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Bacteriological Assessment of the Quality of Raw-mixed Vegetable Salads Prepared and Sold by Street Food Vendors in Korle-Gonno, Accra Metropolis, Ghana

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Abstract: It is known that raw-mixed vegetable salads are essential part of people's diet all around the world. These vegetables salads are consumed raw and often without heat treatment or thorough washing. Raw-mixed vegetable salads therefore can serve as vehicle for the transmission of pathogenic microorganisms associated with human diseases. The aim of this study was to isolate and analyze the common bacterial pathogens associated with raw-mixed vegetable salads prepared and sold by street food vendors in Korle-Gonno, Accra, Metropolis, Ghana. A total of 75 different raw-mixed vegetable salads were sampled from the open places and were subjected to bacteriological analysis using standard Food and Agriculture Organization (FAO) of the United Nations (UN) total aerobic plate count method. A total mean colony count of bacteria ranged 0.87-5.6 $\log_{10}$ CFU/g was isolated. *Escherichia coli* (35%), *Staphylococcus aureus* (33%), *Klebsiella* sp. (17%), and *Bacillus* sp. (15%) were the bacteria isolated from the various raw-mixed vegetables salads investigated. This study has shown that all the raw-mixed vegetable salads sampled from Korle-Gonno, Accra Metropolis, Ghana had a high bacterial contamination and their persistence and proliferation is a reflection of poor hygienic practices by the street food vendors. The use of unsafe or contaminated water to irrigate the vegetables when growing on the farm or garden could also be a contributing factor.

Key words: Quality, raw-mixed vegetable salad, aerobic plate count, *Escherichia coli.*

1. Introduction

Salad is a form of food made primarily of a mixture of raw vegetables and or fruits [1]. Common vegetables used in salad include cucumber, pepper, tomatoes, onions, red onions, cabbages, carrots, spring onions, and radishes. Other ingredients such as olives, mushrooms, hard-boiled eggs, cheese, meat or seafood are sometimes added to salads. There are reports that a large number of vegetables can serve as good sources of antioxidants and phytonutrients, and have health protecting properties, to improve human well-being [2, 3]. However, the salads containing raw vegetables may be unsafe, mainly because of the environment under which they are prepared and consumed [4].

In Ghana there is a report that mixed vegetable salad which is usually prepared with fresh lettuce, tomato, onion, carrots or green pepper, and cucumber is at times added to a local food called 'waakye' i.e., rice and cowpeas boiled with Guinea corn leaves (*Sorghum bicolor* L. Moench) [5]. Vegetable salads added to plain rice eaten with stew are sold by street vendors in

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the restaurants and canteens in most parts of Ghana [5]. Normally, vegetables that are eaten raw without any heating during the processing stages can get contaminated because of the manure and the irrigation water used in cultivation [6]. Contaminated hands of farmers and from contaminated water used to wash the vegetables after harvest. Also bad and unhygienic raw-mixed vegetable salad preparation methods can introduce various bacteria and parasites into the salad and cause diarrheoal diseases. It is known that eating food that is contaminated by bacteria is one of the main reasons for food poisoning. Food poisoning can be caused by several bacterial species that are especially present on the raw vegetables and fruits and also in the dairy products that are used in the salad dressings and toppings. Several food borne bacteria such as *Campylobacter jejuni*, *Escherichia coli*, *Clostridium perfringens*, and species of *Salmonella, Staphylococcus*, *Yesinia* and *Shigella* have been previously reported to be the main pathogens which can contaminate vegetables salads and cause diarrheoal after consumption of these contaminated vegetables salads [7, 8].

