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Bacteriological Quality of Salads Sold at Selected Restaurants in Accra, Ghana

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Abstract:

Background: The increasing prevalence of chronic non-communicable diseases has led to a greater emphasis on the consumption of healthy foods, such as vegetables. Vegetable salads from restaurants are generally perceived as safe. We investigated the bacteriological quality of vegetable salads sold in two popular restaurants in Accra.

Methods: Twenty salad samples were purchased from two popular restaurants (A and B) with two branches each in Accra, Ghana. Restaurant A had branches at Dansoman and North Industrial Area, while B had branches at Osu and Tesano. Total aerobic colony forming unit (CFU) and biochemical assays were performed by standard culture techniques and protocols, to determine the microbial load and species present.

Results: Mean aerobic bacteria count was 1.77E5 and 1.45 E5 CFU/g for Restaurants A, and B respectively. The North Industrial Area branch of A had more CFUs (2.64E5 CFU/g) than the Dansoman branch (0.9E5 CFU/g), and statistically significant ($p=0.0010$). The Tesano branch of restaurant B had higher CFUs (1.9E5 CFU/g) than the Osu branch (1.0E5 CFU/g), and also statistically significant ($p=0.0022$). Furthermore, ANOVA across the four branches showed a significant difference ($p<0.0001$). The main isolates identified from both restaurants were *Enterobacter spp.* (28.7%), *Citrobacter spp.* (20.4%), *Klebsiella ssp.* (18.5%) and *Enterococcus spp.* (7.4%).

Conclusion: *Enterobacter* species was predominant among others. Education of the restaurant staff, and the application of food safety and handling procedures must be established, and food regulatory institutions must carry out routine inspection at these sites to ensure consumer protection and public health.

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1. INTRODUCTION

Salads are made primarily of a mixture of raw vegetables and/or fruits such as lettuce, spinach, cabbage, carrots, onions, tomatoes, green beans, strawberries, apples, pears etc. [1,2].

Vegetables are important for human nutrition and their presence in vegetable salads confers several health benefits. Vegetable salads are rich sources of folate, vitamin A, C, K and minerals such as iron and calcium. Recommendations for increased intake of vegetables is on the rise. Various guidelines for the prevention and management of chronic non-communicable diseases recommend increasing the amount of vegetables in our daily diets [3-5]. Consuming vegetable-based salad is associated with higher nutrient intakes and diet quality among adults [6]. They contain antioxidants and phytochemicals which help to prevent chronic diseases like diabetes, cancers and heart diseases [7,8]. Their rich sources of dietary fibre additionally help in weight management and prevents constipation [9,10]. Due to their highly perishable nature and the fact that they are mostly consumed raw or minimally processed, in order to retain their natural taste and heat labile nutrients, vegetables easily become vehicles of bacterial contamination. This can increase the risk of foodborne diseases associated with the intake of vegetables [11,12]. Environmental and human factors such as poor agricultural practices and unhygienic practices of food handlers during preparation and sale of vegetables are some of the factors that can easily result in their contamination and subsequent threat to public health [10,13]. Various bacterial species including *Staphylococcus aureus*, *E. coli, Klebsiella spp*. and *Bacillus spp.* have been isolated in vegetables sold at the market [10,14] as well as in ready to eat vegetable salads in countries including Ethiopia, Cameroon and Ghana [1,15]. Health promotion exercises addressing the prevention and management of most chronic diseases have targeted urban dwellers especially people in middle to high socioeconomic groups. This is partly because their risk of developing non-communicable diseases are higher. These groups have reported increasing prevalence of hypertension, diabetes and obesity in recent decades [16-18]. Their higher education and income levels make them selective in their choice of eateries, preferring restaurants over street foods or canteens [19]. With the growing awareness of health benefits of vegetables especially among urban dwellers vegetable salads from restaurants are often preferred over those from street vendors due to the perception of greater food safety. Significant amount of work has been carried out on the safety of street foods in Ghana, with the conclusion that most of them are prepared under unhygienic conditions and are contaminated with pathogenic microorganism [15,20]. However, not much work has been published in the country on hygiene and food safety of foods sold at hotels and restaurants. All efforts at ensuring the safety of vegetable salads from restaurants are therefore necessary to prevent foodborne diseases, protect public health and avoid undermining health promotion efforts targeting the reduction of chronic disease incidence and management in Ghana. The aim of this study was to investigate the bacteriological quality of vegetable salads sold in two popular restaurants in Accra. Information obtained from this study will alert food regulatory bodies to improve their surveillance and monitoring of restaurants to ensure the safety of foods they provide. It will further inform food service establishment managers and staff to observe appropriate personal, and food hygiene protocols in order to control occurrences and spread of foodborne diseases.

