



## Assessment of the Microbial Quality of Food Contact Surfaces (Utensils) of Hotels and Restaurants in Addis Ababa

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### Abstract

**Background:** Food contact surfaces are surfacing that encounter food for humans. It can harbor and introduce pathogens into food.

**Objective:** The aim of this study was to assess the microbiological quality of food contact surfaces (utensils) from hotels and restaurants in Addis Ababa.

**Methods:** We used cross-sectional study design in which 12 hotels and 28 restaurants were randomly selected in Addis Ababa. Swab samples from utensils of hotels and restaurants were collected (i.e., four utensils from each establishment) and analyzed over the period of three months resulting in a total of 160 swab samples.

**Result:** Among the hotel and restaurant utensils the highest median count of log total coliform was obtained on trays ( $5.93\log_{10}$  CFU/100 cm<sup>2</sup> in hotels and  $5.00\log_{10}$  CFU/100 cm<sup>2</sup>, in restaurants). *Fecal coliform* and *E. coli* was detected in 14.37% and 3.12% of utensils, respectively. The highest median count of log *S. aureus* was  $5.95\log_{10}$  CFU/100 cm<sup>2</sup> on tray in hotel and  $5.57\log_{10}$  CFU/100 cm<sup>2</sup> on dipper in restaurant and, the median counts of log APC was  $9.37\log_{10}$  CFU/100 cm<sup>2</sup> on tray in hotel and  $8.51\log_{10}$  CFU/100 cm<sup>2</sup> on spoon in restaurant.

**Conclusion:** The finding showed that there is considerable microbial load and inadequacy of washing and cleaning services in hotels and restaurants in Addis Ababa. Hence, there is a need to strengthen monitoring and supervision system in hotels and restaurants.

**Keywords:** Microbial; Utensils; Contact surfaces

### Introduction

Food contact surfaces are surfacing that encounter food for humans. It includes knives, spoons, and tables, cutting boards, trays, flatware, tables, cups/glasses, and highchairs. Surfaces onto which food may drain, drip, or splash, such as the apron of food handlers, inside of refrigerator, desiccators, or a microwave oven are also important [1] food contact equipment can harbor and introduce pathogens into food. Processes such as trimming, slicing, milling, shredding, peeling mechanical abrasion and various methods of disintegration if done with contaminated equipment may introduce contaminants from the equipment involved [2].

Several food spoilage bacteria can attach to food contact surfaces and remain viable even after cleaning and sanitation [3]. Bacteria like *Listeria monocytogenes*, *Salmonella spp.*, and *Campylobacter*, *E. coli* and *Staphylococcus aureus* survive in kitchen utensils, on hands and in bench cover materials [4] and they are the main causes of food borne outbreaks. Contaminated kitchen utensils result in 27% of outbreaks and infection from food borne pathogens [5]. Seventy-Six million people within the United States contract a food borne illness each year [6]. Another route through which foods get contaminated during processing and preparation is infected food handlers and their unhygienic practices [7]. Humans (their skin, mucous membranes and cuts, open sores or a skin infection) can serve as reservoirs of pathogens [8,9]. Improper food handling practices contribute to 97% of food borne illness in food service establishments and at home [10].

A very important characteristic of pathogenic bacteria is forming biofilms on abiotic surfaces [11]

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which can tolerate antimicrobial agents and difficult to eradicate [12]. Water is another important vehicle for the contamination of kitchen utensils and formation of biofilms. Hence, the microbial quality and temperature of water used for washing dish has a detrimental effect [13,14]. In summary, poor quality and unavailability of water, lack of knowledge and experience of food handlers, inadequate monitoring and supervision, poor sanitation practices, lack of proper storage facility and unsuitable environments for food operations are the major contributors to contaminations [15].

Addis Ababa is the capital city and the economic center of the country. Due to urbanization, industrialization and job availability, the flow of population or rural exodus to the city, Addis Ababa, from the different corners of country is increasing overtime. Most of the workers use establishment in the vicinity during working hours. If the hygiene and sanitary conditions of those food establishments are poor, no doubt that there would be an illness due to food borne pathogens. Very few studies were conducted nationwide at large and in Addis Ababa in particular to assess the microbial quality of food contact surfaces (utensils) in food establishments [16-18].

