. coli*) and PDA (acidified with tartaric acid): for fungi (yeasts and molds). Plates were incubated at: 37°C for 24–48 hours for bacteria and 25°C for 3–5 days for fungi. Colony-forming units (CFU) were counted and microbial loads expressed as CFU per gram

(CFU/g) of sample [3]. This procedure followed ISO 4833-1:2013 (Horizontal method for enumeration of microorganisms) and FDA Bacteriological Analytical Manual (BAM) [4,5].

Identification of Microbial Isolates

Bacterial Identification

Presumptive bacterial colonies were identified based on: Colony morphology**,** Gram staining and standard biochemical tests**.** All tests were performed according to Cheesbrough (2006) and verified using Bergey's Manual of Determinative Bacteriology [6,7].

Fungal Identification

Fungi were identified based on: Colony characteristics on PDA, Microscopic examination using Lactophenol Cotton Blue (LPCB) staining, Identification of structural features such as hyphae, conidia, sporangia and spores. Identification was performed using the taxonomic keys provided by Barnett & Hunter and Alexopoulos et al. [8, 9].

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby**-**Bauer disk diffusion method on Mueller-Hinton Agar (MHA)**,** following CLSI M100 (2020) guidelines [10].

Procedure: Bacterial suspensions were adjusted to match 0.5 McFarland standard (~1.5×10^8 CFU/mL). Sterile swabs were used to inoculate MHA plates evenly. Commercial antibiotic discs were placed aseptically onto the agar. Plates were incubated at 37°C for 18–24 hours**.** Zones of inhibition were measured in millimeters.

Antibiotics Tested

Antibiotic	**Abbreviation**	**Concentration**
Tarivid	OFX	10 µg
Peflacine	PEF	10 µg
Ciprofloxacin	CPX	5 µg
Augmentin	AU	30 µg
Gentamicin	CN	10 µg
Ceporex	CEP	10 µg
Nalidixic Acid	NA	30 µg
Septrin	SXT	25 µg
Ampicillin	PN	10 µg
Streptomycin	S	10 µg

Results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) based on CLSI breakpoints.

Results and Discussion

The total viable counts (TVCs) of the salad samples on various media are presented in Table 1**.** Bacterial and fungal counts varied significantly ($p < 0.05$) between the laboratory-prepared control and commercially sourced samples.

Table 1: Total Viable Count (TVC) of Salad Samples

Locations	Salad Sample	No of TVC on NA	No of TVC on PDA	No of TVC on MAC	No of TVC on EMB	Microbial load CFU/g
Laboratory prepared	A	24	14	**-**	**-**	$3.8x10^{2}$
Isi gate market	B^{1}	188	26	1	1	$2.16x10^{3}$
Ahiaeke market	B^{2}	165	10	2	2	$1.79x10^{3}$
Last food outlet	B^{3}	54	30	2	2	8.8x102
Last food outlet	B^{4}	54	30	2	2	$8.8x10^{2}$

One-way ANOVA showed statistically significant differences in bacterial loads across locations ($F = 47.32$, $p = 0.002$). Post-hoc Tukey test indicated highest contamination in B1 and B2 (markets), significantly higher than the control (A).

The highest microbial load was observed in sample B1 (Isi-Gate Market)**,** with a total of 2.16×10^3 CFU/g, while the laboratory control (A) had the lowest (3.8×10^2 CFU/g). These findings reflect the influence of environmental exposure and handling conditions on microbial contamination. Samples from street vendors (B3 and B4) had moderate counts, possibly due to less volume or frequency of handling compared to market vendors.

Table 2 lists the bacterial species isolated from each salad sample. Common bacterial contaminants included *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, *Proteus*, and *Bacillus* species, suggesting potential public health risks associated with poor handling and hygiene practices.

Table 2: Showing Bacteria Isolated from Sample

Samples	Organisms Isolated (Bacteria) from sample
A	Proteus and Staph
B^1	Pseudomonas, Staph, E. coli, Bacillus
B^2	Pseudomonas, Staph, E. coli, Bacillus
B^3	Pseudomonas, Staph and proteus, Bacillus
B^4	Bacillus, E. coli and proteus

All samples except the control showed presence of coliform bacteria such as *E. coli* and *Proteus*, which are indicative of fecal contamination and poor hygiene. *Staphylococcus aureus*, associated with skin flora and food poisoning, was also prevalent. The presence of *Bacillus* spp., while common in the environment, may suggest spore-forming bacteria that can survive mild washing procedures.

Table 3 summarizes the fungal species isolated from the salad samples. Fungi identified included yeasts and molds such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Geotrichum*, indicating possible spoilage and mycotoxin risks.

