Original Research Article

Assessment of Bacteriological Quality of Fresh Vegetable Salads and Associated Risk Factors in Food Service Establishments in Mwanza City, Tanzania

Abstract

This study examined the bacteriological quality of fresh vegetable salads and associated risk factors in restaurants and street food vendors in Nyamagana and Ilemela municipalities, Mwanza City, Tanzania. Thirty samples of vegetable salads (i.e., *Kachumbari*), along with 30 swabs from hands and chopping boards, were collected and analyzed. High contamination levels exceeding acceptable limits were observed with total bacterial counts, *S. aureus*, and *E. coli* ranging from 3.6 to 6.7 log CFU/g. All *Kachumbari* samples (100%) were unsatisfactory, and 17 (56.7%) had unsatisfactory *E. coli* levels. Moreover, 22 samples (73.3%) had unsatisfactory *S. aureus* levels, and 10 samples (33.3%) showed unsatisfactory *Salmonella* spp. contamination. Further, *Kachumbari* from street food vending sites had a significantly ($p < 0.05$) higher TBC mean value (6.5 ± 0.3 log CFU/g) than the one from restaurants (5.2 ± 0.6 log CFU/g). On the other hand, chopping boards and hands had high total counts ranging from 3.5 to 4.7 log $CFU.cm^{-2}$. Also, the type of chopping board was significantly related to the *S. aureus* contamination levels in the *Kachumbari* salads ($p < 0.05$). The presence of both hygiene indicator microorganisms and pathogens indicates a potential public health risk associated with the consumption of *Kachumbari*. Urgent intervention measures are required to enhance handling practices, personal hygiene, and overall safety throughout the food value chain, thus ensuring the quality and safety of vegetable salads in food service establishments.

Keywords: Vegetable salads, *Kachumbari*, bacteriological quality, safety.

1. Introduction

The consumption of fresh vegetable salads as well as mild heat-treated products has increased across the world, including in developing countries (Mir *et al*., 2018), due to their relatively low cost, high nutritious values, and easy preparation. Vegetable salad may be prepared in several ways, either taken as raw without any kill-step or mild heat treated and carry different names depending on the location (Mbae *et al*., 2018; Namukwambi*et al*., 2022; Rakha *et al*., 2022). In Tanzania, vegetable salad is commonly known as *Kachumbari*. Globally, over 2.5 billion people eat ready-to-eat (RTE) street foods such as vegetable salads every day (Ukenna& Ayodele, 2019).

Kachumbari is widely prepared and consumed in various food service establishments (FSE) in Tanzania. This salad is prepared from different types of vegetables, like bell peppers, cabbages, cucumbers, carrots, tomatoes and onions (Mbae *et al*., 2018). Although salads may be mildly heat-treated, they are normally taken when they are cold. They are regarded as among the ready-to-eat (RTE) cold foods (Kapeleka*et al*., 2020). Cooling can be done in a refrigerator, but in most FSE, such facilities are lacking; hence, cooling is done under ambient conditions. For public safety, salads should be prepared and handled under hygienic conditions.

Vegetable salads are rich sources of micronutrients such as vitamins (like vitamins A, B, C and K) and minerals (including iron, calcium, zinc, magnesium and phosphorus) with several health and nutritional benefits to consumers (Charles-Aworh, 2015; Pereira *et al*., 2022). Consumers prefer taking vegetable salads along with their main dish, particularly, fried potatoes, plantain, rice or ugali. However, if not well-handled vegetable salads are frequently associated with microbiological contamination (Mir *et al*., 2018; Ndunguru&Ndossi, 2020; Schuh *et al*., 2020). Consumption of vegetable salads has been implicated with foodborne disease (FBD) outbreaks (Little & Gillespie, 2008; Lynch *et al*., 2009). The outbreaks vary in magnitude from a small number of individuals affected to several thousands (Ebel *et al*., 2019). Previous studies have identified the presence of pathogens like *Escherichia coli* and *Salmonella* spp. in fruit juices and vegetable salads (Kechero*et al*., 2019; Mbae *et al*., 2018; Ndunguru&Ndossi, 2020).

Moreover, vegetables present multiple avenues for attachment and invasion of pathogenic microorganisms at different stages along the food chain, including cultivation, harvesting, processing and marketing (Osafo *et al*., 2022). The utilization of contaminated water during vegetable washing constitutes an additional source of contamination (Aworh, 2020). Other potential sources of contamination are soil, unclean processing equipment, feces (of human or animal origin), and inadequate product handling (Kechero*et al*., 2019).

Furthermore, Mwanza City has numerous street food vendors and restaurants that serve vegetable salads. Although the local food markets in this city are not well organized, they are the major sources of vegetables for *Kachumbari* preparation in the city's food service establishments. Therefore, from the sanitary practices, it is rational to presume the presence of bacterial contamination in vegetables purchased from these markets. Similarly, previous studies in developing countries like Tanzania, have observed inadequate sanitary conditions, unhygienic food handling practices during preparation, and insufficient storage facilities for food in food establishments including, street food vending sites, restaurants, and hotels (Namukwambi*et al*., 2022; Ndunguru&Ndossi, 2020; Rakha *et al*., 2022). Moreover, vegetable salads, like other RTE foods, are commonly consumed without heat treatment or other processing effective to eliminate or significantly reduce harmful microorganisms (Othman, 2014). These factors collectively intensify the risk of contracting food-borne diseases among consumers of fresh vegetables and salads, which raises public health concerns.

