

## Research Article

# Microbiological Quality of *Kachumbari*, a Raw Vegetable Salad Popularly Served Alongside Roast Meat in Kenya

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Raw salads are regularly implicated in food-borne disease outbreaks globally. Consumption of *kachumbari*, a raw vegetable salad, alongside roast meat is widespread in Kenya. The aim of this study was to evaluate the bacteriological quality of *kachumbari* samples ( $n = 39$ ) collected from a cross section of roasted meat eateries in Kenya. The United Kingdom's Health Protection Agency guidelines were used to infer safety of the salads due to lack of local criteria for microbiological safety of ready-to-eat fresh produce placed in the market. Based on *Escherichia coli* counts, 14 (35.9%) of the samples were of satisfactory microbial quality ( $<20$  CFU/g), 7 (17.9%) in the borderline ( $20$ – $10^2$  CFU/g), and 18 (46.2%) unsatisfactory ( $>10^2$  CFU/g). All samples examined for staphylococci had counts falling within the borderline range ( $20$ – $10^4$  CFU/g). Collectively, 3 (7.7%) of the sampled salads were classified as potentially harmful to health and/or unfit for human consumption due to the presumptive presence of 2 (5.1%) *Campylobacter* spp. and 1 (2.6%) *E. coli* O157. *Salmonella* was not detected in any of the samples. The presence of hygiene indicator microorganisms and pathogens demonstrates that *kachumbari* salads present a public health risk.

## 1. Introduction

A ready-to-eat raw vegetable salad locally referred to as *kachumbari* is widely prepared at home and in food outlets around Kenya. Its common fresh ingredients include tomatoes and red bulb onions [1]. The recipe could vary to include bell peppers, coriander, and hot green/red chilies. Occasionally, freshly squeezed lemon juice or vinegar is added to enhance the flavour. The vegetables are normally washed, chopped into small pieces, and mixed to yield the salad (Figure 1). Serving *kachumbari* as an accompaniment to *nyama choma* (roast meat) is a popular cultural practice in Kenya [2]. This combination is offered in a cross section of top-class to low-end roast meat eateries that are ubiquitous in urban centres and the countryside and is popular among locals and tourists visiting the country.

Disease occurrences associated with fresh fruits and vegetables are increasingly becoming a global public health concern [3, 4]. Several recorded food-borne disease outbreaks in the world have been attributed to minimally

processed fresh produce. For instance, in 2011, a Shiga toxin-producing *Escherichia coli* outbreak in Europe was linked to fenugreek sprouts [5]. In 2013, there was a multistate outbreak of Shiga toxin-producing *E. coli* O157:H7 linked to ready-to-eat salads in the USA [6]. Minimally processed vegetables and fruits are the leading causes of food-borne disease outbreaks in the USA [7]. In the USA and Europe, the Centres for Disease Control and Prevention and European Food Safety Authority, respectively, investigate, track, and preserve data on food-borne disease outbreaks [5, 6]. Kenya lacks such elaborate fact-finding mechanisms; hence, precise data relating to outbreaks associated with foods including fresh produce are difficult to obtain. However, perturbing trends in North America and Europe, which are regions with stringent food safety regulations, validate the concern. The most important pathogenic enteric bacteria contaminants of fresh cut produce include *Campylobacter* spp., *E. coli*, *Salmonella* spp., and *Shigella* spp. [8]. Other bacteria of concern are *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Aeromonas* spp., depending on product



FIGURE 1: *Kachumbari* salad served alongside roast meat.

handling conditions during preparation and storage. Spore-forming bacteria of *Bacillus* spp. and *Clostridium* spp. can also persist where fresh cut produce is held in modified atmosphere packs, which can favour germination of spores and growth of vegetative cells [8, 9].

