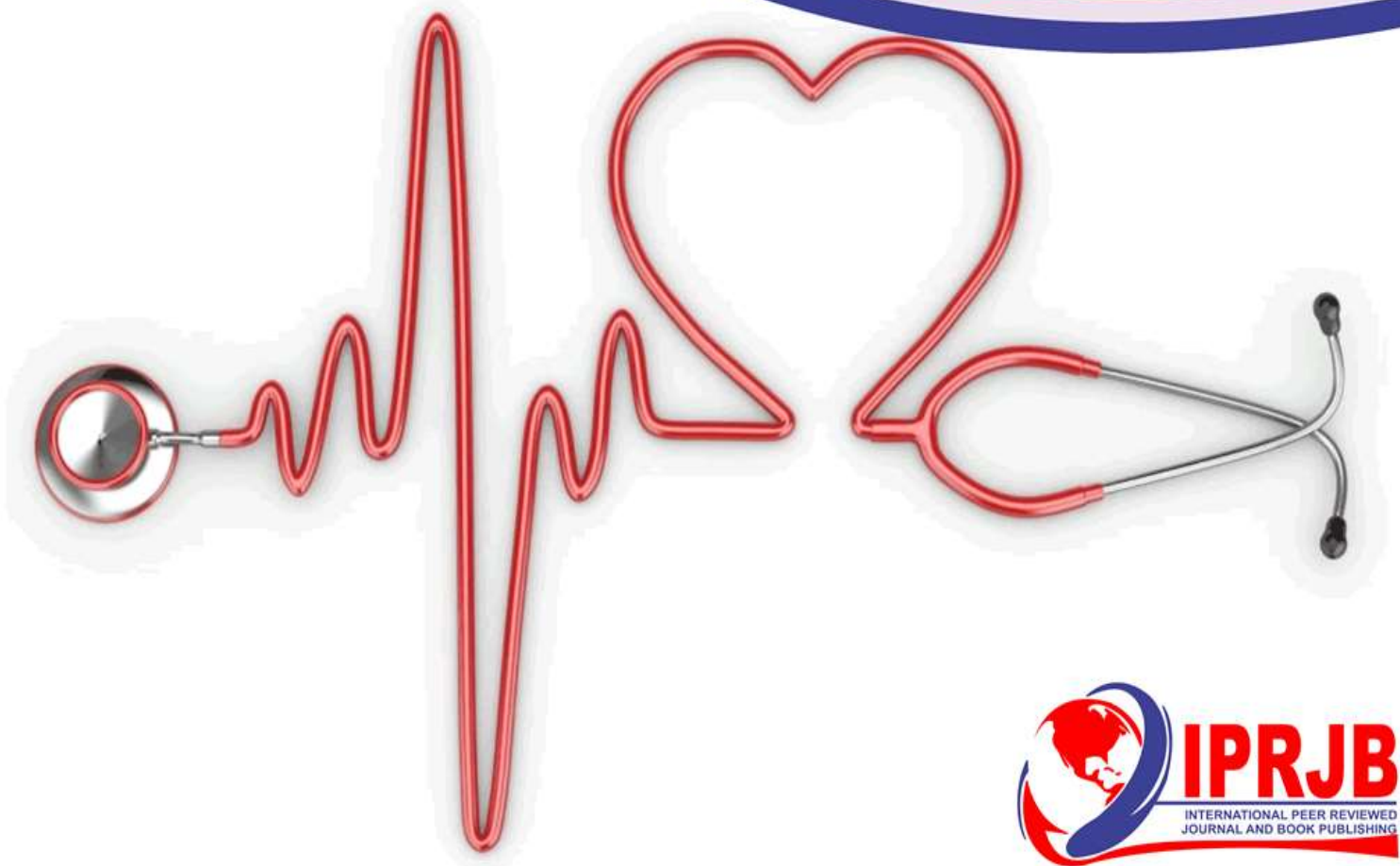


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**Bacteriological Contamination Level of Foods and Water Sold With *Escherichia Coli*,
Salmonella SPP, *Staphylococcus Aureus*, *Coliforms* and *Vibrio Cholera* in Food
Establishments in Nairobi City Kenya**