2. MATERIALS AND METHODS

2.1. Study Design and Sites

The study design was experimental, and was carried out in Accra, the capital city of Ghana. Salad samples were obtained from various branches of two renowned and well patronised restaurants, following a pilot study conducted among college students to ascertain consumer patronage. Ethical approval was obtained within the same period before microbiological data was collected from the respective branches of the restaurants following purchase of the samples and laboratory work. Generally, salad leaves prepared for human consumption are pretreated by washing with potable water. During the pilot study, information obtained at the premises indicated that the salads sold in all the four locations were pretreated before preparation, thus soaked and washed primarily in water to remove inherent physical, chemical and biological contaminants in order to produce a safe intake. A pretest by way of ascertaining this knowledge was also confirmed by the restaurant operators during the aforementioned pilot study. The response obtained was therefore put to a confirmatory experiment by testing for the presence and load of the microbes using various microbial assays. The two brand restaurants were identified as A and B. Two branches of each

brand were also selected. Branches of brand A were coded as D and N for Dansoman and North Industrial Area respectively, whilst branches of brand B were coded as O and T for Osu and Tesano suburbs respectively.

2.2. Sampling, Processing and Analysis

The two branches of each restaurant identified were selected by random sampling. A total of 20 salad samples (5 samples per branch) containing ingredients such as lettuce, cucumber, carrot, onion, sweet pepper, topped with salad cream, were purchased from the designated restaurants at lunch time, over a five-week period. One sample was purchased from each restaurant every week on alternating weekdays. The salads were purchased in disposable packs as served by the restaurants. They were immediately placed in a sterile ice chest with ice packs and transported to the laboratory for immediate processing and analysis. Microbial analyses were carried out at the Microbiology Laboratory of the University of Ghana Medical School by qualified laboratory scientists. Each sample was divided into two for a duplicate assay, where ten grams of the samples was added to a sterile stomached bag and 90 ml of sterile peptone water added and macerated until homogeneous solution was obtained. One millilitre of the resulting solution was serially diluted in 9 ml sterile peptone up to a fifth dilution followed by enumeration using pour plate and streak method as described by [21,22].

2.3. Enumeration and Identification of Species

Colonies on all duplicate plates were counted between 25 and 300 and multiplied by their respective dilution factors to obtain the mean CFU/g. Gram staining, colonial morphology and biochemical tests were used to identify the species found. The Gram stain was employed to pre-screen and then differentiate the species, and was performed alongside the colonial morphology before the final identification of species was carried out using the biochemical assays. With regards to the colonial morphology the shape, colour, texture and overall appearance of each colony were determined as stated by [23]. Following a 24-hour incubation period, samples showing similarities of morphological characteristics were grouped and further tested with gram staining for identification as described by [24]. Further species differentiation was carried out by motility test where the possession of flagella for movement was ascertained [25]. According to the latter, bacterial cells can also be differentiated by the production of indole from tryptophan. This test was performed over a 24-hour period by adding 2 drops of Kovac's reagent, followed by shaking in peptone water and allowing to stand. The production of a red colour, separating out with a layer of alcohol was indicative of positive results. Citrate test was performed to differentiate species that could metabolise citrate as carbon source. In this assay, saline suspensions were prepared with the bacterial colonies that showed Gram negative results with 0.20 ml saline. Citrate slopes were incubated for 48 hours at 35˚C; a bright blue colour action identified those positive species for the test.