Although several studies across the world have assessed the microbiological quality of fresh fruits and vegetable salads (Giwa *et al*., 2021; Kechero*et al*., 2019), very few studies have been conducted in Tanzania (Kayombo & Mayo, 2018; Ndunguru&Ndossi, 2020). Consequently, in Tanzania, there is limited information on the bacteriological quality of the cold RTE foods including *Kachumbari*. Considering the increase in the consumption of vegetable salads in Tanzania and its neighboring countries (Mbae *et al.,* 2018), the information on bacteriological quality of these salads may be useful in enhancing proper food hygiene practices and management for safeguarding public health and strengthening consumer confidence; which could in turn reduce FBD treatment costs and promote sales in FSE like restaurants and street vendors. Therefore, the current study aimed at assessing the bacteriological quality of *Kachumbari* (fresh vegetable salads) and associated risk factors in the food service establishments that prepare and serve this salad in Mwanza City, Tanzania.

2. Materials and methods

2.1. Study area

This study was carried out in Nyamagana and Ilemela Municipalities in Mwanza City. This city is situated in the northern part of Tanzania. It is positioned between $1^{o}30'$ and 3^{o} south of the Equator and longitudinally located between $31^{o}45'$ and $34^{o}10'$ east of Greenwich. The population of Mwanza city is around 1,310,754 inhabitants (World Population Review, 2022).

2.2. Study design

The study utilized a descriptive cross-sectional research design to assess the risk factors of contamination and evaluate the bacteriological quality of *Kachumbari* in FSE. The survey was conducted from March to April 2023.

2.3. Assessment of microbiological contamination

2.3.1. Selection of bacteriological parameters

The four bacteriological parameters comprising indicators of fecal hygiene (*E. coli*), personnel hygiene (*S. aureus*), food safety (*Salmonella* spp.), and general process hygiene (total bacterial counts, TBC) were selected and analyzed (Lambrechts *et al*., 2014; Mbae *et al*., 2018). Total bacterial counts were selected to evaluate the general quality of raw materials, the efficiency of handling methods and hygienic conditions during processing, the hygienic state of the tools and utensils, and the time/temperature profile during storage. *E. coli* naturally grows only in the intestines of human and vertebrate animals (Berthe *et al*., 2013). It is the species of coliform that is considered as the indicator of fecal contamination and the potential presence of human pathogens (Luna-Guevara *et al*., 2019). Therefore, the presence of *E. coli* in food suggests that human pathogens may have accessed the food. The natural habitats of *S. aureus* are human skin, hair and superficial mucous membranes (nose). Thus, the presence of huge populations of *S. aureus* may imply enterotoxin production or a problem with sanitation or manufacturing procedures (Abebe *et al*., 2020). *Salmonella* spp. is the most common pathogenic bacteria in both humans and animals, and they are abundantly present in nature. *Salmonella* spp. has been observed to be the important cause of food-borne diseases that pose a severe public health problem across the world (Asfaw-Geresu*et al*., 2021). Therefore, the detection of *Salmonella* spp. in food implies that the food is unsafe and unsuitable for human consumption.

2.3.2. Sample collection

A total of 90 samples were collected for microbiological analysis from 30 FSE. The number of FSE involved were restricted to 30 due to budgetary and time constraints but also the study aimed to establish the current status. A multistage technique and convenience sampling were used to select the 30 FSE. First, two wards with a high population of street food vendors were randomly selected from each municipality from a list of wards with a high population of street vendors. Then, convenience sampling was employed to select the thirty FSE, proportionally between street food vending sites (SFV) and restaurants, from the four randomly chosen wards; in which 20 SFV and 10 restaurants were drawn for the study. Thereafter, three samples (*Kachumbari*, hand and chopping board swabs) were obtained from each FSE. Overall, 30 *Kachumbari* samples, 30 food handler’s hand swabs and 30 chopping board (CB) swabs were collected for microbiological analysis. The collected samples were aseptically transferred into sterile zip-lock bags to minimize the risk of cross-contamination. The bags were kept in a cool box with ice packs to keep the temperature between 4 and 7℃. Then samples were transported to the Tanzania Veterinary Laboratory Agency (TVLA) microbiology laboratory and analyzed within 2 hours after sampling using methods adapted from the US-FDA Bacteriological Analytical Manual (Bennet *et al.,* 2013; Bird *et al.,* 2018; Hammack *et al.,* 2004). Analytical reagents (diluents) and medium were prepared in accordance with the relevant test method specifications and the manufacturer's instructions.

2.3.3. Analysis of samples

Laboratory analysis samples were prepared aseptically. From each salad 25 g was weighed on analytical balance (Adventurer TM PRO OHAUS) made in China; and transferred into sterile polythene zip-lock bags. Thereafter, *Kachumbari* in each sterile bag was mixed well with 225 mL of buffered peptone water (BPW). The mixture was then homogenized thoroughly by using a stomacher (Seward STOMACHER R 3500 Lab System). Also, the collected swab samples of food handlers' hands and chopping boards were first placed into 5 mL of buffered peptone water for 2 minutes. Then, 10-fold serial dilutions of samples from 10^{-1} to 10^{-10} in the sterile buffer were aseptically performed. After which 0.1 mL from each dilution was inoculated onto Plate Count Agar (PCA, for TBC), MacConkey Agar (MCA for *E. coli*), Baird Parker Agar and Mannitol Salt Agar (BPA and MSA, for *S. aureus*) and Xylose Lysine Deoxycholate (XLD) Agar and Salmonella Shigella (SS) Agar (XLD and SS for *Salmonella* spp.) and incubated at 30 ± 1°C for 24 ± 2 h for TBC, 37 ± 1°C for 24 + 2 h for E. coli, 37 ± 1°C for 48 ± 2 h for *S. aureus* and 37 ± 1°C for 24 ± 2 h for *Salmonella* spp. Then appropriate dilutions were enumerated for presence of TBC, *E. coli* and *S. aureus*, and the presence of *Salmonella* spp. was visually confirmed. After which total number of microbes per gram of salad and per cm^2 of contact surface (hands and chopping boards) were calculated accordingly.