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Bacteriological Contamination Level of Foods and Water Sold With *Escherichia Coli*, *Salmonella spp*, *Staphylococcus Aureus*, *Coliforms* and *Vibrio Cholera* in Food Establishments in Nairobi City, Kenya



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Abstract

Purpose: Food borne illnesses are major health burden leading to high morbidity and mortality. It is a growing public health concerns worldwide resulting from food and water contaminated by pathogenic microorganism, toxins or chemical hazards. It is estimated that 10 to 20% of food borne illness are contracted from food establishments. The main aim was to determine bacteriological contamination levels of foods and water sold with *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*, *coliforms* and *Vibrio cholerae* in food establishments in Embakasi South Nairobi City County Kenya.

Methodology: The study design was descriptive analytical design. The Samples were collected in selected food establishments (Cafeteria, Hotels, Restaurants and food Kiosks) in Embakasi South Sub county Nairobi City County. The study collected 274 samples of food and water randomly sampled and collected using sterile food bags and water bottles within selected establishments and transported to the laboratory in cool boxes packed with ice packs. The samples were analyzed within 6 hours after collection. Microbiological analysis of food and water were borrowed from WHO and bacteriological analytical manual of foods to identify and isolate coliforms, *Staphylococcus aureus*, *Vibrio cholera*, *Escherichia coli* and *Salmonella spp*.

Results: It was found that some foods and water sold and consumed in the selected food establishments was contaminated with food borne microorganisms. *Escherichia coli* isolated in 137 food samples were at 24.1%, *Vibrio cholera* at 23.4%, *Staphylococcus aureus* at 32.8%, and *Salmonella spp* at 4.4%. Total coliforms detected in 137 samples of water were at 43.8% where 32.8% of them were *Escherichia coli*.

Unique Contribution to Theory, Practice and Policy: The study concluded there was high level of bacterial contamination of foods and water consumed in selected foods establishments. Nairobi City County health officers should enhance regular sampling of foods and water for microbial quality, health education on sources of food contamination at food eateries and establishments to prevent food and water contamination which later lead to food borne illness outbreak.

Keywords: *Bacteriological Contamination, Food Establishments, Nairobi City Kenya*

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INTRODUCTION

Food-borne illnesses are a major health burden worldwide leading to high morbidity and mortality. Globally, Diarrhea disease is a leading cause of child mortality and morbidity in the world and mostly results from contaminated food and water sources (WHO, 2017). An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (DALYs). (WHO, 2022) Foodborne diseases are as a result of eating foods and water with pathogenic microorganisms like bacteria, viruses, parasites or with dangerous chemicals and toxins (Newell et al., 2010; WHO, 2018). In developing countries, approximately 10 to 20% of food-borne disease (FBD) outbreaks are due to food contamination (Tessema *et al.*, 2014)

Food borne illnesses poses a great and serious threat to public health in Kenya as evidenced by frequent outbreaks of cholera and other diarrheal diseases associated with contamination of food and water in the country. Eating foods and water in the food establishments with microorganisms results to food borne illness. Food poisoning agents that are associated with foods and water include *Escherichia coli*, *Salmonella spp*, *Vibrio cholera*, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens* among others (Sockett, 1991). Microbiological contamination of foods served in restaurants can occur at any stage during storage, manipulation, and processing, or even at the serving stage. It can be originated by contaminated raw materials or cross-contamination from the air, water, dust, human and animal wastes, and many other sources (Osimani *et al.*, 2013). In Nairobi City County many food handlers do not observe food handling and safety practices and this may have resulted the transmission of food illnesses in food establishments under this study. This study aims to identify the cause of massive increase of food borne illness such as typhoid, cholera and diarrhea as well as baseline references for planning interventions in the study area. The main aim of the study to determine bacteriological contamination level of foods and water sold with *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *coliforms* and *Vibrio cholera* in food establishments in Embakasi south Nairobi Kenya.