2.4. Urease Test

This test was solely carried out to differentiate urease positive proteins from other *Enterobacteriaceae*. A slant of urea agar was inoculated by streaking onto the surface of the test sample and was observed for colour change at 6 and 24 hours at 35˚C incubation temperature.

Cytochrome oxidase test was also performed to determine the presence of oxidase enzyme producers in the salads as in the protocol by [26,27].

2.5. Statistical Analysis

Statistical significance between the mean CFU/g obtained from both branches within each restaurant group (D and N for restaurant A; O and T for restaurant B) was determined separately at $P \leq 0.05$ using unpaired T-test. In addition, One-Way ANOVA was employed to determine statistically significant differences across all four branches of the two restaurants. These analyses were performed using GraphPad Prism 8.

3. RESULTS

The results of aerobic colony count of the salad samples from restaurants A and B were recorded during the laboratory investigations. This was achieved per gram of the salad samples from the four respective branches (D, N, O, and T) based on a total of 5 samples from each location (Table **1**). The North Industrial Area branch (N) of restaurant A recorded the highest mean colony count, followed by the Tesano branch (T) of restaurant B (Table **1**), then the Osu Branch (O) followed with the least being the record from the Dansoman Branch (D).

Table 1: Aerobic Colony Count of Salad Samples from Restaurants A and B

Restaurant	N	CFU/g	CFU/g	Mean CFU/g
Restaurant A				
Branch D	5	0.93E5	0.87E5	0.9E5
Branch N	5	2.62E5	2.66E5	2.64E5
Restaurant B				
Branch O	5	1.03E5	0.97E5	1.0E5
Branch T	5	1.87E5	1.93E5	1.9E5

Where N = number of samples taken from the respective restaurant or branch, CFU = Colony forming unit.

Table 2: Unpaired T-Test between the Two Branches of Restaurant A, and B at p≤0.05

Mean of microbial load	Means	Difference between means ± SEM	p-value	Significantly different (p≤ 0.05)
Restaurant A, branches D and N	Branch D (0.9E5) Branch N (2.64E5)	1.74E5 ± 3606	0.0010	Yes
Restaurant B, branches O and T	Branch O (1.0E5) Branch T (1.9E5)	0.9E5 ± 4243	0.0022	Yes

Where SEM = standard error of mean.

The empirical analysis revealed the presence and numbers of pathogenic species in the salad samples, i.e., in the order of high to low; *Enterobacter spp* followed by *Citrobacter spp.,* were the most prevalent species in the salads from restaurant A, compared to *Klebsiella spp.* for restaurant B followed by *Enterococcus spp*. Throughout the branches, Branch N recorded the highest number of *Citrobacter spp*. Whereas Branch O recorded the highest *Enterobacter* and *Klebsiella spp.* (Table **4**). Furthermore, Branch D, N, and T recorded the same number of samples for *Enterobacter spp.* Whilst D and N, and O and T also recorded the same species in their sample sizes of 2 and 1 respectively (Table **4**).

At p≤0.05, an unpaired T-test between both branches of each of the two restaurants i.e., branches D and N for restaurant A, and branches O and T for restaurant B showed a significant difference between results from branches D and N of restaurant A, and between branches O and T of restaurant B. However, the overall statistical significance and the percentages of total species obtained among the restaurants are shown in Tables **3** and **4** respectively in which variations were observed in the microbial counts among the four branches of both restaurants.

There were statistically significant differences (p≤0.05: p=0.0010 and p=0.0022) between restaurant A, branches D and N, and restaurant B, branches O and T respectively. Analysis of variance on the microbial load among the four branches of the two restaurants also showed a significant difference of p<0.0001, as shown in Table **3**.