2.3.4. Interpretation of microbiological results

Tanzanian standards (TZS) and East African Standards (EAS) were employed to interpret the results. The criteria used to interpret microbiological results are presented in Table 1.

Table 1: Microbiological criteria of vegetables and vegetable salads

S/N	Parameter	Criteria (Maximum Limit)	Source
1	TBC	10^2 CFU/g	EAS 1109:2022
2	*E. coli*	Absent	TZS 730/ISO 16649-1
3	*S. aureus*	10^2 CFU/g	TZS 125/ISO 6888-1
4.	*Salmonella spp*	Absent/25g	TZS 122/ISO 6579-1

2.4. Assessment of pH

The pH was measured in the salad exudate after aseptically taking samples for microbiological analysis and allowing them to stabilize at room temperature. A pH and Conductivity Meter (Benchtop pH Meter, Bioevopeak) was used after being calibrated using standard buffer solutions of pH 7.0 and pH 4.0. The 5 mL of salad exudate was placed into a test tube, the pH tip was submerged approximately 3 cm into the liquid and was permitted to stabilize, then the reading was taken. Distilled water was used to rinse the probe between the measurements.

2.5. Statistical analysis

The data collected were analyzed using SPSS Version 25. Descriptive statistics were employed to compute means, standard deviations, median and range. Whereas, an independent t-test was performed to determine the difference in the mean bacterial counts of TBC, *S. aureus* and *E. coli* between wooden and plastic chopping boards, as well as, between *Kachumbari* from restaurants and street food vendors. On the other hand, a one-way Analysis of Variance was carried out to assess the differences among mean values of TBC, *S. aureus* and *E. coli* in *Kachumbari.* Correlation analysis was conducted to determine the relationship between microbiological counts (TBC, *E. coli* and *S. aureus*) of *Kachumbari* and that of hands. A chi-square test was performed to establish the relationship between independent variables (type of

FSE and CB) and *Kachumbari* contamination with *E. coli* and *S. aureus*. The significance level, $P < 0.05$ was used.

2.6. Ethical considerations

This study was carried out in conformity with the guidelines of the Helsinki Declaration on ethical principles governing research on human populations (World Medical Association, 2013). The objectives of the study were explained to the study participants. They provided consent, and taking part in the study was entirely optional. Every participant was free to end their involvement at any moment. To ensure that the study participants remained anonymous, we did not record their identities on the data gathering tools. Participants were guaranteed confidentiality. Formal approval to conduct the study was received in writing from the authors' university.

3. Results

3.1. Raw materials used to prepare the salad

In this study, raw materials for *Kachumbari* preparation in food service establishments in Mwanza city were investigated. The findings showed that all FSE prepared their *Kachumbari* salads from onions, tomatoes, cucumbers, bell peppers, and carrots; without adding lemon or vinegar (Figure 1). Also, the FSE bought the raw materials (vegetables) from the local food markets within Mwanza city (Figure 2).