Synopsis of gaps that informed the study; Unhygienic environments where food articles are prepared, poor food handling practices by food handlers, unhygienic food establishments and low compliance level to the existing food safety laws and regulation are most contributing and underlying causes of food borne diseases. There are very minimal studies done on bacteriological contamination of foods and water consumed in various structured food establishments. Food borne illnesses continue being a challenge and a burden in the country. Interaction of these factors may lead to spread of food pathogens into water and foods being prepared within the establishments. Past literatures have not fully addressed microbial loads and contamination at food premises. This study sought to investigate bacteriological contamination level of foods and water in food establishments.

METHODOLOGY

Study design: The study design was cross-sectional analytical since it involved the analyses of the laboratory data for establishing baseline level of contamination of foods and water.

Participants: Foods and water samples in the selected food establishments (Kiosks, cafeteria, hotels and restaurants) in Nairobi City, Kenya. The foods obtained were: cooked rice, githeri, fish, ugali, chips, vegetables (Sukuma wiki and cabbages), kachumbari (salads), and boiled beans/green grams.

Settings: The desired numbers of food establishments within the Embakasi South Sub County were selected by probability to size sampling. The selected food establishments were then stratified by type (cafeterias, restaurants, food kiosks, hotels). The selected food establishments represented the number of samples collected in each category. In Cafeteria 36, samples obtained, 52 samples in food kiosks, 15 in Restaurants and 34 samples in hotels

Samples size: Mihret (2016) approximated that 10 to 20% of diseases outbreak emanated from food establishments (Mihret's.G, 2016). There were 689 food establishments according public health data (2020) in Embakasi South Sub County and therefore 20% was used to calculate sample size. Twenty percent of total food establishment in the sub county were calculated as follows: $20/100 \times 689 = 137$. In every food establishment visited a sample of water and food were obtained making a total of 274 samples that is 137 for foods and 137 for water.

Collection, transportation and analysis of food and water samples: Different cooked foods from food establishments were collected using vendors serving spoons and put in sterile containers. Water samples were obtained from same premises using sterile water bottle. The samples were then labeled according to food type, name of food premise and sample reference number then placed into cool box container with ice packs to maintain temperature of between 6°C- 10°C and transported to University of Nairobi Public Health Pharmacology & Toxicological Laboratory.

Microbial Analysis of Foods and Water

Ten grams of food sample was weighed into a sterile stomacher bag and 90ml of sterile buffered peptone water added to it (which is a pre-enrichment media). The mixture was blended for 30 seconds at a speed of 1500rpm using a stomacher to produce a homogeneous mixture of decimal dilution of 1:10. The homogenate mixture was then incubated for 18-24hrs at 37°C for pre-enrichment

Isolation of *Escherichia coli*

MacConkey agar was used to culture food sample from buffered peptone water using streaking method. A loopful of homogenous sample was taken from previously pre-enriched sample and inoculated onto the MacConkey media. The plate was incubated at 37°C for a period of 18-24hrs in order to obtain distinct colonies. The presence of characteristic colonial morphology for *Escherichia coli* on MacConkey plate was noted as medium size pink colonies. The pink colonies were then purified on a clean MacConkey plate and confirmed using Lysine indole motility (LIM) test which is a biochemical test. The media differentiates *E.coli* from other enterobacteraceae depending on different reactions on indole, deaminase, motility and carboxylase. The presence of *E.coli* gives indole positive, deaminase positive, motility positive and decarboxylase positive.