Raw vegetable salads are microbiologically risky foods since they are eaten uncooked [8]. This necessitates enforcement of strict sanitary practices from field to fork because there is no single step that can ensure complete elimination of potentially pathogenic microorganisms from their ingredients as compared to cooked meals. In Kenya, consumer protection against unwholesome food is provided for by several legislations. They include the Food, Drugs and Chemical Substances Act, Chapter 254; the Public Health Act, Chapter 242; and the Meat Control Act, Chapter 316, of the Kenyan laws [10]. The regulations are enforced by the Department of Public Health under the Ministry of Public Health and Sanitation. Additionally, the Kenya Bureau of Standards (KEBS) formulates and enforces specific product standards for processed foods. There exist no guidelines governing the microbiological quality of prepared ready-to-eat raw foods served to consumers in Kenyan public eateries. A number of studies carried out in the country have revealed the presence of pathogenic enteric microorganisms in raw salads, in cooked foods, and on food-handling personnel and also documented poor food-handling practices in food outlets [11–16]. None of the encountered studies have specifically focused on *kachumbari* in roast meat eateries. Preparation of *kachumbari* in a meat-handling environment is potentially hazardous since raw meats are widely recognized as major sources of enteric pathogens [17, 18]. The meats on offer at *nyama choma* outlets include chevon (goat meat), beef, lamb, chicken, and pork.

In view of the aforementioned, the current study was aimed at evaluating the microbial quality of *kachumbari* from a cross section of roasted meat eateries by assaying for hygiene indicator microorganisms and food-borne pathogens occasionally associated with fresh cut vegetables. The ultimate target is sensitizing food handlers, consumers, and food safety enforcement authorities about the health risk posed by *kachumbari*.

## 2. Materials and Methods

**2.1. Sampling.** The study sites comprised 5 towns located in the Mount Kenya region within 80 km radius from the Meru town, which is located 224 km to the northeast of the Kenya's

capital, Nairobi (Figure 2). Akin to many other towns, roast meat eateries are popular.

Three roast meat eateries offering *kachumbari* as part of the menu were randomly selected in each town. Between 2014 and 2015, 3 samples per eatery were procured at 1-week interval except in Maua town to yield a total of 39 vegetable salad samples, that is, Meru ( $n = 9$ ), Chuka ( $n = 9$ ), Nanyuki ( $n = 9$ ), Isiolo ( $n = 9$ ), and Maua ( $n = 3$ ). The salads were put in clear polythene bags as customarily dispensed by the vendors for takeaway packs. The samples were transported to the laboratory in a cool box with frozen gel packs and analyzed on the same day. Additional information collected during field sampling included the animal source of meats served by individual outlets and inclusion of lemon juice or vinegar in the salads. Ingredients in the samples were also noted.

**2.2. Microbial Analysis.** Microbial determinations were carried out according to the International Organization for Standardization (ISO) methods, namely, beta-glucuronidase-positive *E. coli*: ISO 16649-2:2001, coagulase-positive staphylococci: ISO 6888-2:1999, *E. coli* O157: ISO 16654:2001, *Salmonella* spp.: ISO 6579:2002, and *Campylobacter* spp.: ISO 10272-1:2006 [19–23]. Briefly, 10 g of each vegetable salad sample was aseptically weighed into a sterile Pulsifier® plastic bag (Microgen Bioproducts Ltd., UK), and 90 ml of sterile Maximum Recovery Diluent (MRD) (HiMedia Laboratories, India) was added to make a 1 : 10 dilution. The bag was placed in the Pulsifier (Microgen Bioproducts Ltd., UK) and treated for 30 seconds [24]. Serial dilutions of the suspension were made in MRD and analyzed for beta-glucuronidase-positive *E. coli* and coagulase-positive staphylococci using the pour plate method by dispersing 1 ml of dilutions in Tryptone Bile X-Glucuronide Medium (HiMedia Laboratories, India) and Rabbit Plasma Fibrinogen Agar (HiMedia Laboratories, India), respectively, followed by incubation and enumeration as recommended in the standard protocols. For detection of *E. coli* O157, 25 g of each sample was aseptically weighed and placed into a sterile Pulsifier plastic bag, 225 mL of sterile Modified Soyabean Bile Broth supplemented with novobiocin at 20 mg/L (HiMedia Laboratories, India) was added, and an enrichment homogenate was made by treating with the Pulsifier for 30 seconds. The mixture was incubated and subcultured onto Tellurite Cefixime Sorbitol MacConkey Agar (HiMedia Laboratories, India) and HiCrome MacConkey Sorbitol Agar (HiMedia Laboratories, India). Twenty-five grams of test portions for detection of *Salmonella* were pre-enriched in Buffered Peptone Water (ISO) (HiMedia Laboratories, India) supplemented with potassium sulphite 0.5% w/v (final concentration) for all samples containing onions to reduce natural bactericidal properties. They were then enriched in Rappaport Vassiladis Soyabean Meal Broth and Muller-Kauffmann Tetrathionate-Novobiocin Broth (HiMedia Laboratories, India) and subcultured onto Xylose-Lysine-Deoxycholate Agar and *Salmonella* Differential HiVeg Agar (HiMedia Laboratories, India). Finally, a 25 g of test portion for *Campylobacter* was enriched in Blood Free *Campylobacter* Broth (HiMedia Laboratories, India) and