Table 3: Showing Fungi Isolated from Sample

Samples	Organisms isolated (fungi) from sample
A	Yeast and Aspergillus
B^1	Yeast, Penicillium, Aspergillus and Rhizopus
B^2	Penicillium, Aspergillus, Rhizopus, Geotricum
B^3	Aspergillus, Rhizopus and Aspergillus
B^4	Penicillium, Geotrichum and yeast

The identification of filamentous fungi like *Aspergillus*, *Penicillium*, and *Rhizopus* raises concerns about potential mycotoxin contamination, especially under warm, humid storage conditions. *Geotrichum*, a spoilage organism, indicates product deterioration. Though yeasts are less harmful, their presence still signifies reduced shelf life and poor storage practices.

Table 4 displays the antibiotic resistance profiles of bacterial isolates against a panel of commonly used antibiotics. Resistance (R), intermediate (I), and sensitivity (S) patterns are indicated for each organism, highlighting concerns over antimicrobial resistance among foodborne pathogens in RTE salads.

Table 4: Antibiotic Sensitive Test Organisms

Antimicrobial agents	Code	Staph	Pseudo	E.coli	Bacillus	Proteus
Tarivid	OFX	S	S	I	R	R
Peflacine	PEF	R	S	R	R	R
Ciproflox	CPX	R	S	S	I	R
Augmentin	AU	R	S	I	R	R
Gentamycin	CN	R	S	R	R	R
Ceporex	CEP	R	S	R	I	R
Nalidixic acid	NA	R	S	I	R	R
Septrim	SXT	R	S	R	I	R
Ampicilin	PN	R	S	R	I	R
Streptomycin	S	R	I	R	R	R

Multidrug resistance (MDR) was particularly notable in *Proteus* spp. and *E. coli*, both of which showed resistance to at least 6 out of 10 antibiotics tested. This confirms global trends in antimicrobial resistance (AMR), especially among foodborne pathogens.

The findings from this study demonstrate the presence of microbial contamination in pre-packaged vegetable salads sold in Umuahia Metropolis, although at relatively moderate levels. The Total Viable Counts (TVCs) ranged from 3.8×10^2 CFU/g in the laboratory-prepared control sample to 2.16×10^3 CFU/g in salads obtained from Isi-gate Market. These values, while lower than the 5.9×10^6 CFU/g reported by Uzeh et al. [11] in similar studies of open-market salads in Lagos, Nigeria, still indicate lapses in food hygiene and handling. The comparatively lower TVC observed in the laboratory-prepared sample reinforces the role of controlled sanitary environments in significantly reducing microbial load in ready-to-eat (RTE) foods [1-2, 11].

The isolation of bacterial species such as *Escherichia coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp., and *Bacillus* spp. is consistent with previous studies on the microbial safety of fresh salads and street foods [1, 3-4, 20]. The recurrent detection of *E. coli* is particularly concerning, as it is widely used as an indicator of fecal contamination, often suggesting poor handling practices or use of contaminated water during washing or rinsing of vegetables [6, 9]. *Staphylococcus aureus*, typically associated with human skin and nasal flora, suggests contamination during manual handling and packaging [5, 11]. Meanwhile, the presence of *Proteus* and *Pseudomonas* points to environmental and water-related sources [7], while *Bacillus* spp., known for forming spores, may persist even in relatively clean environments [3, 13, 19-20].

Fungal contaminants identified included *Aspergillus, Penicillium, Rhizopus, Geotrichum*, and yeast species. These genera are commonly reported in fresh produce and processed foods and may contribute to spoilage and the potential production of mycotoxins under favourable conditions [11-12]. The frequent detection of *Aspergillus* and *Penicillium* is particularly concerning due to their known ability to produce aflatoxins and other toxic secondary metabolites, which pose risks to human health upon ingestion [8, 21].

The antibiotic susceptibility profiles revealed variable resistance patterns among the bacterial isolates. While detailed zone diameter data were not provided, the results indicate the presence of multi-drug resistant (MDR) strains, with resistance observed particularly toward common antibiotics like ampicillin, streptomycin, and nalidixic acid. These findings are aligned with global and local reports highlighting the rising trend of MDR organisms in RTE foods and environmental samples [10, 16, 18]. The public health implication of this is serious, as the consumption of contaminated RTE salads could contribute to treatment failures, increased morbidity, and the spread of resistant strains within communities [8, 14, 23].

Overall, while the microbial loads observed were not alarmingly high, the diversity of both bacterial and fungal pathogens, alongside the presence of antibiotic-resistant strains, highlights the urgent need for improved sanitary practices throughout the salad preparation and distribution chain. Regular monitoring and adherence to good hygiene practices, especially in markets and roadside food outlets are essential in mitigating microbial risks associated with RTE salads.

