



**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF PUBLIC HEALTH**

**ASSESSING HYGIENE AND SANITARY CONDITION OF STREET FOOD
VENDING IN ADDIS KETEMA SUB-CITY, ADDIS ABABA**

BY:-BIRUK TESEMA (BSc)

**A RESEARCH THESIS TO BE SUBMITTED TO THE SCHOOL OF PUBLIC
HEALTH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTERS IN PUBLIC HEALTH**

**DECEMBER, 2020
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Acronyms

| | |
|-----------|--|
| AMBC | Aerobic mesophilic bacterial count |
| AOR | Adjusted odds ratio |
| COR | Crude odd ratio |
| CfU/g | Colony-Forming Unit per gram |
| FAO | Food and Agriculture Organization of the United Nations |
| FMHACA | Food, medicine, and health care administration and control authority |
| Log CFU/g | Logarithm of colony-forming unit per gram |
| SD | Standard deviation |
| SFVs | Street Food Vendors |
| SS | Sampling site |
| USD | United States dollar |
| WHO | World Health Organization of the United Nations |

Abstract

Background: WHO defined street food as food and beverages prepared and sold in the street and other public places for immediate consumption. It is also described as the food that is prepared either at home or along the street and consumed at the point of purchase or elsewhere without any processing.

Objective: The objective of this study is to assess the hygiene practice of the vendors and sanitary conditions of the surrounding vending site with laboratory analysis of street food in the Addis Ketema sub-city of Addis Ababa.

Methods A total of 301 street food vendors were assessed by a structured questionnaire and direct observation in four selected sampling locations of Addis Ketema sub-city. Additionally, the bacteriological quality of sixty samples of street food was determined using aerobic mesophilic bacterial count, total coliform, and Salmonella test. About 18 questions were set to measure street food vendor's hygiene practices. Vendors who scored less than 12 points were considered poor and those who scored 12 points and above considered good hygiene practice. Independent variables were fit separately into a bivariate logistic model to evaluate the degree of association with the dependent variable. Then variables that have a p-value ≤ 0.2 were exported to a multivariable logistic regression model. The strength of association was measured using OR and 95% CI and a p-value < 0.05 considered statistically significant.

Result: The present study found that food safety knowledge and environmental condition of the vending site shows statistical association with good hygiene practice of the vendors. Vendors who work in the poor environmental condition of vending sites were 74% less likely (AOR=0.265, 95% CI (0.116, 0.607) to have good hygienic practice as compared to those who work in the good environmental condition of the vending site. 86.7% of the sample showed an unsatisfactory level for aerobic mesophilic bacteria count and about 5(9%) of the sample showed the presence of Salmonella.

Conclusion: The level of hygiene practice of the vendors and the environmental condition of the vending site was poor. About 82% of the vendors had poor hygiene practice and 72% of the surveyed participants' carried out their work in poor environmental conditions. Moreover, the bacteriological quality analysis of food also shows the result beyond a recommended limit for the tests conducted.

1. Introduction

1.1 Background

WHO defined street food as “food that is prepared and sold in the street and other public places like a bus station, large construction sites, colleges, health facilities and other similar business centers for immediate consumption” (1). It is also described as the food that is prepared either at home or along the street and consumed at the place of purchase or elsewhere without any processing and preparation (2-4). Street food vendors retail food that is relatively inexpensive and suitable for urban inhabitants and economically poor rural migrants (5).

Street food vendors are of three types based on their mode of vending. These can be mobile vendors which include vendors who retail food on foot and bicycle; semi mobile those who are using pushcart and stationary food vendors those who sell food from a fixed stall. Street food vending is commonly a family or one-person business and often it is unlicensed (6).

Poor personal hygiene of the person involving in food processing signifies a potential threat to food safety and its suitability in consumption (7). So, street food vendors should maintain their personal hygiene regularly. In addition to this, it is necessary to cover hair during food processing to avoid loose hair and dandruff from falling onto food and food preparation materials (8). Food vendors should be cautious during different stages of food processing at the preparation and vending place because of improper practice may compromise food safety at these stages (8, 9). To improve the safety of street-vended food WHO develop five basic rules entitled “WHO five keys to safer food”. These rules include keeping the vending unit and location clean, separating raw and cooked food, using safe water and raw materials, cooking food thoroughly, and keeping food at safe temperatures (10).