Isolation of *Vibrio cholera*

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) was used to test the presence of *Vibrio cholera* in food. A loopful of the previously pre-enriched sample was streaked onto TCBS media and incubated at 37°C for 18-24hrs and then examined for characteristic yellow colonies. This is a selective media for *Vibrio cholera* and therefore yellow tinny colonies indicated the presence of *Vibrio cholera* in the food sample. Presumptive *Vibrio cholera* colonies were later confirmed by gram staining giving gram negative organism with thin short rods

Isolation of *Staphylococcus aureus*

A loopful of the previously pre-enriched sample was taken and streaked on mannitol salt agar which is the selective media for *Staphylococcus aureus* and was incubated at 37°C for 18-24 hours. Presence of characteristic tinny yellow colonies on the media indicated the presence of *staphylococcus aureus* in the food sample. Coagulase test was then done to confirm the presence of *Staphylococcus aureus* in the food sample using fresh rabbit plasma. The plasma was diluted at the ratio of 1:1 with distilled water and 200µl of the diluted placed in a test tubes. Sub cultured, pure colony of the presumptive *Staphylococcus aureus* was suspended in the tube containing diluted rabbit plasma and incubated at 37°C for 18-24hrs, and formation of a clot in the tube indicated the presence of *Staphylococcus aureus*.

Isolation of *Salmonella spp*

A loopful of pre-enriched food sample from buffered peptone water was inoculated in enrichment media (Selenite F broth) and incubated at 37°C for 18-24 hrs. A loopful of it was then streaked using a sterile wire loop onto Xylose Lysine Deoxycholate agar (XLD) media and incubated at 37°C for 18-24 hrs. The presence of *Salmonella spp* was indicated by characteristic pink colonies with black centered. The presumptive *Salmonella spp* colonies were sub cultured on XLD for purification. Purified colonies were confirmed using LIM (motility indole lysine media) which differentiate *Salmonella spp* from other enterobacteriaceae. Biochemical reaction for Salmonella included by indole negative, deaminase positive, motility positive and decarboxylase positive.

Isolation of *Escherichia coli* in Water Samples

Water samples obtained from selected food establishments were tested for total coliforms by passing 100mls of each sample through a filtration unit with a sterile filter membrane size 0.45µm. The filters membrane from the unit was then placed on Eosin Methyl Blue Agar (EMBA) and incubated at 37°C for 18-24 hrs. The presence of metallic green sheen colonies on the filter papers indicated the presence of *E.coli* and pink colonies were the other coliforms other than *E.coli*. The characteristic colonies of *E.coli* were sub cultured on EMBA and upon growth, confirmatory test of Lysine Indole motility (LIM) and were carried out to identify the presence of *E.coli* in water.

Isolation of *Vibrio cholera* in Water Samples

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) media was used to test the presence of *Vibrio cholera* in water samples. Fifty milliliters (50mls) of water was centrifuged at 1500 rpm for 5 minutes, the supernant was poured off then a loopful of the deposit taken streaked on TCBS media and was incubated at 37°C for 18-24hrs. The characteristic tinny yellow colonies indicted the presence of *Vibrio cholera* in water sample. For confirmation the yellow colonies were sub cultured on TCBS then the gram staining was done. A positive *Vibrio cholera* was shown by gram negative organism with thin short rods

Data analysis: The procedure for analysis was adopted from (ANDREWS W.H, 2003) for (biological analytical manual for food) and FSSAI (2012) Manual of methods of analysis of food.

Ethical approval: Approval was sought from Kenyatta University Graduate School, clearance from Kenyatta University Ethical Committee (PKU/2237/113481) and permit from National Council for Science and Technology (NACOSTI P/21/9259, Permit from Embakasi Sub

County Local Authority as indicated in Appendix 5 and 6. Research participants' informed consent and participation was voluntary and the process was guaranteed privacy and confidentiality as in appendix

RESULTS

Table 1 and 2 illustrates the summary of microorganisms isolated in various food establishments. All the microbial isolates were found in sampled foods where 24.1% (33) of food samples had *E. coli*, 23.4% (32) samples were contaminated with *V. cholera*, 32.1% (44) of the samples had *S. aureus* and 4.4% (6) of the food samples were contaminated with *Salmonella spp*. Food cafeteria were most contaminated with food microbes where it lead with highest contamination with *S. aureus*, *V. cholerae*, then followed by Food kiosks, Hotel and Restaurant with least contaminated with few microorganisms.