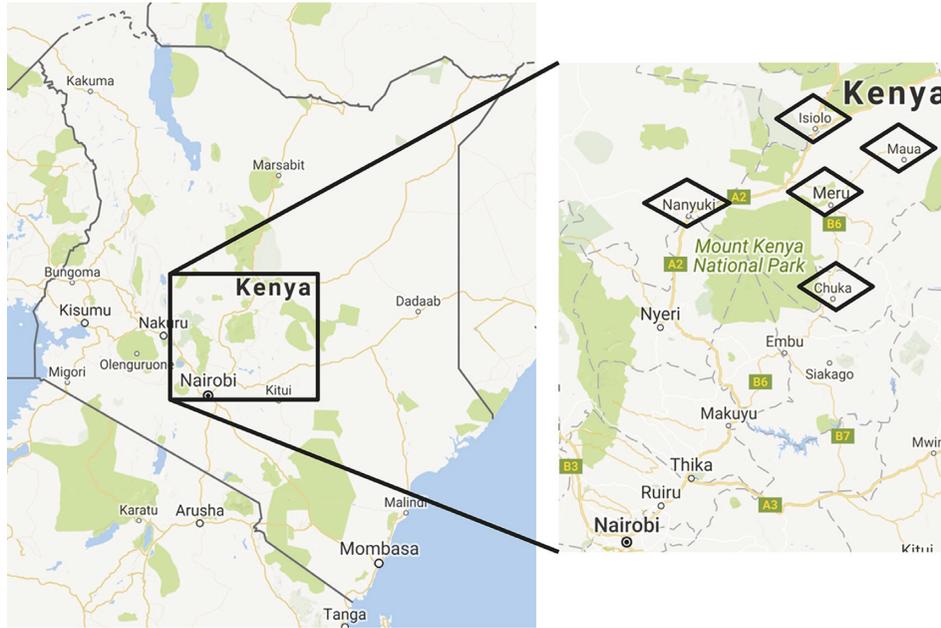


FIGURE 2: Map showing towns where raw vegetable salad samples were sourced.

subcultured onto Blood Free *Campylobacter* Selectivity Agar and Karmali *Campylobacter* Agar (HiMedia Laboratories, India). Microaerophilic condition in a 3.5-litre incubation jar was generated using CampyGen CN0035A sachets (Oxoid Ltd., UK).

Presumptive confirmation of suspect colonies of *E. coli* O157, *Salmonella*, and *Campylobacter* was done using Microgen *E. coli* 0157-CEM44 Latex Kit, Microgen *Salmonella*-CEM42 Latex Kit, and Microgen *Campylobacter*-CEM46 Latex Kit (Microgen Bioproducts Ltd., UK), respectively, as per the manufacturer's instructions.

**2.3. pH Measurement.** The pH was taken in the salad exudate after aseptically drawing samples for microbial analysis and allowing them to stabilize at room temperature. The pH meter (Model pH 211, Hanna Instruments, Italy) equipped with an HI 1131B glass body combination refillable pH electrode and an HI 7669/2W temperature probe for automatic temperature compensation was used according to the manufacturer's instructions. Briefly, a two-point calibration with pH 4.01 and pH 7.01 buffer solutions was performed, the salad exudate was decanted into a plastic 50 ml beaker, pH electrode and temperature probe tips submerged approximately 4 cm into the liquid and were allowed to stabilize, and the reading was taken. Deionized water was used to rinse the probes between the measurements.

