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BACTERIOLOGICAL QUALITY OF SALAD SOLD BY LOCAL FOOD VENDORS WITHIN SELECTED OPEN MARKETS IN ACCRA, GHANA

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ABSTRACT. *This study evaluated the bacterial contamination of salads sold in open markets in Accra, Ghana. Salad samples were gathered from 30 vendors in both Madina and Nima markets. The microbiological analysis included standard plate counts (SPC), Enterobacteriaceae counts (EC), and coliform counts (CC). In total, sixty salad samples were quantitatively assessed for aerobic mesophiles, Enterobacteriaceae, and coliforms using culture-based methods, with identification of bacterial isolates. Data were analyzed using SPSS version 20. It was found that 40% of the samples exceeded acceptable limits for SPC. The mean SPC was significantly higher ($p=0.042$) in unacceptable samples ($3.23x10^8$ cfu/g) compared to acceptable samples ($1.09x10^6$ cfu/g), indicating issues with handling. Both EC and CC means were elevated in both categories but did not differ significantly. Isolated organisms included Salmonella spp. and Staphylococcus spp. Salads served by hand had higher SPC ($3.28x10^8$ cfu/g) and CC ($2.67x10^7$ cfu/g) compared to those served with a spoon, while salads in food flasks exhibited higher counts than those in polythene bags for SPC ($4.53x10^8$ cfu/g, $p=0.021$) and CC ($1.80x10^8$ cfu/g). Enclosed vending areas showed greater microbial loads across all parameters compared to open sites. Poor hygiene practices in salad vending likely contributed to the significant microbial contamination observed. Implementing targeted interventions and regular testing could enhance the safety of street food.*

KEYWORDS: Salad, Bacteriological quality, Local vendor, Contamination, Food safety

INTRODUCTION

Health officials recommend consuming vegetables to bolster the immune system by providing essential nutrients and vitamins, as they are rich in micronutrients vital for biochemical processes that facilitate the formation of antibodies (Calder, 2022; Pecora *et al*., 2020). Salad, typically a combination of raw

vegetables such as lettuce, cabbage, carrots, and onions, is often chopped and sometimes combined with mayonnaise. In contemporary society, salads are convenient and appealing because they require no cooking or preparation. However, children and women of reproductive age in Ghana frequently experience micronutrient deficiencies due to low intake of foods rich in these essential nutrients, adversely affecting their health and hindering social and economic development.

Observational studies have shown that these salads are often stored at inappropriate temperatures, subjected to excessive handling by food vendors, and sold in unsanitary conditions (Kok, 2014; Ramatla *et al*., 2023). Unfortunately, many vegetables are not grown under hygienic conditions, largely due to inconsistent rainfall. Many growers cultivate their crops near polluted water sources within urban areas, where contamination from garbage, as well as human and animal waste, is common. Consequently, these contaminated vegetables make their way to city markets and are prepared into salads for public consumption.

Additionally, the growing preference for fast food, due to its time-saving nature, places strain on fast food operators and local vendors, who often rush to meet consumer demand. This hurried preparation can lead to foodborne illnesses, presenting significant public health challenges that result in human morbidity, mortality, and economic losses. It is crucial to prioritize the hygienic handling and preparation of vegetables, particularly since they are primarily consumed raw. This study focused on evaluating the bacterial quality of salads sold by ready-to-eat food vendors. The research was conducted cross-sectionally and involved samples collected from food vendors in Madina and Nima.

MATREIALS AND METHODS

Samples were collected from vendors in Madina and Nima during mid-morning, using sterile zip-lock bags, and stored in an ice chest for transportation to the laboratory for analysis. A structured questionnaire was employed to gather data on the socio-demographic characteristics of the vendors and the conditions of their surrounding environments.

Sampling technique

A total of 60 vendors were randomly selected, with 30 vendors from each market site in Madina and Nima. All salad vendors at both markets were counted to establish the total number of vendors. The stalls were numbered sequentially, and numbers were assigned by moving through the market following a predetermined pattern, ensuring that each vendor had an equal opportunity for selection. A random number generator was then used to produce 30 random numbers for the Madina market and 30 for the Nima market, aligning with the total vendor count.