Table 1: Microorganisms Isolated in Selected Food Establishments

Food establishments (total samples)	<i>Escherichia coli</i>	<i>Vibrio cholera</i>	<i>Staphylococcus aureus</i>	<i>Salmonella spp</i>
Hotel (36)	12(33.3%)	7(19.4%)	10(28.8%)	2(5.5%)
Food kiosk(52)	13(25%)	8(15.4%)	10(19.2%)	3(5.8%0
Food cafes(34)	6 (17.6%)	13(38.3%)	21(61.8%)	1(2.9%)
Restaurants (15)	2 (13.3)	4(26.7%)	3(20%)	0 (0%)
Total n (137)	33 (24.1%)	32(23.4%)	44(32.1%)	6(4.4%)

Escherichia coli in Food Samples

The presence of characteristics colonies of *Escherichia coli* was noted as medium size pink colonies as illustrated in figure 1. Out of 137 food samples obtained from selected food establishments, cumulatively 24.1% (n=33) were found to be contaminated with *Escherichia coli*. Hotels had high number of samples with *E. coli* at 33.3% (n=12) from hotels followed by food kiosks at 25% (n=13), 17.6% (n=6) from food cafeteria and 13.3% (n=2) from restaurant. Foods obtained from hotels and food kiosks were highly contaminated with *E. coli*



Figure 1: Pink Colonies of Escherichia coli from Food Sample on a Macconkey Agar. Arrow Showing Single Colony of E.coli

Vibrio cholera

The characteristic of *Vibrio cholera* colonies appeared as yellow tinny on Thiosulfate Citrate bile salt(TCBS) plate as demonstrated in figure 2. Cumulatively, 23.4% (n=32) of the total samples had *Vibrio cholera*, where 38.2% (n=13) of samples were from cafeterias, 26.7% (n=4) samples from restaurants, 19.4% (n=7) samples from hotels, 8(15.4%) samples from food kiosks. Food cafeterias had the highest number of positive samples with *Vibrio cholera*



Figure 2: Yellow Tiny Colonies with Opaque Centers and Translucent Edges Colonies of Vibrio cholera. Arrows Showing Single Colonies of Vibrio cholera

Staphylococcus aureus

The colonies of *Staphylococcus aureus* were tinny yellow on mannitol plate as illustrated in figure 3. Thirty-two percent 32.8% (n=45) of samples had *Staphylococcus aureus*. Food cafeterias were with highest percentage of samples with *S. aureus* 61.8% (n=21), followed by hotel at 28.8% (n=10), 20% (n=3) samples were from restaurants and 19.2% (n=10) of samples were from food kiosks. Majority of samples contaminated with *S. aureus* were from food cafeteria.



Figure 3: Tiny Yellow Colonies of Staphylococcus aureus on Mannitol Media Arrows Showing Single Colonies of Staphylococcus aureus

Salmonella spp

A characteristic pink colony with black center indicated *Salmonella* spp on Xylose Lysine Deoxycholate Agar (XLD) media as shown in figure 4. Total number of samples isolated with *Salmonella* spp were 4.4% (n=6) out of 137 food samples. Food kiosks had 5.8% (3) samples contaminated with *Salmonella* spp, 5.5% (2) samples were from hotels and 2.9% (1) of them samples from food cafes. Foods samples obtained from restaurants had no *Salmonella* spp.