Despite few, those few studies indicated the magnitude of contamination and the requirement of further studies in-terms of geographical coverage, variety of food contact surfaces, and parameters measured. This study was designed to assess the microbial quality of utensils of hotels and restaurants in Addis Ababa.

## Materials and Methods

### Study area and sampling unites

This study was conducted in Addis Ababa from February to May 2018. Study design involved a laboratory based cross-sectional survey and only primary data generated from laboratory investigation was used. After a list of 525 establishments were obtained from Food, Medicine and Drug Administration, (205 hotels and 320 restaurants), 40 establishments (12 hotels and 28 restaurants) were randomly selected using lottery method for microbial investigations. Moreover, from a list of food contact surfaces utilized in hotels and restaurants, four kitchen utensils (Dipper, Spoon, Glass and Tray) that are believed to be frequently contacted by food handlers were purposely selected [19]. From these utensils a total of 160 surface swab samples were collected and analyzed.

### Swab sample collection procedure

The method of choice for examination of surfaces is swabbing of a known area (100 cm<sup>2</sup>) using a sterile swab that has been moistened in 10 ml of neutralizing diluents (Buffered peptone water) [20,21]. A sterile cotton swab was used which is made up of wound cotton head on a 12 cm long wooden stick (Meheco, China). It was moistened with a sterile rinse solution and used for rubbing the surface to be examined. Swabbing is the most common method to sample food contact surfaces.

To avoid contamination, sterile gloves (surgical) were used (Anhuizhongjian Plastic and Rubber CO., Ltd, China). Sample collection was performed on working days from utensils which were cleaned and ready to use for lunch time. Food contact surfaces sampling was performed by swabbing a delimited area (100 cm<sup>2</sup>) [20,22]. Swab head was rubbed slowly and thoroughly over an area of about 100 cm<sup>2</sup> of sampled area. Then, the swab head was rinsed into sterile 10 ml of 0.1% buffered peptone water (Sisco Research Laboratories Pvt. Ltd, India) and the excess was pressed out. The swab was broken off, while the head was remained [23]. Collected swabs

were aseptically transferred in a cool box to the laboratory within two-four hours for further analysis.

### Swab sample processing and analysis

Swab samples in tubes were thoroughly mixed for 30 sec using vortex to make initial dilutions. For each sample, 1:10 serial dilutions were made by using peptone water. Then serial dilution was made to 10<sup>-7</sup> for each sample to get appropriate number of colonies which ranges from 30 to 300. The samples were analyzed for aerobic plate count, total coliforms, fecal coliforms, *E. coli*, *Staphylococcus aureus* and *salmonella*. To enumerate the microorganisms, portions of appropriate dilution were poured or spread plated onto duplicate plates of the appropriate culture medium as follows and for all parameters that involved colony count, the best two consecutive dilutions were used, as n<sub>1</sub> and n<sub>2</sub> to calculate the results. A total bacterium colony count was presented as organisms per 100 cm<sup>2</sup> (CFU/100 cm<sup>2</sup>). The average plate count was calculated using this formula:

$$N = C/V (n_1 + 0.1 n_2) d$$

Where C is the sum of colonies on all plates counted; V is the volume applied to each plate; n<sub>1</sub> is the number of plates counted at first dilution; n<sub>2</sub> is the number of plates counted at second dilution; and d is the dilution from which first count was obtained [20,24].

**Aerobic plate count (APC):** For enumeration of APC, 0.1 ml portions of the appropriate dilutions (up to 10<sup>-7</sup>) was spread plated on pre dried surface Of Plate Count Agar (PCA) (PARK Scientific APHA, USA) using a sterile glass rod and incubated at 37°C for 48 h. All available colonies were counted as APC using colony counter (YLN-30, UK) [24].

**Analysis of total coliforms, fecal coliforms and *Escherichia coli*:** The enumeration of total coliforms was done by the pour plate method using Violet Red Bile Agar (VRBA) (HIMEDIA laboratories, India). The media was mixed with 0.1 ml of the sample dilutions and allowed to set. Finally, an overlay was prepared using VRBA and incubated at 37°C for 24 h [25,26]. For confirmation of total coliform and fecal coliform, a typical colony was taken from VRBA and inoculated to Brilliant Green Bile Broth (HiMedia laboratories, India) and EC Broth (HiMedia laboratories, India) and incubated for 37°C and 44°C for 24 h, respectively. For detection of *E. coli*, a loopful from EC broth was taken and inoculated to nutrient broth and incubated at 44°C for 48 h. Then, indole test was performed. Two to three drops were used for each test.