Vendors whose numbers matched those generated randomly were approached to secure informed verbal consent for voluntary participation. If a selected vendor was unavailable or chose not to participate, the nearest vendor was then asked instead. After the vendors were chosen, the available salad was thoroughly mixed using sterile spoons. Three composite portions of approximately 100 grams each

were scooped from different areas of the bulk salad to ensure a representative sample. These samples were immediately placed in sterile bags, labeled, and transported in chilled conditions to the analytical laboratory.

Standard Plate Counts

This procedure provides a systematic approach for estimating the bacterial count in food samples. It is an empirical measurement because no single growth medium or set of physical and chemical conditions can fulfill the physiological requirements of all organisms present in a sample. Additionally, organisms may appear individually, in pairs, clusters, or chains. To perform a tenfold serial dilution and analyze it using the pour plate method, ten grams of each salad sample were blended in 20 ml of phosphate buffered saline (PBS) and then adjusted to a final volume of 100 ml (Łepecka *et al*., 2022). A sterile pipette was used to transfer 1 ml of each diluted sample onto the plates for examination. Subsequently, 25 ml of molten, cooled Plate Count Agar was added to each well-labeled plate. The medium and sample were thoroughly mixed, and the plates were allowed to sit on a flat surface. After solidification, the plates were incubated for 18 to 24 hours at 37°C, with cultures performed in duplicate. Following an overnight incubation period, counts were conducted using a colony counting device and expressed as colony-forming units per gram (cfu/g) (Łepecka *et al*., 2022).

Enterobacteriaceae Count

Enterobacteriaceae are part of the intestinal microbiota, and their presence in food and water worldwide signifies fecal contamination and inadequate hygiene practices. An L-rod spreader was employed to evenly distribute the inoculum across the surface of the agar, ensuring uniform growth after applying 0.1 ml of each dilution onto 25 ml of solidified MacConkey agar. The plates were incubated at 37°C for 18 to 24 hours, with cultures conducted in duplicate. After incubation, counts were performed using a colony counting device. Each distinct colony was enumerated whenever possible and reported in colony-forming units per gram (cfu/g) (Łepecka et al., 2022).

Coliform Count

The spread plate technique using MacConkey agar was utilized to accurately quantify the total coliforms present in the salad samples by facilitating discrete colony formation. This method provided a reliable assessment of fecal contamination levels and the overall bacteriological quality of the salads evaluated. Tenfold serial dilutions were carried out in nine additional sterile tubes, each containing 9 ml of sterile water, using the concentrated solution. To promote confluent growth, an L-rod spreader was employed to evenly distribute the inoculum across the surface of the agar after applying 0.1 ml of the sample from each dilution onto 25 ml of solidified MacConkey agar. The plates were then incubated at 37°C for 18 to 24 hours, with cultures conducted in duplicate. Following incubation, counts were made using a colony counting device that allows for visual inspection of individual colonies. Whenever feasible, the distinct lactose-fermenting colonies were enumerated and recorded as colony-forming units per gram (cfu/g) (Łepecka et al., 2022).

Isolation of Organisms

Bacteriological studies commence with the processes of bacterial isolation, purification, and identification. Isolation was carried out to obtain pure bacterial cultures. The supernatant from a 2 ml aliquot of the macerated material was decanted after being spun at 1100 rpm for 30 minutes in a refrigerated centrifuge. The sediment was then inoculated into Selenite F broth and incubated at 37°C for 18 to 24 hours before being sub-cultured on Salmonella/Shigella Agar. To identify *Escherichia coli*, *Klebsiella* spp., and other enterobacteria, a second loop of sediment was placed onto MacConkey Agar. The sediment was also cultured on blood agar and chocolate agar to promote the growth of additional organisms, including Gram-positive bacteria. All incubations were performed at 37°C under aerobic conditions for 18 to 24 hours. In cases of mixed growth, purity plating was employed to identify suspected colonies, and conventional biochemical techniques were utilized to confirm the identities of the colonies (Mridha *et al*., 2020).

Purity plating

A loopful of the inoculum from an isolated colony on the mixed growth culture was used to inoculate both MacConkey agar and blood agar to obtain a pure culture. The inoculum was then streaked on the plates using a four-quadrant method to create parallel overlapping strokes, while flaming the loop between strokes to ensure the formation of isolated colonies. The plates were subsequently incubated at 37°C for 18 to 24 hours in an aerobic environment. Each distinct bacterial colony identified on the mixed growth culture plate underwent the complete procedure again.