**2.4. Data Analysis.** Microsoft Excel 2010 with data analysis add-ins was used to compute, summarize descriptive statistics, and generate graphical illustrations. Correlation analysis was done to establish the strength of relationship between pH of the samples and *E. coli* counts. Categorized microbial data were compared against the UK Health Protection Agency Guidelines for Assessing the Microbiological

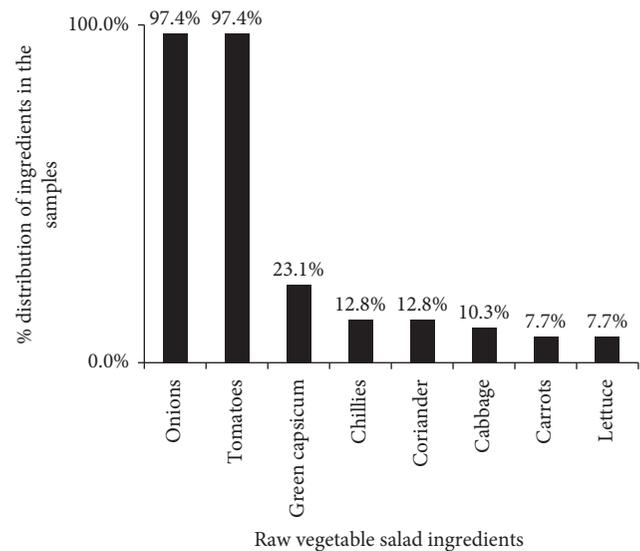


FIGURE 3: Vegetables used in *kachumbari* raw salads served by roast meat eateries ( $n = 39$ ).

Safety of Ready-to-Eat Foods [25], and deductions on the microbial quality and safety of the salads were tabulated.

### 3. Results

Roasted goat meat was served by all sampled eateries (15, 100%), followed by beef (14, 93.3%) and chicken (10, 66.7%). Tomatoes and onions were virtually present in all the vegetable salads. Other ingredients used to a lesser extent included green capsicum, chillies, coriander leaves, cabbage, lettuce, and carrots (Figure 3). It was also noted that ingredients used by the specific eateries varied from one sampling interval to the next. One eatery was recorded to

TABLE 1: Level of contamination by hygiene indicator microorganisms in raw vegetables salads served by roast meat eateries.

Hygiene indicator microorganisms	CFU/g (interpretation)	% of samples ( $n = 39$ )	Samples' pH interval
Beta-glucuronidase-positive <i>Escherichia coli</i>	<20 (satisfactory)	14 (35.9%)	3.95–4.53
	20 – $\leq 10^2$ (borderline)	7 (17.9%)	3.89–4.81
	$> 10^2$ (unsatisfactory)	18 (46.2%)	3.45–4.62
<i>Staphylococcus aureus</i> and other coagulase-positive staphylococci	<20 (satisfactory)	0%	
	20 – $\leq 10^4$ (borderline)	39 (100%)	
	$> 10^4$ (unsatisfactory)	0%	

Satisfactory: suitable for human consumption; borderline: moderate microbiological risk; unsatisfactory: unsuitable for human consumption as per the Health Protection Agency Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Food [25].

TABLE 2: Presumptive positive incidence of food-borne pathogens in the raw vegetables salad samples from roast meat eateries ( $n = 39$ ).

Pathogen	Presence in 25 g (unsatisfactory/potentially injurious to health and/or unfit for human consumption) [25]
<i>E. coli</i> O157	1 (2.6%)
<i>Salmonella</i> spp.	ND
<i>Campylobacter</i> spp.	2 (5.1%)

always incorporate lemon juice in the raw salad, while another occasionally put vinegar.

Levels of contamination with hygiene indicators are presented in Table 1. Categorization into satisfactory, borderline, and unsatisfactory classes was done according to the UK Health Protection Agency Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods 2009 as indicated in the table. In regard to *E. coli* contamination, 25 (64.1%) samples fell in the unsatisfactory and borderline categories. All samples evaluated for coagulase-positive staphylococci had counts falling within the borderline range. The pH of the salads ranged between 3.45 and 4.81, with 10 (25.6%) of the samples having a  $\text{pH} \geq 4.5$ . All satisfactory samples fell below the pH 4.53. However, borderline and unsatisfactory salads were encountered across the entire pH interval (Table 1). There was a weak positive correlation between pH and *E. coli* counts ( $r = +0.21$ ), within the recorded pH range of the salad. High *E. coli* counts were scattered around the upper pH.