Although a number of studies have been done on ready-to-eat (RTE) foods in the Accra Metropolis and Ghana as a whole [6, 9-11], the bulk of fresh vegetables consumed in Ghana's capital Accra come from small scale vegetable growers in the city. For some farmers, growing vegetables is a hobby, for many others it's a main source of livelihood. From observation, most of the vegetable gardens are usually sited along the major drains and are irrigated with the water from these gutters [6]. There is concern that the fresh vegetables become contaminated as a result of untreated water in drains used to irrigate them. Considering that many foodborne pathogens can survive and grow in many fresh vegetables, these can easily be transferred to raw-mixed vegetable salads and pose a health hazard to consumers. Mishandling during processing and transportation, and also cross-contamination at food service establishments from other contaminated food items or infected workers can also contribute to the presence and growth of bacterial pathogens on these raw-mixed vegetable salads. Therefore the objective of this study was to assess the bacteriological safety of raw-mixed vegetable salads prepared and sold with RTE foods in Korle-Gonno, a suburb of the Accra Metropolis, Ghana.

2. Materials and Methods

2.1 Study Area and Design

A cross sectional study was conducted at Korle-Gonno, a suburb of the Accra Metropolis, Ghana from March to August, 2013. The source of water for the community is pipe-borne and dug out wells. Sanitation facilities are not encouraging as the whole community has about three public toilets and only a few houses have their own toilet facilities. There are choked gutters and indiscriminate defecation in the study area. Before the start of the project, a preliminary survey of the various food centers and areas was carried out to gather information on the location of street food centers, type of street foods sold, type of salad, and also to get information on the hygiene and sanitary conditions of the food sellers and the food centers [12].

2.2 Sample Collection

Five (5) different food vendors were chosen by a simple random sampling method, without any order using a modification of the methods by Mensah et al. [13] for various vendors that sells the same foods with raw-mixed vegetable salad along the major streets and open spaces of Korle-Gonno, a suburb of the Accra Metropolis. Each food vendor was given a specific code number which corresponds to the number for each media plate used for the investigations. Raw-mixed vegetable salad samples were purchased from the selected food vendors in the mornings for five consecutive days per week, for a period of 3 weeks; constituting 75 samples as previously proposed by Ameko et al. [5]. The ingredients and description of the

5 street food vendors that were selected for the study are presented in Table 1. Samples were placed into sterilized plastic containers and quickly sent to the laboratory for bacteriological analysis. Ethical clearance with identification number SAHS-ET/10310070/AA/17A/2012-2013 was obtained from the Ethics and Protocol Review Committee, School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences, University of Ghana before the start of the project.

Table 1 Selected vendors and the different salad ingredients sold.

Vendors	Constituents
1	L, Ca, O, Cu, T
2	L, Ca, O, Cr, T
3	Ca, Cr, O
4	Ca, Cr. O, T
5	L, Ca, Cr, O

L: lettuce; Ca: cabbage; O: onion; Cu: cucumber; Cr: carrot; T: tomato.

2.3 Bacteriological Analysis

2.3.1 Total Viable Bacterial Counts

All the samples were processed in the Microbiology Laboratory (ML), SBAHS, College of Health Sciences, University of Ghana, Korle-Bu, Accra. The total viable counts of bacteria from the raw-mixed vegetable salad samples were evaluated using a modification of the Food and Agriculture Organization (FAO) of the United Nations (UN) standard methods [14]. Twenty five grams (25 g) each of the raw-mixed vegetable salads samples were weighed on a beam balance and transferred into a plastic "Stomacher" bag. Then 100 mL of sterile distilled water (H_2O) were added into each sample and rinsed vigorously for 10 min for homogenization to obtain 10^{-1}. Ten-fold serial doubling dilutions of the samples through to $10^{-}5$ were made as follows: 4 additional sterile test tubes were appropriately labelled and serially arranged on the test tube rack for each sample. Sterile distilled H_2O (9 mL) was introduced into each test tube with the aid of micropipette with sterile tips. Using separate sterile pipette tips, 1 mL of the rinsed test sample was introduced into the first test tube (10^{-2}) and mixed thoroughly. One milliliter (1 mL) of the contents of 10^{-2} test tube was pipetted and introduced into the second test tube (10^{-3}) and mixed thoroughly. The same procedure was repeated for the rest of the tubes. Then 0.1 mL of each dilution were pipetted using a micropipette with sterile tips and dropped on the surface of a pre-labelled plate count agar (PCA: Oxoid Limited, Basingstoke, UK) and Difco MacConkey agar (DMA: Becton, Dickinson and Company, Sparks, MD 21152, USA) plates in accordance with the labelling on the containers containing the raw-mixed vegetable salad samples. A sterile glass spreader was used to spread the sample dilutions uniformly over the surface of the agar plates and incubated at 37 °C for 24-48 h. After overnight and subsequent incubations, the plates were examined for evidence of bacteria growth and the number of colonies counted. The isolated bacterial index on each agar plate was expressed as CFU/g by multiplying the number of colonies with the dilution factor. Counting was done with the aid of a hand lens.