Ethical approval and informed consents

The vendors were chosen in consultation with the market leaders at each study site, considering the vendors' willingness to participate. While no ethical approval was necessary, verbal consent was obtained from each vendor during the sample collection process.

Analysis

All collected data was securely locked and coded for confidentiality. The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20, and results were compiled into tables with Microsoft Office 2010. Two-tailed t-tests were the primary statistical method used to identify quantitative differences between independent categorical groups related to salad vending conditions, storage practices, service methods, and overall microbiological quality in this study, with a significance threshold set at a p-value of <0.05. Descriptive statistics were employed to analyze frequency tables and measures of central tendency, including mean and standard deviation.

RESULTS AND DISCUSSION

According to microbiological standards, the mean standard plate count (SPC), Enterobacteriaceae count (EC), and coliform count (CC) for salad samples were classified as either acceptable or unacceptable (see Table 1). The mean SPC was significantly higher ($p=0.042$) in samples deemed unacceptable compared to those considered acceptable. This substantial difference indicates improper handling and a

lack of hygiene during salad preparation, as elevated SPC levels suggest significant bacterial contamination (Kibret, 2012; Postollec *et al*., 2012; Samapundo *et al*., 2016). An unacceptable SPC value corresponds to counts exceeding 1,000,000 CFU/g (Campos *et al*., 2013; Gilbert, 2000).

Samples with EC of 100 CFU/g or higher are deemed unacceptable for consumption, while samples with CC of 10 CFU/g or more are also considered unacceptable (Campos *et al*., 2013; Gilbert, 2000). In the present study, high levels of EC and CC were observed in both acceptable and unacceptable groups; however, these differences were not statistically significant (see Table 1). Elevated levels of EC and CC, even in acceptable samples, indicate unsanitary practices in production and vending since Enterobacteriaceae and coliforms signify fecal contamination (Figueras & Borrego, 2010; World Health Organization, 2017). Similarly, high levels of EC and CC were reported in salad samples in studies from Saudi Arabia and Ghana (Mensah, 2002). The significant microbial loads highlight the necessity for good agricultural practices during salad vegetable production, as well as hygienic handling and vending, to enhance microbiological quality and safety.

Table 1: Mean count across the various study parameters

1	**Variables**	**Acceptable Mean Count**	**Unacceptable Mean Count**	**p-value**
	SPC	1091363.64	322902631.6	0.042
	EC	1043000.00	242780000.0	0.152
	CC	905945.95	323139130.4	0.36
2	**Mode of serving**	**Hand**	**Spoon**	**p-value**
	SPC	328046666.7	174119791.7	0.43
	EC	1862783333	2065435417	0.89
	CC	266946666.7	88799166.67	0.34
3	**Temperature**	**Warm**	**Cold**	**p-value**
	SPC	239273333.30	124712777.80	0.498
	EC	250900238.10	89534444.44	0.241
	CC	97569523.81	187100000.00	0.589
4	**Nature of site**	**Open**	**Enclosed**	**p-value**
	SPC	103912903.20	312862413.80	0.176
	EC	131878709.70	277972069.00	0.247
	CC	136701935.50	111308965.50	0.868

There were no significant differences between hand and spoon serving methods for any of the parameters assessed. However, the elevated mean levels of SPC and CC associated with hand serving

are concerning, as hands can act as vectors for cross-contamination if proper hygiene is not maintained (Todd *et al*., 2010). Various studies involving street food vendors have detected Staphylococcus aureus in hand swabs, highlighting poor hand hygiene practices (Abdul-Mutalib *et al*., 2012; Kibret, 2012). Spoon-served salads exhibited higher EC counts, potentially due to improper utensil usage or inadequate cleaning. Research underscores the necessity of sanitizing utensils between uses and designating specific serving utensils to prevent cross-contamination (Todd *et al*., 2010).