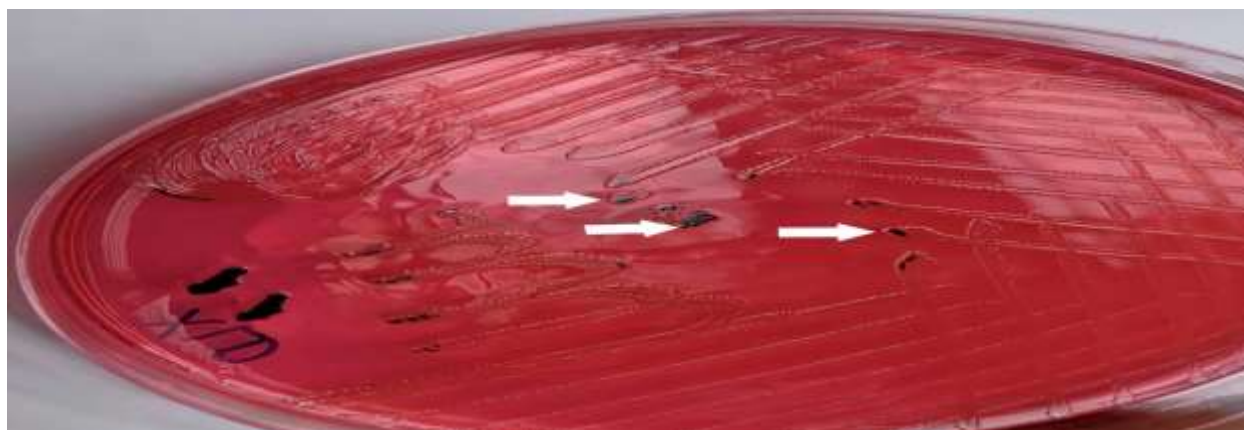


Figure 4: Pink Black Centered Colonies of *Salmonella* spp on XLD Media Arrows Showing Single Colonies for *Salmonella* spp

Microbial Analysis of Water Samples

Escherichia coli, coliforms and *Vibrio cholera* were the microorganisms isolated in 137 samples of water obtained in the selected food establishments. No single colony of *Vibrio cholera* was isolated in all the samples of water tested, however *E. coli* and other coliforms were isolated and they were found to be above WHO acceptable limits as shown in Table 2

Table 2: *Escherichia coli* and Other Coliforms Isolated from Water Samples

s/no	Randomly selected Food Establishments	No of samples with <i>E. coli</i> per CFU/100ml	No of samples with >20 CFU/100ml of total coliforms
1	Cafeteria (34)	13(38.2%)	13(38.2%)
2	Food kiosks (52)	13(25%)	19(36.5%)
3	Restaurants (15)	5(33.3%)	8 (53.3%)
4	Hotels(36)	14(38.9%)	13(36.1%)
5	Total (137)	45(32.8%)	53(38.7%)
	WHO acceptable limits	Nil <i>E. coli</i> per 100ml	<20CFU/100mls

Escherichia coli in Water

The characteristic green metallic sheen colonies of *E. coli* were observed and counted on Eosin Methyl Blue agar (EMBA) media as shown in figure 5. Cumulatively, 43.7% (60) of water samples examined were contaminated with coliforms and 32.8% (45) of water samples obtained from selected food establishments had *E. coli*. Hotels had highest number of water samples with *E. coli* at 38.9% (14), 38.2% (13) water samples were from cafeteria, 33.3% (5) samples from restaurants and 25% (13) water samples were from food kiosks. Total coliforms above acceptable limits of 20 CFU per 100 mls of water were at 38.7% (53), where restaurants had highest percent of samples with above 20 CFU per 100ml at 53.3% (8).



Figure 5: Green Metallic Sheen of *E. coli* on EMBA Media. Arrow Pointing Colonies of *E. coli* in an EMBA Media

Discussion

Foodborne diseases encompass a wide spectrum of illnesses and are a growing public health problem worldwide (WHO, 2022). They are the result of ingesting contaminated foodstuffs, and range from diseases caused by a multitude of microorganisms to those caused by chemical hazards. In this study all the bacteria isolated from foods or water has been implicated in foodborne illnesses. *Escherichia coli* contaminations were at 24.1% out of samples collected, *Vibrio cholera* at 23.4%, 32.8% *Staphylococcus aureus* and *Salmonella spp* isolated were 4.4% and this could be linked to use of contaminated water used for washing utensils and poor hygiene practices of food handlers such as failure to wash hands after visiting toilets. The findings of this study were similar to a study carried by (EMMAH NYAMBURA KARIUKI, 2018) in which *E. coli* was found to be 25.2%. This was also similar to a study carried out in Nigeria in fast food establishment where it was found 20% of samples were contaminated with *E. coli*, 97.3% with *Staphylococcus* and 86.7% of samples with *Salmonella* (Shehu Idris College of Health Sciences and Technology & Usman, 2019). Presence of *E. coli* in food is an indication of fecal contamination occurring from food preparation by food handlers or from materials used (Yeboah-Man et al., 2010). Comparable results from a study done in urban restaurants in Nakuru Kenya by (Muinde & Kuria, 2005) observed *Staphylococcus aureus*, *E. coli* and aerobic bacteria counts were isolated in foods and water. *Staphylococcus spp* has been reported to cause food poisoning due to the heat stable staphylococcus enterotoxin which is resistant to the gastrointestinal enzymes.