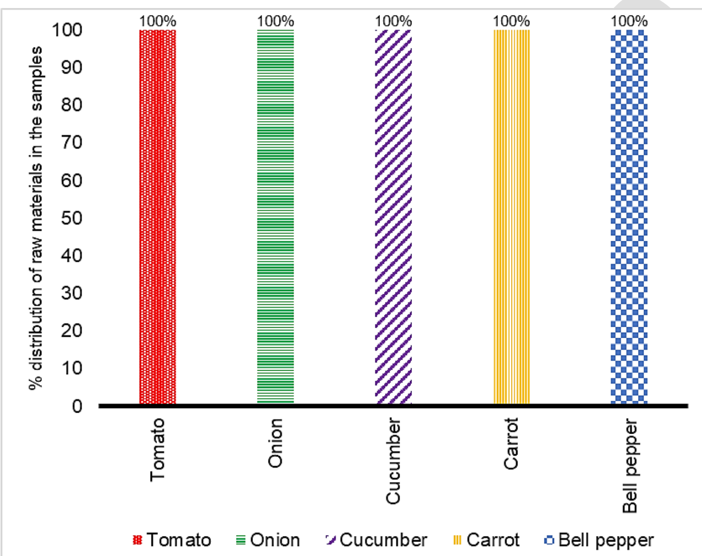

Figure 1: Raw materials for *Kachumbari* preparation

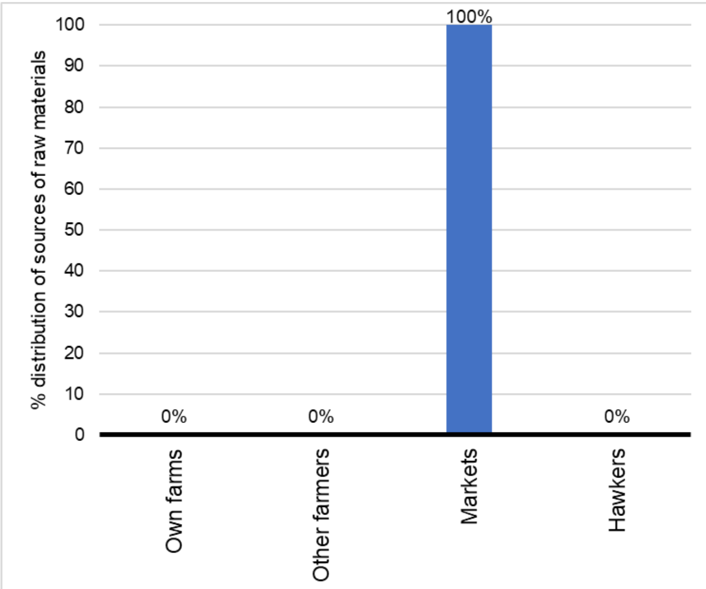

Figure 2: Sources of raw materials for *Kachumbari*

3.2. pH of *Kachumbari*

pH values of *Kachumbari* are presented in Table 2. The pH of the salad samples ranged from 3.0 to 5.7. The majority (24, 80%) of the samples had slightly low pH values, which ranged from 3.0 to 4.5 and only six (20%) samples had pH values between 4.8 and 5.7.

Table 2. pH of *Kachumbari*

pH range	Number of samples (%)
0 – 2.9	0 (0)
3 – 4.5	24 (80)
4.8 – 5.7	6 (20)

3.3. Microbiological quality of *Kachumbari*

The microbiological quality was assessed in all (30) *Kachumbari* samples, 10 from restaurants and 20 SFVs. High total bacterial counts (ranging from 4.8 to 6.7 log CFU/g, Table 3) were recovered in vegetable salads. The TBC counts in all salads exceeded the set limit in vegetables and vegetable salads (2 log CFU/g, Figure 3). However, *S. aureus* counts were between <1 and 6.7 log CFU/g (Table 3). Most (22) salads had excessive *S. aureus* contamination levels above the established legal limit (2 log CFU/g) in vegetable and vegetable salads, of which 6 were from restaurants and 16 were from SFVs (Figure 3). On the other hand, *Kachumbari* had *E. coli* contamination levels ranging from <1 to 6.7 to log CFU/g (Table 3). Of these, 17 samples had high counts (4.7 – 6.7 CFU/g) beyond the set standard (i.e., *E. coli* absent); 7 were from restaurants and 10 from SFVs (Figure 4). *Salmonella* spp. Contamination was detected in vegetable salads from 2 restaurants and 8 SFVs (Figure 4).