2.3.2 Identification of Isolates

The identification and characterization of the isolated bacterial species in the present study were done using colonial morphology, gram staining reactions, catalase, indole, oxidase, motility, citrate utilization, methyl red (MR), Voges Proskauer (VP), triple iron sugar (TSI), and coagulase tests according to the FAO of the UN standards [14] and cross referenced with Bergey's manual of determinative bacteriology [15].

2.4 Statistical Analysis

Results obtained from the experiments were entered into a database and analyzed statistically using descriptive statistics such as means and percentages. Also the student's *t*-test was used to find out significant difference between the parameters studied.

3. Results

It is known that vegetables can be exposed to pathogenic microorganisms in a multitude of ways

during growth, processing, and distribution. Before harvest one possible source of contamination with bacteria, viruses, and parasites may be the soil where the produce is grown in which may be contaminated by animals, insects, dust or irrigation water [16]. In the present study, a total of 75 raw-mixed vegetable salads were obtained from 5 different street food vendors. Although the selected vendors sold raw-mixed vegetable salads together with their respective foods, it can be observed that vegetables like cabbage (*Brassica oleracea* var. *capitata* L.) and onion (*Allium cepa* L.) were common amongst all the 5 different street food vendors (Table 2). No two vendors sold the same vegetable salad ingredients or constituents.

The mean total bacterial colony count ranged 0.87-5.6 $\log_{10}$ CFU/g for all the raw-mixed vegetable salad samples obtained from the 5 different street food vendors in Korle-Gonno, a suburb of Accra, Metropolis, Ghana (Table 2). Four different bacterial species namely *E. coli*, *Staphylococcus aureus*, *Klebsiella* sp. and *Bacillus* sp. were isolated from the raw-mixed vegetable salads investigated (Fig. 1). *E. coli* was the most predominant bacteria with mean viable counts of 1.6 $\log_{10}$ CFU/g representing 35% of all the bacteria isolated. The second most predominant bacterium was *S. aureus* with mean viable counts of 1.5 $\log_{10}$ CFU/g representing 33% of the isolated bacteria. Mean total viable counts for species of *Klebsiella* and *Bacillus* were 1.3 $\log_{10}$ CFU/g and 1.0 $\log_{10}$ CFU/g representing 17% and 15% respectively of all the bacterial isolated in this study (Fig. 1). However, there was no significant association between the selected food vendors, the individual vegetable salad ingredients, and isolated bacterial pathogens (P-value > 0.05).

4. Discussion

Freshly consumed vegetables especially those used in salad mixtures, have been implicated in food

Table 2 Mean total colony count values ($\log_{10}$ CFU/g) of isolated bacteria in the raw-mixed vegetable salads.

Sampling times	Vendors				
	1	2	3	4	5
Week 1	1.96	1.64	0.29	1.04	0.85
Week 2	1.84	1.76	0.28	1.12	0.64
Week 3	1.88	1.92	0.29	1.80	0.72
Total count	5.6	5.3	0.87	3.9	2.21