The street food vending activity is flourished due to the rapid urban population growth that results from high movement from rural areas to urban centers searching for jobs (11). Consequently, many people working outside prefer to consume ready-to-eat foods. The street food vending also serves as a way of employment creation especially for the majority of low-income women and a reflection of the traditional culture of a certain community. For example in Greece and Thailand, they form an important part of community life (12, 13).

1.2 Problem statement

WHO reports that unsafe food results in more than a half of billion cases of foodborne infection and 420 000 deaths annually. From this Africa share the highest-burden with greater than 91 million cases and 137 000 death every year and Ethiopia is listed among the top ten countries in Africa (14). Yet a total of 2.5 billion people globally eat street foods every day and it provides an income for millions of less-educated people (6). In contrast to its benefits street food is often considered as one of the major public health concerns because it is recognized that street food vendors are indigent, unlearned, and lack appreciation for safe food handling (5). Besides this, it contains pathogenic organisms (15-18).

From the reviewed articles, the majority of them included a very small number of participants (9, 19-21) particularly less than sixty vendors. For example, in the study conducted in Amhara regional state, Dessie town involved only nineteen street food vendors (22). In a similar way, concerning bacteriological quality assessment, the study done in Gondar town analyzed thirty food samples (17). This may distort the conclusion derived from the limited study participant. In addition to this, it is not comfortable to do regression analysis. The other thing that was raised in some articles is that they assess only the registered street food vendors (19). This again excludes the existing true pictures of street food vending activities. Moreover, it confuses a decision-maker to decide the rational decision-making process in order to implement a tough street food vending regulation system. The other thing that was revealed from the articles is that most of them did not show appropriate sample size calculation and sampling procedures.

Hygiene practice combined with microbiological quality analysis gives a more clear picture of this subject area. Up to the level of my knowledge, no research incorporates both the factors associated with the hygiene practice of the street food vendors and laboratory analysis of the street food. The studies conducted on street food vendors assessed either the hygiene practice of the vendors or the bacteriological quality analysis of food. However, this specific study assessed both the hygiene practice of the vendors and the bacteriological quality analysis of street food combined with the observation results.

1.3 Significance of the study

This study is crucial to assess the hygiene practice of the vendors and to identify the degree of microbial contamination of street food in urban areas in relation to food quality. In doing such an assessment hygiene practice and environmental condition of vending sites can be pointed out in order to adopt tough regulations on the mechanism of how to regulate street food vendors. If such measures were taken appropriately, the harmful effect of street food that causes food poisoning to the customer will highly be diminished.

The finding of this survey will, therefore, serve as an input in the process of developing strategies on how to regulate street food vending activities for the government and concerned bodies. In addition to this, the finding of this study will lay a foundation for the researchers who are interested to do more on the raised issue.

2. Literature Review

2.1 Hygiene Practice Of Street Food Vendors

Street food loses its safety during a different chain of food processing stages. Perhaps, this is attributed to inappropriate handling and neglecting hygienic measures by the street food vendors which leads to contamination of food (20). Moreover, Liu revealed that the main sources of contamination of street food are poor quality raw material, lack of proper vending site, poor personal hygiene, improper handling, and sanitation practice in the stage of food preparation, storage, and vending (9). Different studies confirm these findings as follows; street food vendors purchase vegetables from the informal market because of their low price (13), food can be prepared by traditional methods, raw meat and vegetables washed carelessly, food can be adulterated intentionally (9), money is handled during food processing (21), about 90% food to be vended throughout the day is cooked in the morning (23), leftover food is mixed with freshly prepared food which can increase the microbial load in food (8). Even though, only 42% of the vendors use cutting boards without washing them before use(13). Street food vendors used the same utensil at home and vending sites for serving the consumer. In addition to this, they use substandard facilities that promote the infestation of cockroaches, rodents, flies and dust which in turn increase the likelihood of contamination (8).