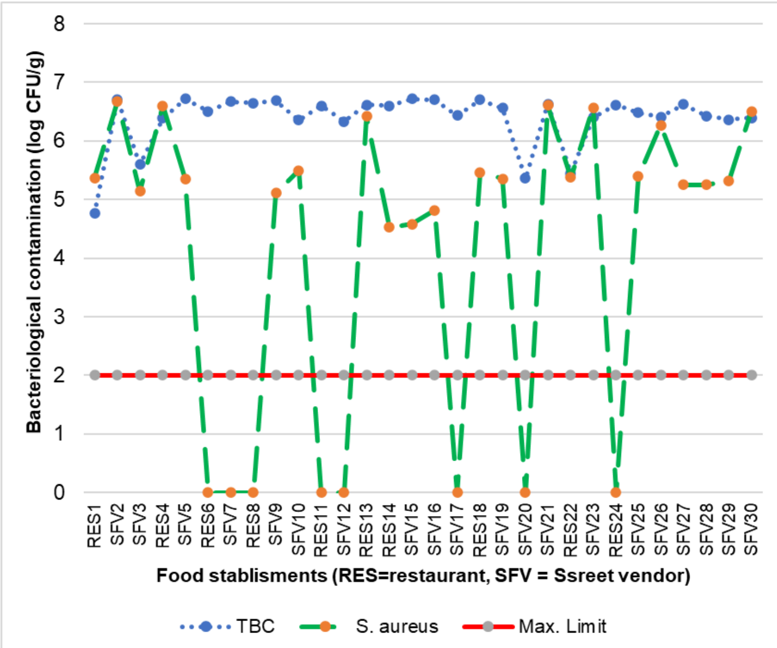

Figure 3: TBC and *S. aureus* contamination levels of *Kachumbari*

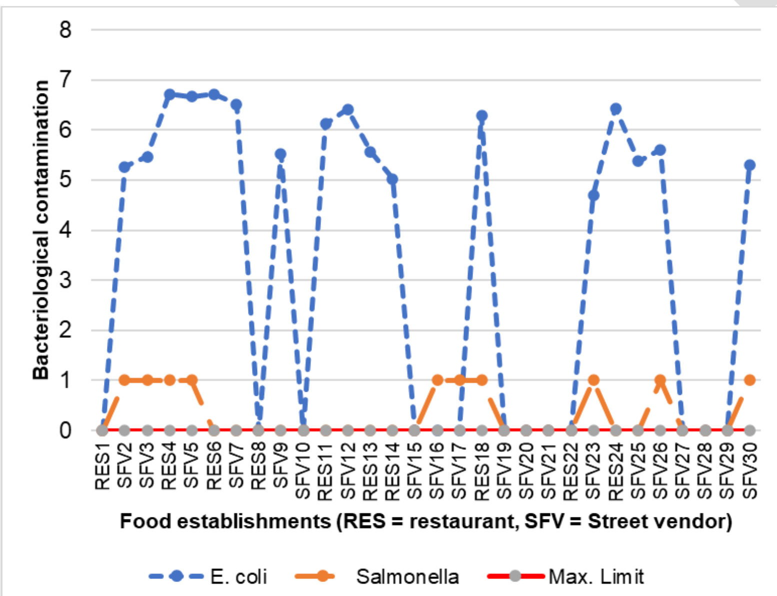

Figure 4: *E. coli* and *Salmonella* contamination levels of *Kachumbari*

An independent t-test was conducted to assess the differences between microbiological contamination levels in vegetable salads from street food vending sites and those from restaurants. The findings revealed that the mean value for TBC (6.5 ± 0.3log CFU/g) in *Kachumbari* from SFV was statistically significantly ($p < 0.05$, Table 3) higher than that of salads from restaurants (log CFU/g 5.2 ± 0.6). However, no significant differences ($p > 0.05$, Table 3) were observed in microbiological contamination with *S. aureus* and *E. coli* betweenvegetable salads from restaurants and street food vendors (Table 3). On the other hand, a one-way analysis of variance was performed to determine the differences among TBC, *S. aureus* and *E. coli* in *Kachumbari* salads. The ANOVA results showed that TBC contamination levels (6.4 ± 0.5 log CFU/g) were significantly ($p < 0.05$, Table 4) higher than *S. aureus* (4.5 ± 1.9 log CFU/g)and *E. coli* (4.0 ± 2.2 log CFU/g). Nonetheless, there were no statistically significant ($p > 0.05$, Table 4) differences between the microbiological mean counts of *S. aureus* and *E. coli.*

Table 3: Differences in microbiological contamination levels of *Kachumbari* salads between restaurants and street food vending sites

Microorganisms	**Mean ± SD (log CFU/g)**		**Range (log CFU/g)**
	FSV (N=20)	**RES (N=10)**	
Total bacterial counts	6.5 ± 0.3^{a}	5.2 ± 0.6^{b}	4.8 - 6.7
S. aureus	4.4 ± 2.4^{a}	3.4 ± 2.9^{a}	<1 - 6.7
E. coli	2.8 ± 2.9^{a}	4.3 ± 2.9^{a}	<1- 6.7

RES = restaurant, SFV = street food vending site. Means with different superscripts in the same row are significantly different at $P < 0.05$.

Table 4: Prevalence of TBC, *S. aureus,* and *E. coli* in *Kachumbari*

Microorganism	**N**	**Mean ± SD (log CFU/g)**
Total bacterial counts	30	6.4 ± 0.5^{a}
S. aureus	30	4.5 ± 1.9^{b}
E. coli	30	4.0 ± 2.2^{b}

Means with different superscripts in the same column are significantly different at $P < 0.05$.

3.4. Microbiological quality of salad chopping boards

In our study, 15 FSEs used wooden chopping boards (WCB) to prepare *Kachumbari*, while the other 15 opted for plastic chopping boards (PCB). High contamination levels of TBC ranging from 3.5 to 4.7 and 4.1 to 4.7 log CFU.cm^{-2} were recovered on WCB and PCB, respectively (Table 5). *S. aureus* contamination levels on WCB and PCB ranged from <1 to 5.6 and <1 to 4.8 log CFU.cm^{-2}, respectively (Table 5). On the other hand, the mean value (3.4 log CFU.cm^{-2}) of *S. aureus* recovered on WCB was significantly ($p < 0.05$, Table 6) higher than on PCB (1.4 log CFU.cm^{-2}). The majority (26) of salad chopping boards had *E. coli* counts less than 1 log CFU.cm^{-2}, and the remaining four had counts ranging from 3.1 to 4.7 log CFU.cm^{-2} (Table 5). *Salmonella* contamination was recorded only on one salad chopping board (Table 5).

Table 5: Microbiological quality of salad chopping boards

FSE	Board type	Counts of microorganisms (Log cfu.cm^{-2})			Presence [+/-]
		TBC	***S. aureus***	***E. coli***	***Salmonella* spp.**
RES11	PCB	4.7	2.7	*	-
RES13	PCB	4.4	*	*	-
RES18	PCB	4.7	*	*	-
RES24	PCB	4.1	*	*	-
RES8	PCB	4.7	*	*	-
SFV10	PCB	4.5	3.1	*	-
SFV12	PCB	4.7	3.4	*	-
SFV19	PCB	4.7	1.8	*	-
SFV2	PCB	4.7	4.8	*	-
SFV20	PCB	4.4	*	*	-
SFV25	PCB	4.7	3.4	4.7	-
SFV26	PCB	4.3	*	*	-
SFV28	PCB	4.6	*	*	-
SFV5	PCB	4.1	*	3.5	-
SFV9	PCB	4.4	2.1	*	+
RES1	WCB	4.7	2.5	4.7	-
RES14	WCB	3.6	2.7	*	-
RES22	WCB	4.7	4.1	*	-
RES4	WCB	4.6	*	*	-
RES6	WCB	3.5	*	*	-
SFV15	WCB	4.3	5.6	*	-
SFV16	WCB	4.5	3	*	-
SFV17	WCB	4.6	4.1	3.1	-
SFV21	WCB	4.7	5.3	*	-
SFV23	WCB	4.1	3.4	*	-
SFV27	WCB	4.7	4.8	*	-
SFV29	WCB	4.6	4.8	*	-
SFV3	WCB	4.7	3.4	*	-
SFV30	WCB	4.4	4.6	*	-
SFV7	WCB	3.9	3.4	*	-

FSE = food service establishment, RES = restaurant, SFV = street food vending site, PCB = plastic chopping board, WCB = wooden chopping board, + = present, - = absent *Microbial count < 1.0 x 101 Log CFU.cm^{-2}

3.5. Association between type of chopping board and *Kachumbari* contamination

An independent t-test was performed to determine the difference in the mean bacterial counts of TBC, *S. aureus* and *E. coli* between WCB and PCB. *Kachumbari* sliced on WCB had significantly ($p < 0.05$) higher *S. aureus* contamination levels (5.8 log CFU/g) than in the salads prepared on PCB (3.4 log CFU/g) (Table 6). No significant differences ($p > 0.05$, Table 6) were observed in microbiological contamination with TBC and *E. coli* between *Kachumbari* chopped on WCB and the one sliced on PCB (Table 6).