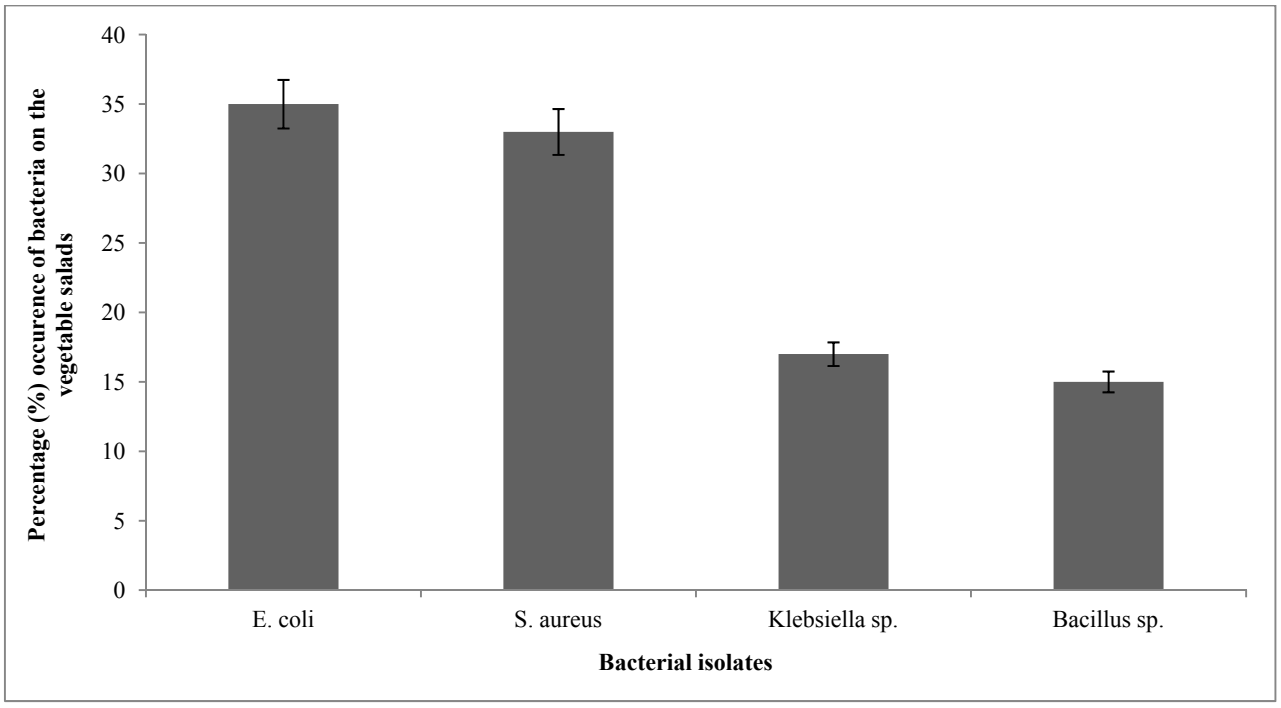

Fig. 1 Percentage distribution of bacterial contaminants isolated from the raw-mixed vegetable salads investigated.

poisoning and thus hazardous to the health of the consumers and the society as a whole [17]. Generally, this study has shown that most raw-mixed vegetable salads that is sold together with street foods at Korle-Gonno, a suburb of the Accra Metropolis are contaminated with various bacterial types. From observations and interviews during the course of this study, it was observed that the selected street vendors sold different salad constituents or ingredients with cabbage and onions being the major ingredients present in all the samples investigated (Table 1). In this investigations majority of the raw-mixed vegetables salads analyzed were contaminated with bacteria pathogens and this could be attributed to poor personnel hygiene, mishandling of the vegetable salads during the washing, cutting and preparation processes. However, factorial analysis performed to verify the significant of the difference in the rate of bacterial contamination of the vegetable salads were statistically insignificant ($P > 0.05$) since each vendor sold different salad mixtures.