A One-Way ANOVA test on the number of occurrences of the various microbial species identified from the two restaurants at p≤0.05 showed p=0.9979 indicating no

Table 3: Summary of One-Way ANOVA on Microbial Load (CFU/g) Obtained from the Salad Samples from the Four Branches of Restaurants A and B at p≤0.05

Treatment	SS	DF	MS	F (DFn, DFd)	p-value
Treatment (between columns)	4.04E+10	3	1.35E+10	F (3, 4) = 869.3	p<0.0001
Residual (within columns)	6.2E7	4	1.55E7		
Total	4.05E+10	7			

Table 4: The Number of Occurrences of the Various Microbial Species Identified in the Salads from the Four Branches of the Two Restaurants

Microbial species	Branch D	Branch N	Branch O	Branch T	% of total *spp.*
Citrobacter spp.	7	10	5	0	20.37
Citrobacter freundii	0	4	0	0	3.7
Enterococcus spp.	4	3	0	1	7.41
Enterobacter spp.	7	7	10	7	28.7
Klebsiella oxytoca½	1	0	0	5	5.56
Klebsiella spp.	5	2	10	3	18.51
Proteus miribilis	0	0	0	5	4.63
Staphylococcus spp.	0	0	2	4	5.56
Streptococcus spp.	2	2	1	1	5.56

significant difference in the number of occurrences of the microbes identified in the two restaurants and their respective branches (Table **4**).

4. DISCUSSION

Nine species were collectively isolated from the salad samples used for the study; six of the total species were Gram negative and 3 were Gram positive. From all the restaurants, *Enterobacter spp.*, *Citrobacter spp*. and *Klebsiella spp*. were the three most prevalent (28.7%, 20.37 and 18.51% respectively), whilst *Streptococcus spp., Staphylococcus spp., Klebsiella oxytoca*$^{1/2}$*, Proteus miribilis* and *Citrobacter freundii* were below 6% in prevalence. According to the International Commission for Microbiological Specification for foods [24], the levels of aerobic colony counts for salads are as follows: 0 -1000 CFU/g – acceptable; 1E4-5 tolerable; ≥ 1E6 is unacceptable. From Table 1, restaurant A had a mean colony counts of 1.77E5 and B 1.45E5, both showing tolerable levels according to Cheesbrough [27]. Statistical analyses on the results inferred that the microbial counts within the two branches (D and N) of restaurant A, and the other two branches (O and T) of restaurant B were statistically significant ($p=0.0010$; $p=0.0022$ respectively, Table **2**), and also across the four branches altogether ($p<0.0001$, Table **3**). However, even though the species identified across the four branches of the two restaurants represented biological hazards of food safety importance, their number of occurrences obtained across the four branches as shown in Table **4**, was not statistically significant ($p=0.9979$). The outcome is thus suggestive of unsatisfactory hygienic conditions and practices by both restaurants, a possible outcome of poor employee hygiene, unhygienic raw materials and or insanitary cooking environment [28]. A similar study conducted in Lagos by Moayed *et al*. [29] reported total aerobic counts ranging from 3.3 x 10^3 to 5.9 x 10^6 CFU/g. Similarly, Cheesbrough [27] found aerobic colony counts in salad samples ranging from 3.1 to 7.8 x 10^5 CFU/g, while Oranusi and Braide [30] reported aerobic colony counts from 9 to 9.8 x 10^6 CFU/g.

The mean bacterial counts reported by Uzeh *et al*., [31], for raw salads sold with *Waakye* (cooked rice and beans) on and around the University of Ghana campus was 8.54 to 8.69 log10 CFU/g, and 6.41 log10 CFU/g from off-campus restaurants. In their study, the salads were raw with added salad cream. These were similar to salad samples studied in this current study. The salad cream present in the samples obtained for this research contained egg yolk, which is a good medium for supporting microbial growth [32], and may have contributed to the high microbial levels recorded in this current study.

The presence of *Citrobacter spp.* is indicative of contamination of salad with faecal material, blood, pus or urine. This could have occurred at any step of the food handling, including the use of contaminated raw materials, unhygienic packaging material, use of unclean water in food preparation, inadequate washing and cleaning of vegetables and other cooking, and cutting surfaces and utensils [14-16].

The standard aseptic practices ensured from the point of purchase of the salad samples through the laboratory procedures used, makes it unlikely that these organisms were introduced by the researcher. Good laboratory practices and standard operating procedures for microbiological work were adhered to.

Any counts and species identified were therefore obtained from the salad samples tested. Samples were processed on same day of purchase, and refrigerated. The latter action might have inactivated or even killed majority of the mesophilic microbes, such that the results under discussion may have been underreported.