Results of the presumptive presence of pathogenic food-borne enteric microorganisms are presented in Table 2. Collectively, 3 (7.7%) of the sampled salads were classified as potentially harmful to health and/or unfit for human consumption due to the presumptive presence of the thermo-tolerant *Campylobacter* spp. and *E. coli* O157. There was no detection overlap between the samples that presumptively tested positive for either of the pathogens. *Salmonella* spp. was not detected in any of the *kachumbari*.

#### 4. Discussion

With respect to available published information, this is the first report in Kenya specifically evaluating the microbial quality of *kachumbari* using hygiene indicator microorganisms and common enteric pathogenic microorganisms that have been associated with disease outbreaks arising from consumption of fresh produce. Overall, our results

point to poor sanitary practices as indicated by high levels of *E. coli*. Contamination and carryover to the final salads could have arisen from poor field sanitation, unhygienic practices at harvesting, transport, and marketing stages, absence of surface decontamination, and cross-contamination at eateries. The high count of coagulase-positive *Staphylococcus* corroborates the poor level of personal hygiene. The presumptive presence of enteric pathogens *E. coli* O157 and *Campylobacter* spp. signifies the potential danger that could arise from consumption of *kachumbari*.

Other studies in Kenya focusing on the microbial quality of food have also observed the presence of hygiene indicators of faecal contamination, particularly in raw vegetable salads. During investigations carried out in Githurai and Gikomba markets within Nairobi County to assess the quality of street-vended foods, where *kachumbari* (mixture of raw tomatoes and onions) was evaluated alongside other foods, 14 (14.3%) of *kachumbari* samples were confirmed to be positive for faecal coliforms [11]. Notable similarity of the aforementioned study and our investigation is that *Salmonella* spp. was not detected. Subsequent analysis of data emanating from the abovementioned study revealed significant negative association of food contamination with good hygienic practices and conducive environmental practices including washing of hands before handling food, use of an apron, availability of toilet facility with water, the presence of fresh running water in the food preparation area, and pest control [26]. A microbial quality evaluation of food served by street vendors in the industrial area Nairobi by Githaiga [12] reported high levels of total coliforms (63.6%), *Enterococci* (18.2%), and *S. aureus* (54.5%) in raw and partially cooked vegetable salads ( $n = 11$ ), but *Salmonella* was not detected. All vegetables samples positive for *S. aureus* exceeded the KEBS guidelines on cooked foods by harbouring  $> 10^4$  CFU/g [12]. However, the study did not alienate the vegetables subjected to heat treatment from raw ones. Additionally, poor personal and environmental hygiene was reported in the sampled outlets. In neighbouring Uganda, where *kachumbari* (fresh salad dish of tomatoes and onions) was served alongside cooked meat by 90% of pork joints in Kampala, 5.2% of tomato samples ( $n = 77$ ) were contaminated by *Salmonella enteritidis* and 2.6% by *Salmonella gallinarum*, while onions ( $n = 77$ ) had the presence of *S. Gallinarum* at 2.6% [27].

The pH range, that is, 3.45–4.81, observed during our studies did not have a strong influence on *E. coli* counts to a large extent in the raw salads as demonstrated by a small

correlation coefficient ( $r = +0.21$ ). An assessment of raw fruit juice sold in Dares Salaam city in Tanzania, another member of the East African Community, demonstrated distinctively lower counts of *E. coli* and total plate counts in juices with a pH of  $\leq 3.1$  when compared to those whose pH  $> 3.1$  [28]. Probably, acidification of the salad resulting in a lower pH would have led to lower counts.