Most of the street food vendors aim to make a profit at the expense of standard food hygiene and sanitary practice (5). This is because there is no sufficient rule and regulation and makes them operated haphazardly without noticing what they are prepared and how they are prepared (20). This creates another public health problem. For example, street food was the cause of 691 food poisoning outbreaks and 49 deaths from 1983 to 1992 in China. Additionally, Rane reviewed that epidemiological information about infection caused by this subject matter is not really sufficient, because an outbreak of such type is infrequently reported (23). The other thing that worsens the situation is that people did not stop consuming street food due to its inexpensiveness and served quickly even if it is prepared and sold inappropriate conditions (2).

2.1.1 Personal Hygiene Practice

This is deals with keeping the cleanliness of the vendor's body and clothing to maintain overall health and well-being. For example, washing and drying hands frequently, not wearing jewelry or fabricated nails that could contaminate foodstuff, and covering wounds appropriately (24). Street food vendors may be one source of contamination of food because they harbor bacteria on their skin, hair, eye, nose, mouth, and hands. This leads to the appearance of pathogenic microbes in the food if they have not kept their hygiene regularly (9).

Concerning the personal hygiene practice of food vendors, different studies report different results. For example, the study done in China revealed that about 46% of study participant did not exercise hand washing at critical points such as before, during and after preparing food; cleaning tasks, after blowing the nose, coughing, or sneezing, after touching waste, pre and post-operation of the kitchen activities (9). Another study reported that 67% of street food vendors wash their hands quarterly per day at the workplace without using detergents while 33% did not wash their hands and about 95% of them can handle food after touching money without sanitizing their hands (19). In contrary to this Lues found that only 4% wash their hands appropriately with detergents while the rest of them used only water and the most of vendors reported as they wash their hands after using the toilet (13). Barro et al depict that when vendors use bare hands to serve vending activities, the risk of food contamination and transmission of feaco-oral diseases are highly increased (21). In relation to this Isaac Monney, reported that 26% of street food vendors did not use any equipment to handle food and 21% of them observed dirty fingernails (25). Nureddin et al found that 73.6% did not wash their hands before preparing food and 53.6% did not cover their hair. Greater than 50% did not use an apron (5).

2.2 Factor Associated With Hygiene Practice

2.2.1 Sociodemographic Factor

Socio-economic and cultural backgrounds have a direct effect on the behavioral and hygienic practices of street food vendors (26). Similarly, Reang revealed that the level of socio-economy and awareness determines food handling practice. This is because a vendor with poor socioeconomic status and awareness level may be hindered from performing

appropriate food hygiene practices due to the inability to fulfill those required materials and utensils (3). In addition to this, the other factors that associated with food quality are gender, educational status, age, and income of the vendors (7, 26). For example, the vendors who have monthly income greater than 21 USD had 3.2 times more likely to have better hygienic practice as compared to those with lower income (22). Moreover, the other study also showed that vendors with a secondary level of schooling give more attention to food hygiene as compared to those who are less educated (3).

2.2.2 Food Handling Training

Training is very important in the process of maintaining food hygiene. That is why it is emphasized by different studies as it influences food handling practice (7, 12, 27). The study done in Kuching City revealed that vendors who are trained about food safety were four times higher in performing good practice in comparison to those who did not take the training (7). The study conducted in Dessie town also found that the odds of having food safety practice were 6.7 times higher among vendors who receive food safety training than those who did not receive the training (22). The other study reported the importance of training as; the female vendor who did not take food safety training may perform hygiene practice lower than her male counterpart who has taken the food safety training. Thus attention should be given to training the vendors irrespective of gender (12). Training is also one of the factors that enhances the potential of street food vendors in addition to instructing and supervising them. This probably improves the whole vendor's hygiene practice (7).

Omemu revealed that only 12% of the vendors acquired formal training while the rest got knowledge on how to process food through observation (28). Nureddin report that 63% of them acquired skills from parents and 33.7% acquired skills by self-practice (5). Chukuezil also found that 47.62% of the street vendors' knowledge of food vending by trial and error, 47.62% acquired by observation, and 4.7% of them attend formal training (29).