For SPC, EC, and CC, mean counts were greater in warm samples compared to cold ones, although not significantly. Higher microbial growth is anticipated at elevated temperatures, as bacteria multiply rapidly between 4°C and 60°C (Korajkic *et al*., 2013). Maintaining proper cold storage below 5°C is essential to inhibit pathogen growth (Pham, 2014). The absence of significant differences suggests potential temperature abuse, as samples sold warm would likely have considerably higher counts if they had been adequately refrigerated prior to sale. Although differences were not significant (p=0.758, p=0.161, and p=0.618), mean counts for SPC, EC, and CC were all higher for samples obtained from dirty locations (Figure 1). The environments were categorized as neat or dirty based on a structured scoring system that evaluated the cleanliness and maintenance of food premises (Park *et al*., 2010; Tan *et al*., 2013). Factors such as surfaces, floors, and drainage were assessed, and establishments were classified as dirty or neat based on these evaluations. Contamination of produce in dirty environments can occur through dust, pests, and cross-contamination (Imathiu, 2017). Clean food preparation surfaces are critical for reducing microbial contamination (Kramer & Assadian, 2014; Rusin *et al*., 2002). The lack of significant differences between both groups indicates that sanitation remains inadequate, even in those areas presumed to be clean.

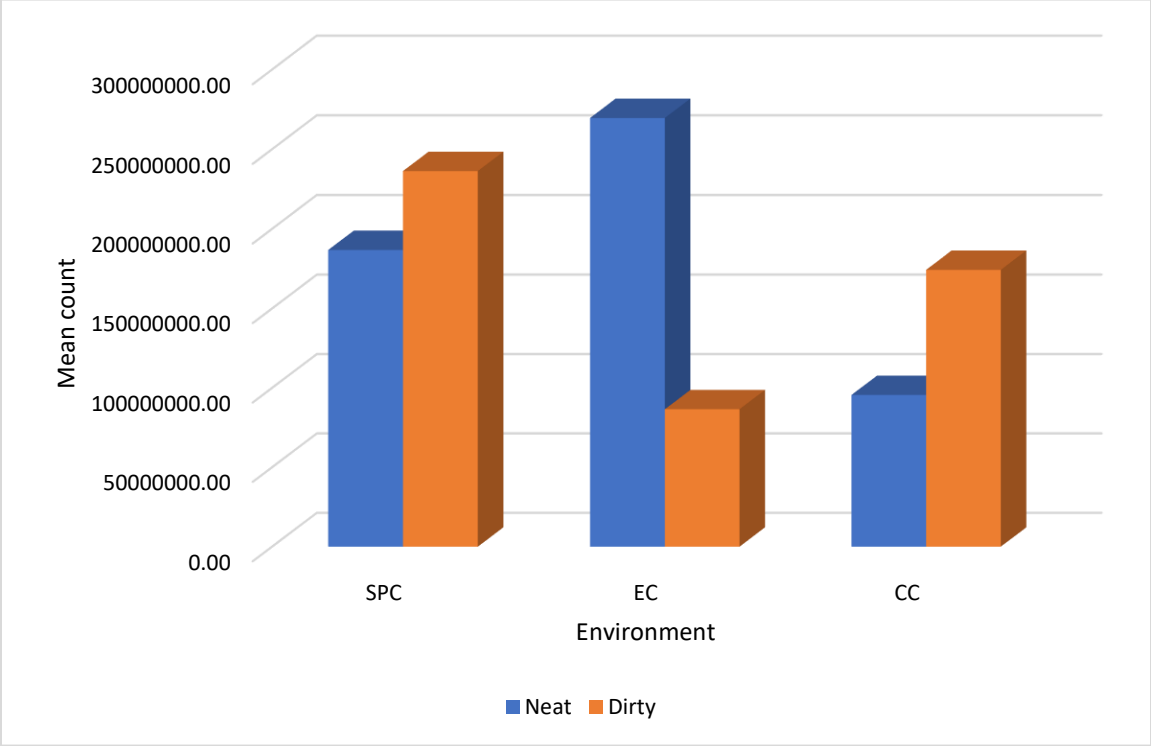

Figure 1: Total bacterial counts of samples per the environment of sampling

For all parameters (SPC, EC, CC), the mean microbial loads were greater in salads sold at enclosed sites compared to those at open sites, although the differences were not statistically significant. The heightened contamination observed in enclosed environments is concerning, as it suggests specific risks associated with closed spaces. Enclosed stalls may be more prone to accumulating dirt, pests, and microbes than open areas, which benefit from better airflow. Vendors operating in enclosed stalls might also engage in riskier practices, such as inadequate washing and refrigeration, compared to their open counterparts who, despite lacking facilities, must keep their produce cool and protected. However, the absence of significant differences between open and enclosed vendors indicates widespread mishandling and poor hygiene practices across both types of vending sites. There is likely a need for all vendors to receive improved food safety training and access to essential sanitation facilities, including running water, refrigeration, and waste disposal (Hill *et al*., 2022; Mosupye & von Holy, 2000; Nkosi & Tabit, 2021). Installing washable surfaces in stalls may also help mitigate contamination risks specifically in enclosed locations.