***Staphylococcus aureus*:** For enumeration of *Staphylococcus aureus*, 0.1 ml portions of the appropriate dilutions was spread plated on pre dried surface of mannitol salt agar (Difco Laboratories (USA) using a sterile glass rod and incubated at 37°C for 48 h [27,28].

**Isolation and identification of *Salmonella* sp.:** Detection of *Salmonella* was carried out according to the Bacteriological Analytical Manual, established by USDA (USDA, 2001). Initially, 0.1ml enriched swab samples were transferred from Buffered Peptone Water to Selenite Cystine Broth (HiMedia laboratories, India). The mixtures were vortexed gently and incubated at 37°C for 24 h. After incubation, 1 loopful of Selenite Cysteine Medium was transferred and streaked into Xylose Lysine Deoxycholate (XLD) agar (BIOMARK Laboratories, India) and incubated at 37°C for 24 ± 2 h. Lastly, biochemical identifications were performed using Triple Sugar Iron (TSI) agar and Lysine Iron (LI) agar.

## Data management and quality control

Sample collection format which contains type of sample, location of sample site, date and time of collection, name of sample collectors and number of samples collected was prepared. All materials used for lab investigations (bottles, petri dishes, test tubes, pipettes) were washed properly, rinsed for any residues that may have bacteriological effect and were sterilized. Qualified public health professionals were used for sample collection. Swab sample collectors were identified and oriented about sample collection procedures for a half day. The collected samples were transported using triple package. Improperly labeled and broken containers, last arriving samples and samples with insufficient volumes were excluded.

Media preparation was conducted according to manufacturer's instruction on the container. Media sterility was checked by overnight incubation prior to use and quality control was used in all batch of the samples.

## Data analysis

Data was entered in to excel sheet and cross checked with the hard copy for its consistency. Data analysis was performed using SPSS version 23 and STATA version 12. Since the data was a count and yields large number, it was log transformed to attain a manageable form of data. Descriptive statistics including frequencies, percentage, mean, and median were used to summarize the data. Nonparametric test (Mann-Whitney Test) was used to compare median values of the outcome variables between the two establishment types.

## Results

### Total and fecal coliform count

The frequency of growth and median count of total coliform is indicated in Table 1. The growth frequency of coliform on hotel utensils ranged from 6 (50%) on spoon to 9 (75%) on glass and tray. The growth frequency of coliform on restaurants utensils ranged from 15 (53%) dipper, glass and spoon to 19 (68%) on tray. Total coliform growth is highest on glass and tray in hotels and restaurants, respectively. The median count of log total coliform in hotel utensils ranged from  $4.85\log_{10}$  CFU/100 cm<sup>2</sup> on dipper to  $5.93\log_{10}$  CFU/100 cm<sup>2</sup> on trays. Similarly, the median count of log total coliform in restaurant utensils ranged from  $4.35\log_{10}$  CFU/100 cm<sup>2</sup> on glass to  $5.00\log_{10}$  CFU/100 cm<sup>2</sup> on tray.

Moreover, fecal coliform was detected in 25% of hotels and 32.14% restaurants and *E. coli* was detected in 25% of hotels and 7.14% restaurants. With regard to utensils, fecal coliforms and *E. coli* was detected in 12.5% and 6.25% of hotel utensils and 15% and 1.78% of restaurant utensils. In general, fecal coliform was detected in

14.37% of utensils and *E. coli* was detected in 3.12% of utensils.

### *S. aureus* count

The growth frequency of *S. aureus* on hotel utensils is indicated in Table 2 and shows 4 (33.3%) on glass to 7 (58%) on tray. The growth frequency of *S. aureus* on restaurants utensils showed 14 (50%) on glass to 19 (68%) on dipper. The median count of log *S. aureus* in hotel utensils ranged from 0  $\log_{10}$  CFU/100 cm<sup>2</sup> on dipper and Glass to  $5.95\log_{10}$  CFU/100 cm<sup>2</sup> on tray. The median count of log *S. aureus* in restaurant ranged from  $2.02\log_{10}$  CFU/100 cm<sup>2</sup> on glass to  $5.57\log_{10}$  CFU/100 cm<sup>2</sup> on dipper.