2.2.3 Food Safety Knowledge

The other factor that is linked with the hygiene practice of the street food vendors is food safety knowledge. Food safety knowledge of street food vendors is one of the risk factors in the transmission of foodborne disease (2, 8). Studies were done to assess the food safety

knowledge of the vendors. For example, Liu found that about 66% of the vendors lack food safety and about 12% of the vendors found it to be aware of certain sanitation technique and the way of reducing cross-contamination (9). In the other study, most of the vendors understand as microorganism contaminate food while limited respondents assumed ingredients that change food color and flavor during preparation and preservation can cause food contamination (28).

The study done in Kenya reported that most of the vendors perform without food safety knowledge that food could be a source of pathogen microbes (30). To fill the gap that exists in such circumstance food handling training play a crucial role in equipping vendors with the right knowledge to assure food safety and quality. Lack of training is linked with poor knowledge of food safety and inappropriate hygiene behavior (30). The other studies also showed a strong linkage between food safety knowledge with hygiene practice (31-33). For example, a vendor who has a good level of knowledge is two times higher to have good practice in comparison to those who have a poor knowledge level (33).

2.2.4 Availability of the Sanitary Facility

Lack of basic infrastructures such as water supply, wastewater drainage system, garbage disposal and latrine facility is a major challenge at the selling point of street food vending (2, 9, 23). From these infrastructures, due to limited access to water supply, street food vendors reuse water several times to clean utensils (9, 13). Additionally, because of a lack of garbage disposal, street food vendors are forced to dump waste on the street, major road, and drainage channels (5, 8). On the other hand, the delivery of these sanitary facilities reduces the gap between knowledge and safe hygiene practice in the vending sites (3). This is because sometimes food vendors may not change their knowledge into practice if these basic infrastructures are not available (28).

2.3 Environmental Condition of the Vending Site

The vending site should be clean and located far away from the waste dumpsite, wastewater, dust, and animals (34). Vending stalls must be built in such a way that enables the cleaning and maintenance process (8). In contrary to this principle the existing reality on the ground is very much different from this issue. For example, open-air vending or uncovered structures

such as wheelbarrows, carts, tables and car trunks were very dominant in most vending sites (5, 8, 30). This results in contamination of food with dust and exhaust fumes from a vehicle which may be exposed to chemical hazards such as polycyclic aromatic hydrocarbon, lead, and arsenic (35). Liu also reported that because of street food can be prepared and sold in poor environmental condition, they have the potential to get contaminated and cause foodborne illness (9). This is because pathogenic microorganisms might be transported by the dust particle and fall on the food when it is prepared on the street(8). Rane also found that place of food preparation is the main contributory factor for the contamination of food by microbes (23). Therefore,itis recommended that the place of food preparation should be always tidy entirely during working periods (34). Unclean surrounding environment leads to dirty working surface (30). Nurreddin reported that 60% of the vendor perform their job in an unclean environment with the presence of flies at all places around the vending unit. This is because of the proximity of the vending site to the waste dump site (5). Vending site close to dumping site favors the breeding of insects and rodents (24). The presence of insects around the vending site was found to increase the contamination of street vended food (36) by harboring food-borne pathogenic microbes (23).

2. 4 Bacteriological Quality of Food

Concerning the bacteriological quality of street food, many works of literature majorly determined total aerobic count (total viable count), total coliform, *Staphylococcus aureus*, and *Salmonella*. The total viable count ranges from 7.7×10^3 to 9×10^8 CFU/ml (37-40). Total coliform isolated 5.4×10^2 CFU/mo and thermotolerant coliform was 4.6×10^1 CFU/mo. The total coliform count was between the log of 3 to 4. About 67% had a total coliform count beyond the recommended level while 33% had acceptable limits (38). Khan reported that all sample contains a significant amount of microorganism and the presence of total coliform have negatively related to food quality and safety (39). Similarly, Barro et al found pathogenic microorganisms presented on utensils that street food vendors used (21) and *E.coli* was the most dominantly identified species in most of the food samples (16, 21, 37, 41). Ossai ochonogor also revealed that 69% of sampled food that greater than $\times 10^4$ CFU/g considered “unsatisfactory” while the value less than this number considered “satisfactory”(38).