Globally and more specifically in developing countries, microbial quality evaluation of raw vegetable salads and their intact ingredients singled them out as high-risk foods. A study on the prevalence of microbial contamination on the surface of vegetables used to prepare raw salads, namely, cucumber, tomato, carrot, coriander, cabbage, beetroot, radish, and spinach (total  $n = 480$ ), in Dhanbad city, India, revealed 16.7% *E. coli*, 1.3% *E. coli* O157:H7, and 4.0% *Salmonella* spp. contamination [29]. Assessment of salads served alongside street foods in Hyderabad, India, showed a high incidence of food-borne pathogens. In carrots ( $n = 53$ ), 73.6%, 58.5%, and 67.9% of the samples were contaminated with *Staphylococcus*, *Salmonella*, and *Yersinia* spp., respectively, while the corresponding incidence was 56.4%, 45.5%, and 23.6% in onions ( $n = 110$ ). Associated evaluation of sanitary practices revealed poor adherence to personal and environmental hygiene; 98% of the vendors did not wash the vegetables before preparation and serving, while about 56.6% of the vendors did not peel them [30]. In another recent study in Abidjan, Ivory Coast, raw mixed vegetable salad samples ( $n = 306$ ), whose ingredients included tomatoes, onions, cucumbers, and lettuce, were seasoned or unseasoned; 100%, 77.8%, and 2.6% were contaminated with Enterobacteriaceae, *E. coli*, and *Salmonella*, respectively. Moreover, lack of training on hygiene practices, the absence of decontamination, and cross-contamination of vegetables via contact with fresh chicken or fish were identified as the most important risk factors [31].

In the current study, we presumptively detected *Campylobacter* spp. in two samples obtained from the same eatery on two consecutive sampling intervals. None of the documented studies about the microbial quality of raw vegetables in Kenya had examined the occurrence of *Campylobacter* spp. Our quest to include it in the study was informed by isolation from chicken and beef samples from retail outlets in Nairobi [18]. *Campylobacter* spp. has been highlighted as a leading aetiology of bacterial enteritis [8, 9]. Additionally, *Campylobacter* spp. has been found in raw vegetables. Chai et al. [32] encountered contamination of raw vegetables used in *ulam*, a popular Malaysian raw salad dish, from three locations which included a wet market and two supermarkets, with an average prevalence of *Campylobacter* spp. ranging from 29.4% to 67.7%. The high levels were postulated to stem from cross-contamination with poultry products during holding at packing stages.

Increasing fruit and vegetable consumption is a priority of the World Health Organization due to its role in ameliorating incidences of micronutrient deficiencies and chronic diseases [33]. Unlike most of the fruits which can be washed and peeled, vegetables destined for raw salads including tomatoes and onions (main ingredients in

*kachumbari*) are consumed whole upon some form of size reduction. Rendering them safe while preventing the loss of nutrients is vital. Suitable methods of preparing vegetables before consumption, for example, steaming, warrant consideration. Other techniques of surface decontamination such as use of hyperchlorinated water, chlorine dioxide, and peracetic acid normally used at the industrial scale by fresh produce companies may be too complex for food service personnel to implement. Additionally, they are usually preceded by good agricultural practices and followed by elaborate handling after sanitization to prevent postprocess contamination.

## 5. Conclusions

Our study, those of other authors, and outbreaks associated with minimally processed fresh produce warrant consideration of *kachumbari* for frequent microbial quality monitoring to avert food-borne illness. It affirms that hygienic practices leading to presentation of raw vegetable salads in Kenyan roast meat eateries are compromised to a large extent. This calls for intense training of food service personnel on sanitary practices. However, food safety of fresh produce is a concerted effort from field to fork, and it is rather difficult for the vendors to achieve this goal alone since they have no control over field production, transport, and marketing channels. Appropriate decontamination methods that are easy and safe to use, without adversely altering the organoleptic quality of the salads, should be applied to reduce the risk. Public health enforcement authorities ought to consider banning the sale of raw salads in establishments where microbial quality cannot be guaranteed to safeguard the consumer. Additionally, the information herein serves to caution consumers so that they can avoid being victims of food-borne infections while indulging in this popular accompaniment of roast meat. This study evaluated a limited number of salad samples as offered to the consumers in selected roast meat eateries to give a snapshot of microbial quality. The authors did not have control over numerous variables, which could have influenced the quality of the salads including the origin of raw materials, transportation, and sanitary practices during preparation. Therefore, it should not be broadly concluded that the microbial quality of *kachumbari* was predominantly a result of cross-contamination from meats.

## Data Availability

All data arising from this study are contained within the manuscript.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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