### Determination of aerobic plate count

The growth frequency of APC on hotel utensils was indicated in Table 3. It showed 12 (100%) growth on all utensils. The growth frequency of APC on restaurant utensils showed 24 (86%) on glass to 27 (96%) on dipper. The median count of log APC in Hotel utensils ranged from  $9\log_{10}$  CFU/100 cm<sup>2</sup> on spoon to  $9.37\log_{10}$  CFU/100 cm<sup>2</sup> on tray. The median count of log APC in restaurant utensils ranges from  $8.37\log_{10}$  CFU/100 cm<sup>2</sup> on glass to  $8.51\log_{10}$  CFU/100 cm<sup>2</sup> on spoon. *Salmonella* was not detected in any of the samples.

The median comparison of microbial counts from utensils in hotels and restaurants are indicated in Table 4. All microbial parameters across all utensils were compared between hotels and restaurants. The result show that there is no statistically significant difference between microbial load of utensils measured at hotels and restaurants except for APC analyzed for glass (at  $p=0.016$ ). From all hotel and restaurants kitchen utensils where microbial growth was observed, except for *S. aureus* found on dipper and glass, all microbial load is beyond the acceptable limit stated by European Community [29] and Center of Disease Control [30]. For cleaned and ready to use surfaces, the load of aerobic plate count should be below 10CFU/cm<sup>2</sup> and for Enterobacteriaceae and other pathogenic bacteria, it should be below 1CFU/cm<sup>2</sup> [31].

## Discussion

In this study the frequency of growth of coliform ranges from 53% on restaurant to 75% on hotel utensils. It was found that the mean log count of coliforms ranges from  $3.30 \pm 3.24$  on restaurant dipper to  $4.57 \pm 2.85$  on hotel tray. The frequency of growth of *S. aureus* ranges from 33.3% on hotel to 68% on restaurant dipper. The mean log count of *S. aureus* ranges from  $3.20 \pm 3.37$  on restaurant glass to  $4 \pm 3.59$  on hotel tray. Similarly, the frequency of growth of aerobic plate count (mesophilic plate count) ranges from 86% on restaurant glass to 100% on hotel utensils. The mean log APC (Aerobic Mesophilic Bacteria) count ranges from  $7.17 \pm 3.33$  on restaurant glass to  $9.37 \pm$

**Table 1:** Frequency of growth, mean, median, interquartile range, Geo. Mean and range of log count of coliform from hotel and restaurant kitchen utensils (n=40).

Log Count of Coliforms/100 cm <sup>2</sup>							
Premises	Utensil	Freq. of Growth (%)	Mean $\pm$ SD	Median	Inter Quartile. Range	Geo. Mean	Range
Hotel (12)	Dipper	8 (66)	$3.77 \pm 2.87$	4.85	6.00	5.61	6.78
	Glass	9 (75)	$4.44 \pm 2.78$	5.37	3.52	5.87	7.49
	Spoon	6 (50)	$3.93 \pm 2.90$	5.00	5.33	5.78	7.49
	Tray	9 (75)	$4.57 \pm 2.85$	5.93	3.99	6.05	7.55
Restaurant (28)	Dipper	15 (53)	$3.30 \pm 3.24$	4.68	6.2	6.05	8.00
	Glass	15 (53)	$3.35 \pm 3.31$	4.35	6.26	6.13	8.00
	Spoon	15 (53)	$3.45 \pm 3.15$	4.93	6.00	5.95	8.00
	Tray	19 (68)	$3.91 \pm 2.98$	5.00	8.00	5.59	8.00

**Table 2:** Frequency of growth, Median, Interquartile range, Geo. Mean and range of log count *S. aureus* from hotel and restaurant kitchen utensils (n=40).

Log Count of <i>S. aureus</i> /100 cm <sup>2</sup>							
Premises	Utensil	Freq. of Growth (%)	Mean ± SD	Median	Inter Quar. Range	Geo. Mean	Range
Hotel (12)	Dipper	5 (41)	2.48 ± 3.08	0.00	5.84	5.94	6.65
	Glass	4 (33.3)	2.33 ± 3.45	0.00	6.73	6.98	7.51
	Spoon	6 (50)	3.38 ± 3.58	2.65	7.12	6.72	7.46
	Tray	7 (58)	4.01 ± 3.59	5.95	7.05	6.83	7.69
Restaurant (28)	Dipper	19 (68)	4.05 ± 2.97	5.57	6.39	5.87	7.30
	Glass	14 (50)	3.20 ± 3.37	2.02	6.14	6.28	8.15
	Spoon	17 (60.7)	3.69 ± 3.19	4.78	6.86	5.94	8.55
	Tray	18 (64)	3.86 ± 3.09	4.77	6.70	5.88	7.85

**Table 3:** Frequency of growth, median, interquartile range, Geo. Mean and range of log count of APC from hotel and restaurant kitchen utensils (n=40).