Chandi revealed that using water from an open well, involving more people in processing food and residing in a house without toilet facilities, and a greater number of bacteria in sampled food are significantly associated (42). Reddi also found about 96% of the food sample were containing various pathogenic microorganisms. Besides he found that even though food handlers have food safety knowledge, there is a huge gap between knowledge and practice (43). Mekelele found that 90.5% were found to be “unsatisfactory quality”withthe total aerobic plate count test. 57.1% and 50% of Salmonella and S. aureus were reported non-compliant with French standard for cooked food respectively and unfit for consumption (44).

The study conducted in Gondar town revealed that 82% of the food sample contained different pathogenic microorganisms and about 2/3rd of them are found above the reference value when counted by the standard plate method. S.aures and Salmonella were the major species isolated (45) while the study done in Jimma town predominately found bacillus species followed by staphylococcus species (46).

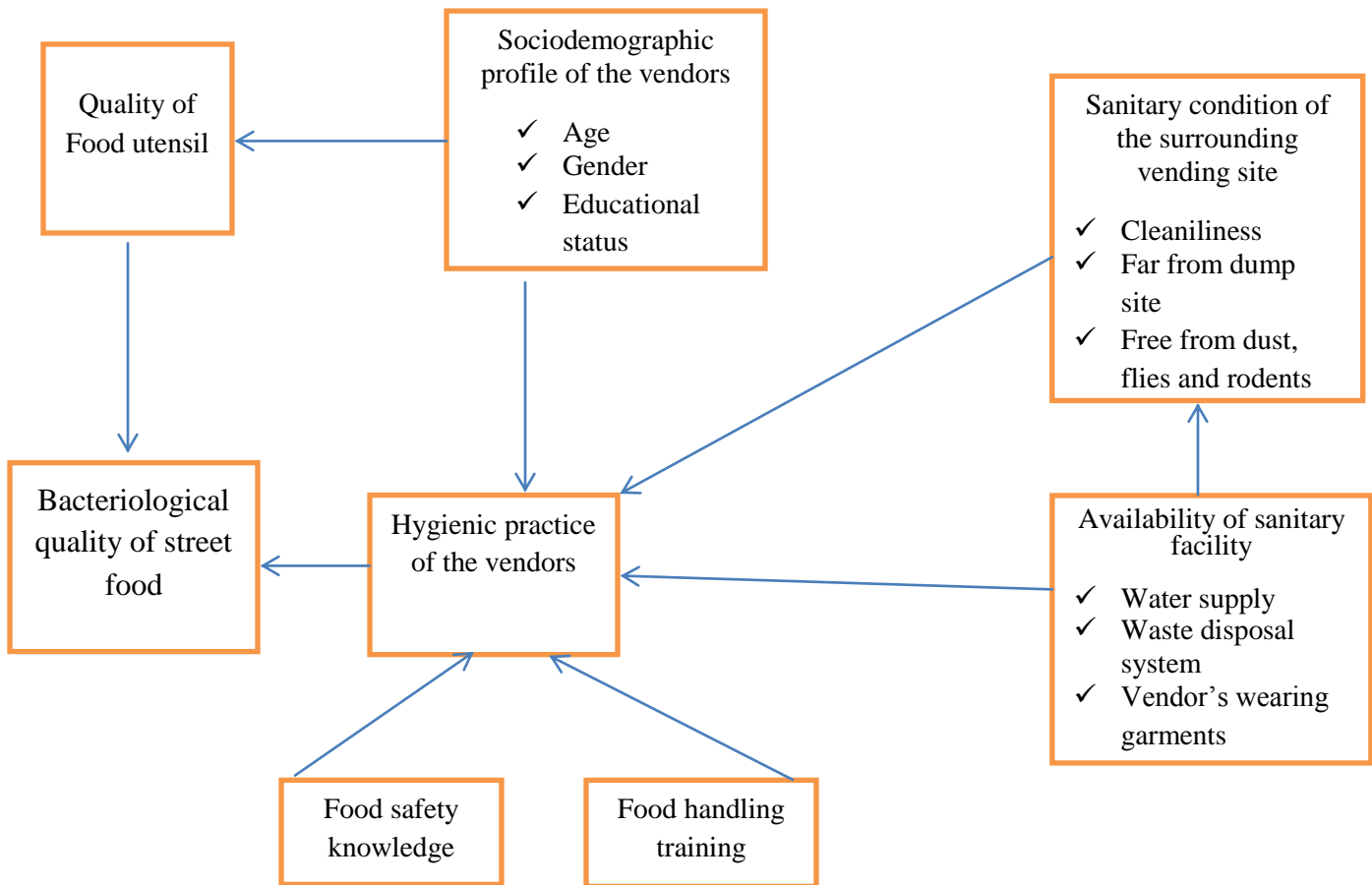


Figure 1: Conceptual framework showing the factors that affect the quality of street food.

The quality of street food is affected by different factors as it is shown in the conceptual framework. The major factors are the hygiene of the vendors, the cleanliness of the food utensils, socio-demographic profile of the vendors, availability of the sanitary facility, and sanitary conditions of the vending environment. The factors are developed from a different article related to the topic of the research. For example, some kinds of literature say that the hygiene practice of the street food vendors is affected by socioeconomic factors and other factors like age, gender, and educational level. In addition to this, different factors can determine the safety of the food starting from the quality of raw material, the way food is handled and stored. This can be shaped by food handling training and food safety