The high microbial counts observed even in open vending settings highlight potential risks at all sites, not solely those that are enclosed, underscoring the necessity for proper monitoring of microbiological quality across various vending environments. Implementing targeted interventions that consider vending conditions and vendor practices may prove to be more effective than a generalized approach to enhancing food hygiene and safety.

Figure 2 illustrates the microbial counts associated with various types of salad containers—food flasks compared to polythene bags. For both SPC and CC, the mean bacterial loads were significantly higher for salads sold in food flasks than those in polythene bags, with the difference in SPC being statistically significant ($p=0.021$). The increased contamination found in food flasks is likely attributable to cracks, scratches, or worn areas that can harbor microbes even after cleaning. Bacteria such as Listeria species can easily adhere to surfaces of materials like stainless steel, rubber, and glass used in flasks (Soni & Nannapaneni, 2010). Additionally, the humid and enclosed environment within flasks may facilitate bacterial survival and growth compared to more breathable bags. However, the elevated counts observed in polythene bags also point to improper handling practices that result in cross-contamination. While food-grade plastic offers an impermeable barrier, these bags can still facilitate the transmission of pathogens through moisture if they become contaminated during filling and storage (Gilbert, 2000). Vendors should utilize new bags for each batch of salad and exercise caution during the filling process to mitigate risks. The findings emphasize the importance of thorough cleaning and replacing damaged food containers. However, improper handling and hygiene can compromise even the cleanest containers, making it crucial to provide training for workers on safe preparation practices.