Table 6: Mean values (log CFU/g) of TBC, *S. aureus* and *E. coli* in *Kachumbari* and chopping boards

	TBC	*S. aureus*	*E. coli*
	Mean values on chopping boards		
PCB	4.5^a	1.4^b	0.5^a
WCB	4.3^a	3.4^c	0.5^a
	Mean values in *Kachumbari* per chopping board type		
PCB	6.5^a	3.4^a	4.1^a
WCB	6.3^a	5.8^b	2.9^a

PCB = plastic chopping board, WCB = wooden chopping board. Means with different superscripts in the same column are significantly different at P < 0.05.

3.6. Correlations between microbiological contamination levels of *Kachumbari* and food handlers' hands

Correlation analysis was conducted to determine the relationship between microbiological counts (TBC, *E. coli* and *S. aureus*) of *Kachumbari* and hands of food handlers. Significant ($p < 0.05$) correlations between different types of indicator organisms on handlers' hands and in *Kachumbari* were observed during the analysis. The correlation between TBC in the salad and *E. coli* on hands was ($r = -0.398$, $p < 0.05$, Table 7) and between *E. coli* in the salad and *S. aureus* on hands was ($r = -.451$, $p < 0.05$, Table 7). However, all the observed positive correlations were not statistically significant ($p > 0.05$, Table 7).

Table 7: Correlations between microbiological contamination levels of *Kachumbari* and hands

			Microbiological counts					
			***Kachumbari* (Log CFU/g)**			**Hand (Log CFU.cm^{-2})**		
			TBC	***S. aureus***	***E. coli***	**TBC**	***S. aureus***	***E. coli***
***Kachumbari* (Log CFU/g)**	**TBC**	R	1	.066	.173	.256	-.149	-.398**
		P		.729	.360	.172	.433	.030
	S. aureus	R	.066	1	-.014	.096	.125	.069
		P	.729		.940	.614	.511	.717
	E. coli	R	.173	-.014	1	-.025	-.451**	.269
		P	.360	.940		.897	.012	.151
Hand (Log CFU.cm^{-2})	***TBC***	R	.256	.096	-.025	1	.246	.378**
		P	.172	.614	.897		.190	.040
	S. aureus	R	-.149	.125	-.451**	.246	1	.129
		P	.433	.511	.012	.190		.498
	E. coli	R	-.398**	.069	.269	.378**	.129	1
		P	.030	.717	.151	.040	.498	

r = Pearson correlation coefficient, **Correlation is significant at the 0.05 level (2-tailed).

3.7. Relationships between independent variables and *Kachumbari* contamination with *E. coli* and *S. aureus*

The outcomes of the chi-square test revealed a statistically significant relationship between chopping board type and contamination of *Kachumbari* with *S. aureus* ($p < 0.05$, Table 8). *S. aureus* was recovered in all 15 (100%) of the salads that were sliced on WCB and only in 10

(66.7%) of the *Kachumbari* prepared on PCB (Table 8). No significant differences in the frequency of the *Kachumbari* contamination with *E. coli* were noted based on both type of FSE and material of the salad chopping board (p > 0.05, Table 8).

Table 8: Relationship between independent variables and *Kachumbari* contamination with *E. coli* and *S. aureus*

Variable		***SS. aureus* contamination**				
		Positive (%)	**Negative (%)**	χ^2	**Df**	**P-value**
FSE type	RES	8 (80.0)	2 (20.0)	0.12	1	0.73
	SFV	17 (85.0)	3 (15.0)			
Board type	PCB	10 (66.7)	5 (33.3)	6.00	1	0.01*
	WCB	15 (100)	0 (0)			
		***E. coli* contamination**				
FSE type	RES	2 (20.0)	8 (80.0)	1.79	1	0.18
	SFV	11 (55.0)	9 (45.0)			
Board type	PCB	11 (73.3)	4 (26.7)	1.29	1	0.26
	WCB	8 (53.3)	7 (46.7)			

Chi-square test at α = 0.05, P values with * denotes significant relationships, FSE = Food Service Establishment, RES = restaurant, SFV = street food vending site, PCB = plastic chopping board, WCB = wooden chopping board, Df = degrees of freedom.

4. Discussion

This study assessed the bacteriological quality of fresh vegetable salads prepared and served in restaurants and street vendors in Mwanza City, Tanzania alongside associated risk factors for contamination and quality deterioration. Our findings revealed that *Kachumbari* from all study FSEs, both restaurants and street food vendors showed high bacteriological contamination levels beyond the maximum limits in vegetable salads intended for human consumption as per Tanzania Bureau of Standards and East African Standards, thus questioning its safety to the consumers. The bacteriological load may indicate the safety (microbiological contamination level) of a product or its quality including the degree of spoilage (Łepecka*et al.,* 2022). However, the bacteriological quality and the extent of deterioration of RTE products like vegetable salads may be influenced by several factors such as source and nature of raw materials (vegetables), pH, FSE type, hygiene of food handlers' hands and type of chopping board used (Kussaga and Nziku, 2023; Mbae *et al.,* 2018; Mohammad & Al-Taee, 2018).