Log count of APC/100 cm <sup>2</sup>							
Premises	Utensil	Freq. of Growth (%)	Mean ± SD	Median	Inter Quar. Range	Geo. Mean	Range
Hotel (12)	Dipper	12 (100)	9.28 ± 0.39	9.31	0.61	9.27	0.84
	Glass	12 (100)	9.20 ± 0.55	9.23	0.67	9.19	1.14
	Spoon	12 (100)	9.08 ± 0.48	9.00	0.32	9.07	1.88
	Tray	12 (100)	9.37 ± 0.49	9.37	0.52	9.35	1.68
Restaurant (28)	Dipper	27 (96)	8.19 ± 1.94	8.49	1.92	8.42	10.36
	Glass	24 (86)	7.17 ± 3.33	8.46	2.24	8.16	10.36
	Spoon	26 (93)	7.97 ± 2.49	8.51	1.76	8.51	10.56
	Tray	26 (93)	7.98 ± 2.55	8.37	2.57	8.51	10.61

**Table 4:** Comparison of microbial counts obtained from kitchen utensils of hotel and restaurant.

Utensils	log Coliform		Log <i>S. aureus</i>		log APC	
	Median df	p-value	Median df	p-Value	Median df	p-Value
Dipper	0.17	0.73	5.57	0.73	0.82	0.094
Glass	1.02	0.446	2.02	0.553	0.87	0.016
Spoon	0.06	0.73	2.13	0.73	0.49	0.658
Tray	0.95	0.094	1.17	0.73	1	0.214

0.49 on hotel tray. Fecal coliforms and *E. coli* was detected in 12.5% and 6.25% of hotel utensils and 15% and 1.78% of restaurant utensils.

The finding of present study is comparable to study made at University of Tennessee by Cosby et al. [32] where the mean log APC ranges from 3.55log<sub>10</sub> CFU/50 cm<sup>2</sup> to 3.81log<sub>10</sub> CFU/50 cm<sup>2</sup>, the mean coliform counts ranges from 35.62CFU/50 cm<sup>2</sup> to 10.72CFU/50 cm<sup>2</sup> and *E. coli* was detected in 1.6% in the range of 1 to 35CFU/50 cm<sup>2</sup> except that the present study use count per 100 cm<sup>2</sup>. Similarly, the APC log count of the present study is in harmony with a study made in Chinese households in which the aerobic plate counts for dishcloths were 10-10<sup>9</sup> cfu/cm<sup>2</sup> in the range of 150 cfu/cm<sup>2</sup> to 1.776 × 10<sup>9</sup> cfu/cm<sup>2</sup> (Beijing) and 62.5 cfu/cm<sup>2</sup> to 8.75 × 10<sup>8</sup> cfu/cm<sup>2</sup> (Shanghai) regardless of institution involved, material type and sample size which was very large in Chinese study [33].

The APC in the present study is similar with study of Rossi et al. [34] and Abubakar et al. [35] in which the APC count ranged from 3.4 to 10.4 log<sub>10</sub> CFU/sponge, with an average of 9.1log CFU/sponge. With regard to fecal coliforms and *S. aureus*, high count was observed in the study of Rossi et al. [34]. Kitchen sponges harbor large number of microorganisms in terms of type and number if not appropriately washed and can cause cross contaminations [36].

The study highlighted insufficiency of food handler's adherence to good manufacturing practice and sanitation standard operating procedures.