It is important to note that these raw vegetables are usually harvested from the soil, hence can become contaminated by pathogenic organisms in soil [18]. Also irrigation water that is commonly used in raising these vegetables and sprinkling to keep them fresh may also be potential source of contamination because one might not know the source of the water used in washing of these vegetables [6]. Repeated spraying of crops with contaminated irrigation water has been found to increase the chances of crop contamination [19]. In the present investigations the highest mean total aerobic plate count was 5.6 $\log_{10}$ CFU/g for all the raw-mixed vegetable salad samples which is higher than the accepted limits reference value of < 5.0 $\log_{10}$ CFU/g as proposed by Mensah et al. [13]. In a similar related work it has been found that the bacterial contamination of salad samples in Kumasi, Ghana was 5.13 $\log_{10}$ CFU/g [11]. Another work on the prevalence of bacterial contamination of RTE foods sold around the University of Ghana campus, Accra, Ghana found mean bacterial contamination of salad as 4.86 $\log_{10}$ CFU/g [13]. Ameko et al. [5] also observed total mean viable counts of 5.17 $\log_{10}$ CFU/g in raw-mixed vegetable salads sold as an accompaniment to street vended cooked rice analyzed from Kokomlemle, a suburb of the Accra Metropolis. In Nairobi, Kenya mean viable bacterial counts of 4.7 $\log_{10}$ CFU/g of vegetable salad samples has been previously reported [17]. The differences in the total mean viable counts might be due to the number of samples used in the present investigations.

Among the isolated bacterial pathogens *E. coli*, *S. aureus*, *Klebsiella* sp. and *Bacillus* sp. were the predominant bacterial species found to be associated with all the samples of raw-mixed vegetable salad analyzed. The most predominant bacterium was *E. coli* (35%) as presented in Fig. 1. The presence of *E. coli* on the salads samples investigated may be as a result of faecal contamination because the bacterium is present in sewage, faeces, soil, water, and commonly come in contact with vegetables as result of the water used during the growing processes of the vegetables [6]. Therefore proper washing of the fresh vegetables used in preparing these raw-mixed vegetable salads are recommended before preparation and consumption.

S. aureus was found in 33% of all the raw-mixed vegetable salad samples investigated (Fig. 1). The high prevalence of *S. aureus* in the samples may be due to pre- and post-harvest handling. For *S. aureus* is an opportunistic pathogen found living in the nasopharynx and skin of up to 50% of normal people [20]. Therefore the high frequency of the bacteria found in this study may be attributed to the bacteria being present as a normal flora of humans and can contaminate the raw-mixed vegetable salads as a result of poor hygiene practices of farmers, retailers, and the food vendors. There are also reports that *S. aureus* can spread by direct contact during harvesting, processing, packing, and exposure of vegetables during the selling process [21].

The detection of *Klebsiella* sp. (17%) can be

probably as a result of ambient temperature for the bacteria is well known in the environment and can be transmitted from the soil, water, and vegetable's when consumed raw as in salads [11]. Also the presence of *Bacillus* sp. (15%) of the raw-mixed vegetables salads investigated may be related to environmental factors. Work on RTE vegetables in Sabon-Gari, Zaria, Nigeria also observed the presence of *Bacillus* sp. [22]. The isolation of *Bacillus* sp. may be due to environmental factors and the ability of the bacteria to form spores. The bacteria are able to survive in the environment, particularly in the air for longer time because of their spore forming properties [23, 24]. It will have been interesting to attempt to isolate *Salmonella* sp. and other deadly pathogens but the small sample size couldn't make it possible. Also another limitation of this study was the inability to analyze the water used for washing the raw vegetables before processing or preparation. It was found out through oral interviews with the selected street food vendors used in the study that washing vegetables thoroughly and increasing the contact time in sodium chloride (NaCl) solutions will help reduce the amount or load of bacterial pathogens present on the surface of these raw vegetables so that consumers can have safe vegetable salads to eat at all times as has also been previously proposed by other research workers [5, 6].

5. Conclusions

From the study it can be concluded that all the raw-mixed vegetable salads investigated from Korle-Gonno, Accra Metropolis were contaminated with one or more bacteria including *E. coli* which gives an indication of faecal contamination of the salad samples possibly from the water used in watering the vegetables during the growing processes in the farm or may be from the retailers during the displaying of the vegetables in the market places and the processes of preparation of these raw-mixed vegetables salads for human consumption by the street food vendors. It is therefore recommended that future studies should use large samples size to be able to isolate *Samonella* sp. and other dangerous pathogenic bacteria if present in these raw-mixed vegetable salad samples before they cause an outbreak.

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