Regarding raw materials for *Kachumbari* preparation, in this study, all food service establishments prepared their vegetable salads from onions, tomatoes, cucumbers, bell peppers, and carrots; and added neither lemon nor vinegar. In line with our findings, Mbae *et al*. (2018) in Kenya found that most (97.4%) of the food establishments prepared their *Kachumbari* from onions, tomatoes, and bell peppers, while only a small proportion of them included chili pepper. This signifies that tomatoes, onions, and bell peppers are the major raw materials for *Kachumbari* preparation in Tanzania and its neighboring countries. Nonetheless, these vegetables can be contaminated with harmful microorganisms, including *E. coli* and *Salmonella* spp. (Akoachere*et al*., 2018; Mbae *et al*., 2018). In our study, all FSEs obtained the vegetables (raw materials) from local food markets in Mwanza City. However, in these markets, the vegetables are not hygienically handled, and are often placed on unclean mats or tables and sprinkled with non-potable water. Although hygienic preparation of salads could completely or significantly reduce microorganisms of concern, recontamination may occur. Whyte (1986) reported that food recontamination through air and dust is a critical issue when products stay for a long time in areas with the possibility of airborne contamination. Therefore, observing hygienic handling practices will prevent food safety hazards in the salads.

pH is another factor that may influence microbiological growth and quality of foods including vegetables and vegetable salads (Gonelimali*et al*., 2018). Most vegetables have pH values near the neutral region, 4.8 – 6.5 (Beuchat, 2002), which favors the growth of most spoilage bacteria and fungi. Thus, vegetables are easily spoiled by both bacteria and fungi. Luckily, in our study, the majority of the samples had low pH values ranging from 3.0 – 4.5, which could inhibit the growth of some bacteria, thereby slowing or hindering spoilage. Nevertheless, it is important to note that pH normally interacts with other parameters like temperature and water activity in foods to cause or prevent spoilage (Moss, 2008; Narayanan, 2020; Odeyemi *et al*., 2020).

On the other hand, all (100%) of the *Kachumbari* salads were unsatisfactory. They had high contamination levels of TBC (4.8 – 6.7 log CFU/g) exceeding the established legal limit in vegetable and vegetable salads, which demonstrates inadequate hygiene of the food business. In agreement with our findings, a study in Addis Ababa, Ethiopia observed excessive TBC contamination levels (6.06 log CFU/g) in vegetable salads (Kechero*et al*., 2019). Further, Faour-Klingbeil *et al*. (2016) in Beirut, Lebanon found that fresh-cut vegetables had TBC counts varying from 2.90 to 7.38 log CFU/g. Also, *S. aureus* (4.5 ± 1.9 log CFU/g) were excessively recovered from both restaurants and SFV above the set standard (2 log CFU/g). Equally, high counts of *E. coli* were recorded in the majority of vegetable salads from both restaurants and SFV, beyond the stipulated legal limits (i.e., *E. coli* absent) in fresh vegetables and salads. *Salmonella* spp. contamination was also detected in *Kachumbari*. In conformity with our findings, Kayombo and Mayo (2018) in Dar es Salaam, Tanzania, recorded high contamination levels in fresh vegetable salads with *E. coli* and *Salmonella* beyond the established limits. Also, Kothe *et al*. (2019) in Brazil and Mbae *et al*. (2018) in Kenya observed high counts of *E. coli* (3.0 – 3.5 log CFU/g) in vegetables and vegetable salads, which exceeded the established standards (2 log CFU/g). Likewise, Aggarwal *et al*. (2020) in India found that 10% of salad samples exceeded the set limits for *Salmonella* spp. in fresh vegetables. Thus, the occurrence of *S. aureus*, *E. coli* and *Salmonella* ssp. in the analyzed vegetable salads could be attributed to inadequate cleaning and improper food handling practices among the study FSEs. Moreover, adhering to the recommended food hygiene and safety standards is particularly vital in food service facilities where large quantities of food are prepared and served every day in a short time, as there is a high possibility of food service workers making errors in the food handling (Kechero*et al.,* 2019). Therefore, it is essential to implement the recommended food hygiene and safety measures in both restaurants and SFVs to ensure the quality and safety of *Kachumbari* and other RTE foods.

Nevertheless, *Kachumbari* from restaurants had a significantly ($p < 0.05$) lower TBC mean value (5.2 ± 0.6 log CFU/g) than the one from SFV (6.5 ± 0.3 log CFU/g). Similarly, Kussaga and Nziku (2023) in Morogoro, Tanzania, recorded relatively higher TBC counts (5.4 log CFU/g) in RTE foods from street vending sites than restaurants (5.2 log CFU/g). This indicates that restaurants are better at observing the recommended food hygiene rules and practices than street food vendors. On the other hand, contamination levels of TBC (6.4 ± 0.5 log CFU/g) in *Kachumbari* salads were significantly ($p < 0.05$) higher than that of*S. aureus* (4.5 ± 1.9 log CFU/g)and *E. coli* (4.0 ± 2.2 log CFU/g). This finding demonstrates that TBC is the most prevalent food hygiene indicator organism in RTEs including vegetable salads (Faour-Klingbeil *et al*., 2016), and high counts of microorganisms of this group in food signifies insufficient food handling as well as the potential presence of food borne pathogens.