The presence of the observed pathogens in the current study are due to poor sanitary conditions of catering establishments and personal hygiene of food handlers which also corroborated with (Haileselassie et al., 2013) study that indicated the presence of *Staphylococcus aureus*, *Salmonella*, *E. coli* and *Campylobacter* as organisms that associated with poor sanitary condition of catering establishments. This study found that 43.8% (60) of water samples examined were contaminated with coliforms where 32.8% of samples were of *E. coli*. this was in agreement with the findings of (Alam, 2006) on his study of study of water quality of Sylhet city and its restaurants found that water was contaminated with fecal coliforms. (WHO, 2006) Had guided that water for drinking should not detect any *E. coli* and thermotolerant coliform

bacteria in a 100ml of water sample. Coliforms and fecal *E. coli* are used as indicator organisms to test water for fecal contamination(Motlagh & Yang, 2019).

The presence of these organisms is an indication that the water is contaminated and may contain pathogens that are harmful to human health. The results of this study were also similar to a study on microbial load of drinking water at point of use in food establishments in Addis Ababa where drinking water was contaminated with *Escherichia coli* which was way above WHO guidelines for drinking water (Girmay *et al.*, 2020). Comparable study by(Nkere *et al.*, 2012) in Nigeria on bacteriological quality of foods and water sold by vendors and restaurants found to be contaminated with coliforms which were above the acceptable limits of 3 CFU/MI where *E. coli* were isolated. (Wandolo, 2016)reported that the quality and safety of food prepared was determined by water sources and its therefore necessary to ensure that contaminated water is not used in the kitchen since water used for washing eventually become part of food

CONCLUSION AND RECOMMENDATIONS

Conclusion

The presence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and *Vibrio cholera* demonstrates a potential health risk as these organisms are pathogenic and have been implicated in foodborne diseases outbreak. There was high level of bacterial contamination of foods and water consumed in selected foods establishments. The prevalence of *Escherichia coli* was at 24.1%, *Vibrio cholerae* at 23.4%, 32.8% were of *Staphylococcus aureus* and *Salmonella spp* at 4.4% in food samples. In water samples analyzed, 43.7% (60) had coliforms where 32.8% (45) of them were of *Escherichia coli*. *Vibrio cholera* was not isolated in any all samples.

Recommendations

1. Nairobi City County health officers should enhance regular sampling of foods and water for microbial quality, health education on sources of food contamination at food eateries and establishments to prevent food and water contamination which later lead to food borne illness outbreak.
2. Nairobi County Government through enforcement officers like public health officers should ensure full compliance to existing food safety laws and public health regulations by food handlers and in all food establishments in order to minimize unsafe food practices.

What is already known on this topic

1. Food borne illnesses poses a great and serious threat to public health in Kenya as evidenced by frequent outbreaks of cholera
2. Biological contamination of foods and water are precursor to food borne illness occurrences in food establishments

What this study adds

1. The findings of the study will be used to explain reasons behind frequent and increase in food borne outbreaks in Nairobi City county Kenya.
2. The study brought out specific policies and recommendations on how to reduce the burden of food borne illness brought out by contamination in food establishments.

Competing Interests

The authors declare no competing interest.

Authors' Contributions

All authors are responsible for conceptualization, data curation, formal analysis, investigation, methodology, research administration, resources, visualization, writing original draft, writing review and editing.

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