knowledge. The other thing is the case of the environment in which food is sold/ prepared. If food is sold/prepared in a poor environmental condition they have the potential to be contaminated. The availability of basic infrastructure plays a great role in the hygiene practice of the vendors which in turn has a direct relationship with the quality of street food.

3: Objective

3.1 General Objective

To assess the hygiene and sanitary conditions of street food vending in Addis Ketema sub-city, Addis Ababa.

3.2 Specific objectives

- ✓ To assess the hygiene practice of street food vendors with their sanitary condition of the vending environment in Addis Ketema sub-city.
- ✓ To determine factors associated with hygiene practice of the street food vendors in Addis Ketema sub-city.
- ✓ To determine the bacteriological quality of street food in Addis Ketema sub-city.

4. Methods

4.1 Study Area

The study was conducted in the Addis Ketema sub-city of Addis Ababa city administration. Addis Ketema Sub City is one of the ten Sub Cities of Addis Ababa city bordered by Gulele Sub City in North, Lideta Sub City South, Kolfe Keranio Sub City West, and Arada Sub City in the East. It has a 7.41 sq. km area and a Population of 304,694 with a Population density per sq.m 36,659.1. Addis Mercato, Africa's largest open-air market place, is found in Addis Ketema. Because of this big market and bus station, people are traveling from different sides of the country to this place and make it overpopulated. In addition to this, through the expansion of the place, different products began to have their specific selling places. Because of this, the sub-city experienced the highest growth of street food vending. However, street food vendors carry out their work in an open environment exposed to sunlight, dust, and flies on the major roadside. Zeru Birhane in his thesis estimated 1500 street food vendors in this study area by revealing that the number of street food vendors changed from time to time and they did not vend in a similar place constantly (47).