The findings from this study demonstrate the presence of microbial contamination in pre-packaged vegetable salads sold in Umuahia Metropolis, with statistically significant differences observed across sample sources. Total Viable Counts (TVCs) ranged from 3.8×10^2 CFU/g in the laboratory-prepared control sample to 2.16×10^3 CFU/g in the salad obtained from Isi-Gate Market. Although all samples remained within the acceptable limit for ready-to-eat (RTE) foods as set by the

World Health Organization (10^4 CFU/g), statistical analysis using one-way ANOVA revealed that the differences in microbial load between sample locations were highly significant ($p = 0.001$). Post-hoc

Tukey tests confirmed that the laboratory control sample was significantly less contaminated than all commercial samples ($p < 0.01$), while salads from open markets (Isi-Gate and Ahiaeke) had notably higher contamination levels than those from street vendors ($p < 0.05$). These results quantitatively reinforce concerns about poor hygiene practices in open markets compared to more controlled environments [1, 2, 11].

The presence of bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus* spp., *Pseudomonas* spp., and *Bacillus* spp. further validates earlier findings in similar studies across Nigeria and sub-Saharan Africa. The repeated isolation of *E. coli*, a strong indicator of fecal contamination, suggests the possible use of unclean water or inadequate handling during salad preparation. Similarly, *Staphylococcus aureus* contamination likely results from direct human contact, such as improper glove use or lack of hand-washing, while *Proteus* and *Pseudomonas* spp. indicate potential environmental or waterborne contamination. Although *Bacillus* spp. are spore-formers commonly found in soil and plant material, their presence even in low numbers in RTE foods warrants attention due to their ability to survive basic washing processes [5, 11].

Fungal species isolated from the samples included *Aspergillus*, *Penicillium*, *Rhizopus*, *Geotrichum*, and various yeast species. The predominance of *Aspergillus* and *Penicillium* is particularly concerning given their capacity to produce harmful mycotoxins such as aflatoxins and ochratoxins under suitable conditions. The presence of these fungi, even in moderate numbers, points to improper post-harvest handling, high ambient humidity, and possibly extended exposure to open air during retail [7].

Antibiotic susceptibility testing revealed variable resistance patterns among bacterial isolates, with particular resistance noted against commonly used antibiotics such as ampicillin, streptomycin, and nalidixic acid. Although detailed inhibition zone measurements were not recorded, qualitative interpretation showed that multi-drug resistance (MDR) defined as resistance to at least three classes of antibiotics was evident in isolates of *Escherichia coli, Staphylococcus aureus,* and *Proteus* spp [10, 16]. This aligns with global trends highlighted by the World Health Organization, where foodborne MDR organisms are increasingly implicated in community-acquired infections and therapeutic failures. The identification of MDR strains in consumer-ready salads raises public health concerns, as it indicates not only contamination but also the potential for these organisms to spread antimicrobial resistance within the human population.

The overall microbial loads in the salad samples were below international safety limits, the statistically significant variation across sources combined with the presence of pathogenic and drug-resistant organisms reveals clear lapses in hygiene, particularly in open market settings [24, 30-32]. These findings underscore the need for strict enforcement of food safety regulations, vendor education on proper handling practices, and routine microbial surveillance of RTE food products in the region.

Addressing these issues is critical in preventing foodborne illness and controlling the spread of antibiotic resistance through the food chain [33].

Conclusion

This study demonstrated that pre-packaged vegetable salads sold in Umuahia Metropolis are moderately contaminated with a range of bacterial and fungal organisms, including known pathogens such as *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Aspergillus* spp. While the total viable counts (TVCs) observed were lower than those reported in similar studies from open markets in other regions, the detection of these organisms still poses a notable public health concern. Importantly, some of the bacterial isolates exhibited resistance to multiple antibiotics, indicating that ready-to-eat (RTE) salads could serve as a potential vehicle for the transmission of antimicrobial-resistant pathogens.

The likely sources of contamination include the use of unclean or untreated water for washing vegetables, poor personal hygiene among handlers and exposure of ingredients to open-air environments during preparation or display, and cross-contamination from utensils, packaging materials, or surfaces. Fungal presence suggests possible storage under high humidity or inadequate refrigeration, particularly during transport or at the point of sale.

To reduce microbial risks, it is essential to enforce strict hygiene standards across the entire food supply chain from harvesting to final packaging. Routine microbial quality surveillance, proper disinfection of raw materials, and regular training of food vendors and handlers on good hygienic practices are strongly recommended. Further studies should focus on pinpointing specific contamination sources through environmental sampling (e.g., water, surfaces, tools, and hands of food handlers) and tracing critical control points in the production and distribution process. These steps are vital in enhancing food safety, protecting consumer health, and curbing the spread of antimicrobial resistance via the food chain.

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