Chopping boards are also an important source of cross-contamination to food (Aviat*et al*., 2016). In our study, high contamination levels of TBC (ranging from 3.5 to 4.7 log CFU.cm^{-2}) were observed on both WCB and PCB, indicating inadequate cleanliness and sanitization procedures among the food establishments. In line with this, a previous study by Giwa *et al*. (2021), found high TBC counts (4.2 log CFU.cm^{-2}) on food chopping boards. On the other hand, excessive counts of *S. aureus* were recovered on both PCB and WCB. Nevertheless, *E. coli* contaminations remained generally low. In consistent with our findings, Bukhari *et al*. (2021) in Mekkah City, Saudi Arabia, observed a prevalence of 17.7% for *E. coli* on food contact surfaces, including cutting boards. Also, Faour-Klingbeil *et al*. (2016) reported the presence of *E. coli* in 31% of the analyzed cutting boards, with contaminations between 2.7 and 7.0 log CFU.cm^{-2}.

Interestingly, in our study, only one cutting board (plastic) was contaminated with *Salmonella* spp. However, some previous studies found that *Salmonella* spp. can readily be transferred from contaminated contact surfaces to food (Jensen *et al.,* 2013, Mohammad & Al-Taee, 2018). This suggests a high possibility of cross-contamination to food if a contact surface is contaminated with *Salmonella*. Chopping boards contaminated with pathogenic bacteria like *E. coli* and *Salmonella* spp. have been linked with food-borne disease outbreaks (Little & Sagoo, 2009; Lynch *et al*., 2009). Therefore, it is critical to ensure that they are thoroughly and frequently cleaned before and after use, with food grade disinfectants.

In this study, facilities that used WCB to prepare *Kachumbari* provided more opportunities for cross-contamination compared with establishments that used PCB, as indicated by the significantly ($p < 0.05$) higher prevalence of *S. aureus* recovered in *Kachumbari* prepared by using WCB. A possible explanation for this is that the plastic surface could have been smooth and intact, facilitating easy removal of contaminants. On the contrary, the frequently used wooden surface could have developed scratches and crevices that would have retained contaminants. Likewise, a previous study in Iraq observed a statistically significant ($p < 0.05$) higher prevalence of *S. aureus* on WCB (29.20%) that was used to chop vegetables than on PCB (19.8%) (Mohammad & Al-Taee, 2018). Although, no significant differences ($p > 0.05$) were observed in microbiological contamination with TBC and *E. coli* between *Kachumbari* which was chopped on WCB and the one that was sliced on PCB, the type of chopping board used to prepare food could have an impact on the microbiological quality of RTE foods, especially raw vegetable salads as they are neither heated nor processed further before consumption.

Microbiological cross-contamination from food contact surfaces like chopping boards can occur, if such surfaces are not cleaned effectively or remain wet between cleaning and use (Evans *et al*., 2004). Of note, food cutting boards may harbor considerable amounts of microbiological contaminants, including food-borne pathogens, even after they have been cleaned with portable water and soap (Sekoai*et al*., 2020). Moreover, the cleaning and maintenance of hygiene standards of food cutting boards are often neglected by the handlers (Aviat*et al*., 2016). In our previous study, we observed that FSEs did not properly wash their chopping boards as there were no cleaning schedules for chopping boards (Magambo*et al*., 2023). Besides, the clean as you go principle is not commonly used which may result in multiplication of microorganisms.

Also, food handlers' hands may be a critical source of human pathogens (Yap *et al*., 2019). Hands are in regular contact with the environment including nose, raw foods, and unclean contact surfaces, and as a result, a number of pathogens can reach food through hands (Lee *et al*., 2017; Rodríguez-Caturla*et al*., 2012). Food can become contaminated via contaminated hands if proper hand hygiene is not practiced among the food handlers when handling food

(Rodríguez-Caturla*et al*., 2012; Yap *et al*., 2019). In our study significant correlations between different types of indicator organisms on handlers' hands and in *Kachumbari* were observed during the analysis. Ironically, all the significant correlations were negative. However, correlations between microorganisms in food and their contact surfaces are normally positive (Bartz *et al.,* 2017), which indicates a positive relationship in contamination levels between a food contact surface and the food that was prepared on it. This signifies that proper hygiene can reduce or prevent cross-contamination of food. Although in this study some positive correlations were observed, they were not statistically significant ($p > 0.05$). In contrast to our findings, a study by Bartz *et al*. (2017) in Mexico found a significant ($p < 0.05$) positive correlation between concentrations of *E. coli* on hands and fresh produce including vegetables. On the other hand, Rodríguez-Caturla*et al*. (2012) in Spain found a significant negative correlation between the presence of *E. coli* in food and the cleanliness of the food handlers' hands ($r = -0.363$, $p < 0.05$), suggesting that cleanliness can play an important role in reducing or preventing cross-contamination from hands to food.

5. **Conclusion**

The findings of our study revealed that the food service establishments that prepare and serve fresh vegetable salads in Mwanza City displayed a high presence of hygiene indicator organisms (TBC) as well as foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* ssp.) in vegetable salads, handlers' hands and chopping boards. Consequently, the consumption of fresh vegetable salads has the potential to cause illnesses in humans, as they were found to be contaminated with disease-causing organisms. It is likely that the bacterial contamination in the salads originated from the hands of the food handlers or the chopping boards, as both were found to be significantly contaminated with pathogenic bacteria. Therefore, it is critical to thoroughly clean vegetables and dip them in food-grade antimicrobial chemicals for sufficient time to eliminate pathogens and significantly reduce the microbiological load. Also, it is important to emphasize the practice of thorough hand and chopping board washing after each stage of food processing. Generally, proper sanitization and hygienic handling should be implemented to minimize the risk of contamination during the preparation, storage, and serving of food.

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