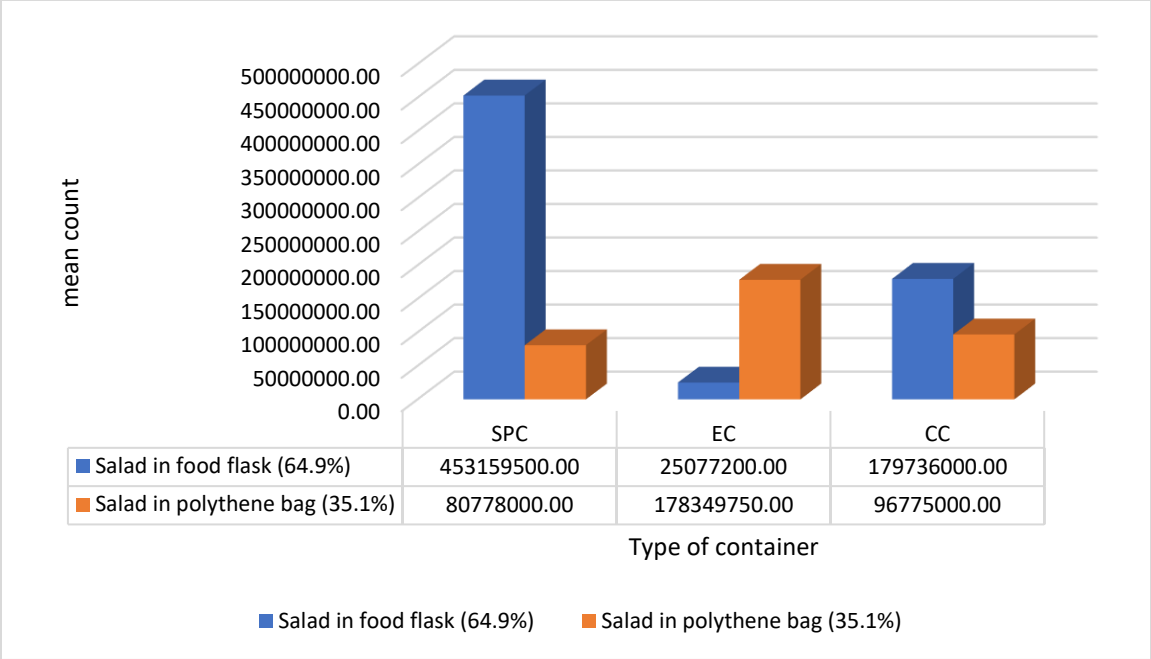

Figure 2: Types of containers used by vendors

The findings of this study highlighted concerning levels of microbial contamination in salad samples collected from street vendors in Accra, Ghana. The average standard plate count surpassed acceptable thresholds in 40% of the samples, suggesting poor microbiological quality. Various factors likely contributed to the elevated contamination levels observed. Salad preparation methods were recognized as a significant source of contamination, with salads served by hand displaying higher microbial loads compared to those served with utensils (Todd *et al*., 2010), indicating risks associated with bare hand contact. Improperly cleaned utensils also served as conduits for microbes (Shayeghi, 2020). Additionally, warm salad samples exhibited higher microbial counts than refrigerated samples, indicating potential temperature abuse (Pham, 2014). Enclosed vending locations were found to contain a greater microbial load, possibly due to inadequate ventilation and hygiene infrastructure (Mosupye & von Holy, 2000). Also, damaged food containers like cracked flasks supported microbial survival and growth more than polythene bags (Soni & Nannapaneni, 2010). The presence of *Salmonella* spp. And *Staphylococcus* spp. Points to fecal contamination and inadequate personal hygiene, respectively (Girma & Aemiro, 2022). Similar pathogens were detected in salads in studies conducted in Saudi Arabia and Thailand (Khiyami *et al*., 2011; Minami *et al*., 2010). Unhygienic handling of produce, washing with contaminated water, and exposure to unsanitary environments likely introduced these pathogens.

In summary, the results underscore the urgent need for vendor training, facility improvements, and enhanced produce handling practices to minimize salad contamination. Interventions should prioritize hand hygiene, temperature regulation, sanitary utensils, protective packaging, and access to clean water. Routine microbiological testing and monitoring of salads sold by street vendors is also crucial to protect public health.

CONCLUSION

The findings of this study highlighted alarming levels of microbial contamination in salad samples from street vendors in Accra, Ghana. The average standard plate count surpassed acceptable food safety limits in 40% of the samples, indicating a significant lack of microbiological quality. Various factors likely contributed to the high levels of contamination, including salad preparation practices, conditions at vending sites, temperature abuses, and damaged food containers. Notably, the method of salad preparation and serving was identified as a primary source of contamination. While no statistical significance was detected, salads served by hand exhibited higher standard plate counts and coliform levels compared to those served with utensils, suggesting potential risks from bare-hand contact. Without thorough handwashing with soap and clean water before and after serving each portion, there is a risk of transmitting pathogens between salads. Furthermore, inadequately cleaned utensils were also found to transmit microbes. The high levels of Enterobacteriaceae in both serving methods highlighted the insufficient hygiene at vending locations.

Temperature abuse was another likely factor contributing to microbial growth. Warm salads generally showed higher bacterial counts than refrigerated ones, although the difference was not statistically significant. If produce had been properly stored below 41°F prior to sale, we would expect substantially lower contamination levels due to inhibited pathogen growth. The lack of a notable difference implies that salads were likely kept at ambient temperatures for extended periods, allowing for microbial proliferation. It is crucial to maintain appropriate temperature controls throughout the entire process, from harvest and transportation to washing, preparation, storage, and serving.

The cleanliness of vending environments also affected produce contamination due to factors such as dust, pests, runoff, and cross-contact. Although the differences were not statistically significant, all microbial parameters showed elevated mean levels in unclean locations. Ensuring proper sanitation is essential to reduce these risks. Maintaining clean food preparation surfaces, washable floors and walls, protected displays for produce, and facilities equipped for cleaning, waste disposal, and ventilation can significantly lower contamination risks.

Additionally, storing salads in damaged containers such as chipped flasks and plastic bags increased bacterial counts, particularly for standard plate counts and coliforms. Cracks or scratches can harbor biofilms even after cleaning, while breathable plastic provides inadequate protection. Nevertheless, high counts in bags indicated that handling practices can overshadow the benefits of proper packaging. Utilizing new food-grade bags for each batch of salad and ensuring careful filling and sealing could mitigate risks more effectively than using pristine containers under poor hygiene practices.

The presence of Salmonella spp. and Staphylococcus spp. suggests inadequate personal hygiene and potential fecal contamination. Possible sources of these pathogens include contaminated irrigation water, soils enriched with manure, and unsanitary practices during post-harvest washing and handling.

Comparable pathogens have also been identified in street-vended salads worldwide. These significant risks highlight the urgent need for interventions throughout the entire supply chain.

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