Klebsiella spp. was the third most prevalent in this study. The species has similarly been reported in salad sources in Ghana by Ameko *et al*., [33]. The current study recorded an overall prevalence of 18.5%, being lower in occurrence at restaurant A (7) compared to B (13). In other parts of the world including Nigeria and India, 6.8% and 5.4% have been reported by Aikins *et al*., [18] and Steele-Dadzie *et al*., [19] respectively. Aboh *et al*., [34] also reported prevalence of 26.7% in salad vegetables in Abuja Municipal Area Council, Nigeria. The threat posed by salads to health is thus not limited to Ghana and Africa but other regions globally. *Klebsiella spp*. is implicated in urinary tract infections, chest infections and also severe bronchopneumonia with lung abscesses [15,27]. It is commonly spread in the environment and can be cultured from soil, water and vegetables when consumed raw. Its presence in the salad thus suggest a compromise of good sanitation practice in both restaurants. The other strain of Klebsiella called oxytoca½ which is associated with hospital-acquired infections [27], was recorded in both restaurants in this current study and may be suggestive of contact of salad with infected persons. The relatively lower levels of *Enterococcus spp., Staphylococcus* and *Streptococcus spp.* recorded in this current study agrees with reports by Moayed *et al*., [29] in Ilam city in Iran and with Oranusi & Braide [30] in Ghana. These organisms are distributed across the environment, thus raises questions about hygienic practices in food handling at these restaurants.

Salads present a rich source of micronutrients necessary for good health, growth and disease prevention. They are also invaluable in the fight to curb the increasing prevalence of chronic non-communicable diseases. These benefits will however be lost if care is not taken by food producers, food regulators, governments as well as consumers to eliminate all sources of microbial contamination and the accompanying threat to public health. Also in particular, the significant difference in microbial load observed across the branches of the restaurant implies the need to standardise food safety practices to reflect quality food service delivery to consumers.

CONCLUSION

The work being reported here has identified the microorganisms that are particularly associated with salads served at various branches of two renowned and well patronised restaurants within Accra, the capital city of Ghana. This study recorded tolerable levels of aerobic bacteria in salads from the selected popular restaurants. The most common organism identified was *Enterobacter spp*., and most of the organisms are a threat to food safety.

With limited knowledge of the general public, the consumption of salads in the sampled locations poses food safety risk due to the fact that pathogenic strains of the organisms could cause foodborne illness and may consequently lead to acquisition of long-term diseases by consumers. Therefore, dissemination of findings and education of the restaurant staff using food production management protocols would reduce or eliminate the potential biological hazards that may confront consumers on a daily basis due to their high patronage.

We recommend that food regulatory institutions conduct regular and stringent inspections, particularly in high-risk locations such as restaurant branches with elevated bacterial counts to ensure food safety, protect public health and promote the consumption of vegetables among the population.

This study thus, revealed that vegetable salads sold at popular restaurants in Accra could contain significant levels of potential pathogenic bacteria including *Entero-bacter spp.* As vegetable salads are increasingly being consumed in efforts to maintain healthy diets, ensuring the microbiological safety of such salads in restaurants is critical for preventing foodborne illnesses.

LIMITATIONS OF THE STUDY

The study encountered resource challenges due to self-funding, hence, gene expression of conserved regions and sequencing of test positive samples were not carried out.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this paper.

FUNDING

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ETHICAL CONSIDERATION

All ethical considerations were fulfilled for this work and a certificate issued by University of Ghana, School of Allied Health Sciences.

Ethics Identification Number SAHS – ET./1035290/AA/ 9A/2013-2014.

AUTHOR CONTRIBUTION

Rebecca K. Steele-Dadzie: Conceptualization, Methodology, Formal Analysis, Writing – Review and Editing

Hannah Asare: Conceptualization, Writing – Original Draft, Methodology, Data Curation, Formal Analysis, Investigation

George Aboagye: Supervision, Writing – Review and Editing, Validation, Visualization

Eric S. Donkor: Conceptualization, Methodology, Supervision, Writing – Editing and Review

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