In the study made in University of Technology, Yola, total bacterial count was found which ranges from 1.0 × 10<sup>4</sup> cfu/ml in knives and cups to 2.5 × 10<sup>6</sup> cfu/ml in plates [37]. Other study made in Terengganu, Malaysia, Lani et al. [23] revealed that the mean log<sub>10</sub> CFU/cm<sup>2</sup> APC ranges from 1.37 ± 1.45 on surface griller to 4.68 ± 0.43 on table of preparation. Similar result was obtained by Cunningham et al. [38]. Other related study revealed the Total Bacterial Count (TBC) per ml of the samples ranges from 1.8 × 10<sup>3</sup> to 7.7 × 10<sup>3</sup> cfc/ml. But, in contrast to the present study, *E. coli* was detected in higher frequency (66.67%) [39]. Study made in the sultanate of Oman and others, Sudheesh et al. [40] and Nhlap et al. [41] revealed that high plate readings of Total Colony Count (TCC) and indicator organisms such as total coliforms, yeasts and molds and *Escherichia coli* were obtained from samples collected from most food contact surfaces.

The growth frequency of Coliforms, *E. coli* and *S. aureus* and the mean log count of APC are in line with the study conducted in Brazil where Coliforms, *Escherichia coli* and *Staphylococcus aureus* were detected in 24 (40.7%), 2 (3.3%) and 13 (22.0%) of the food contact

surfaces, respectively and the mean aerobic mesophilic bacteria count was 3.1log CFU/100 cm<sup>2</sup> of surface area [42]. Human skin is the ideal habitat for *S. aureus* and fecal contamination is also common. The factors that could play an important role in food borne illnesses are the worker training, awareness of handling food and hygiene, correct techniques and implementation of quality standards in food premises [43].

In other study in Accra, Ghana, Addo et al. [44], thirty-seven (37) (35%) of the swab samples showed the presence of coliforms and in contrast to the present study, *Escherichia coli* was absent in all the 105 samples. The total count of aerobic bacteria was high in the swabs from the working surfaces and cutting boards (>10<sup>3</sup> cfu/ml). In the study made in Serbia, take-away food establishments had the highest share of results =2 log<sub>10</sub> CFU/cm<sup>2</sup> for both stainless steel and plastic surfaces. Highest share of stainless-steel surfaces with microbial load =2 log<sub>10</sub> CFU/cm<sup>2</sup> were cutlery, dishes and knives. Plastic dishes had the highest share of results =2 log<sub>10</sub> CFU/cm<sup>2</sup> while cutting boards had the majority of results between 1 log<sub>10</sub> CFU/cm<sup>2</sup> and 2 log<sub>10</sub> CFU/cm<sup>2</sup> [45].

In contrast to the current study, the study conducted in South Africa, revealed that *E. coli* was detected in 50%, 30%, 30%, and 50% of samples from cutting boards, knives, plates and spoons, respectively [46]. Similarly, in other related study, out of the 50 samples analyzed, 13 (26%) were positive for *Escherichia coli* and 0% was positive for *Staphylococcus aureus*. Out of the 13 samples that were positive for *Escherichia coli*, 8 (61.5 %) were from plates, 3 (23.1 %) were from chopping boards and 1 (7.7 %) was from table and spoon each [47].

In this study we found that except for *S. aureus* count on hotel dippers and glass; all other counts are beyond the acceptable limit. In contrast, in follow-up study in United Kingdom, from 585 swabs examined 68% (397) were of satisfactory microbiological quality and 32% (188) were unsatisfactory. Swabs from chopping boards gave a significantly higher proportion of unsatisfactory results (84/141; 60%) compared to those from all other surfaces (104/444; 23% [31].

## Conclusion

The results showed that contamination rate with aerobic plate count, total coliform, fecal coliform, *E. coli* and *Staphylococcus aureus* on washed and ready-to-use utensils were relatively high. This also showed the presence of other pathogenic microorganisms. This can result in the contamination of food with pathogenic bacteria and food borne illness. This in turn implicated for the requirements of improving washing and cleaning in compliance with standard procedures and strengthening the monitoring and supervision system in hotels and restaurants.

The study revealed the presence of considerable number of different bacteria in utensils of hotels and restaurants of Addis Ababa. Therefore, it can be utilized by Addis Ababa City Food, Medicine and Drug Administration Authority and hotels and restaurant managers to strengthen the supervision and monitoring system.

## Authors' Contribution

Abiyot Tenna, designed the study, analyze the data and drafted the MS. KAS, YWW, HTM, organize data collection and critically reviewed the draft MS and contributed significantly ideas that improved the MS. DMD and GM critically reviewed the draft MS and improved its content. All co-authors read and approved the final version of the MS for submission to the journal.

## Ethical Consideration

The study was approved by Institutional Review Board of Addis Ababa City Administration Health Bureau and informed consent was obtained from all study participants.

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