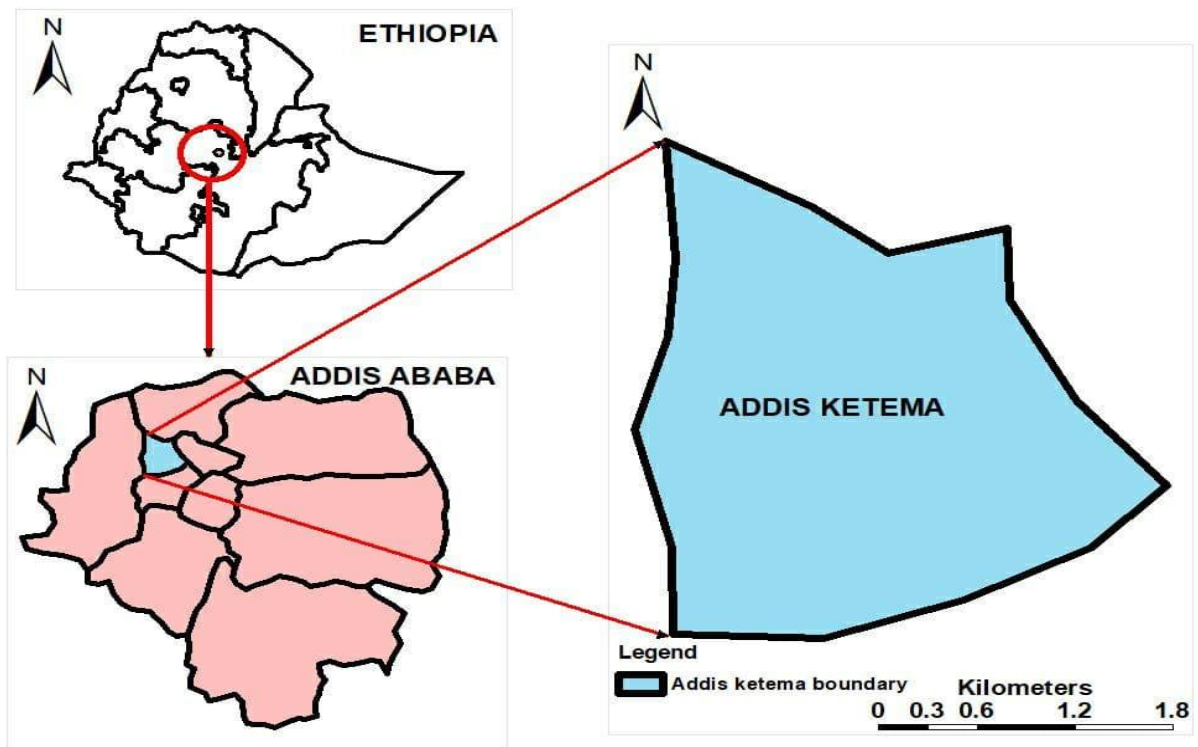


Figure 2: Map of Addis Ketema Sub-city, Addis Ababa.

4.2 Study Design

A cross-sectional study was implemented by using a structured questionnaire with face to face interview and direct observation of the surrounding environment of street food vendors to collect data.

4.3 Populations

4.3.1 Source Population

All stationary street food vendors which are found in Addis Ketema sub-city were the source population.

4.3.2 Study population

All randomly selected street food vendors of the four chosen sites of Addis Ketema sub-city (i.e. Autobus Tera, Gojjam-Cew Berenda, Sebategna, and Mesalemia) were the study population.

4.4 Eligibility criteria.

4.4.1 Inclusion criteria.

Stationary street food vendors who prepare/sell street food were included in the study.

4.4.2 Exclusion Criteria.

Stationary street food vendors who sold packed food and mobile street food vendors were excluded.

4.5 Sample Size

For the first objective, the sample size was determined using the single proportion formula with a 95% confidence interval to estimate a population proportion.

$$n = \frac{Z^2 pq}{d^2}$$

Where, n = minimum sample size Z = Z score corresponding to a 95% level of significance i.e., 1.96

P = estimated environmental sanitation status from a previous study = 24.8% (24)

q = complementary probability of $P = 1 - p$ d = degree of precision required = 5%

Substituting the above values in the formula gives

$$n = \frac{(1.96^2 \times 0.248 \times 0.752)}{(0.05)^2} = 286$$

5% of anticipated non-respondent rate was added and the final sample size will become:-
= 286 + (286 x 5%) = 286 + 14.3 = 301

For the second objective, the sample size was calculated by using the double population proportion formula.

$$n = \frac{(Z_{\alpha/2} + Z_{\beta/2})^2 \times (p_1(1-p_1) + p_2(1-p_2))}{(p_1 - p_2)^2}$$

From the works of literature, it was found that food safety knowledge, income status and food handling training are the factors associated with hygiene practice of the street food vendors (22, 48). The following assumptions were made for a specific factor to calculate sample size separately as it is displayed in the table. A type I error of 5% and power to detect the assumed difference of 80% were considered for all factors.

| factors | Z α /2 for 95% CI | Z β /2 for 80% power | P1 | P2 | n |
|------------------------|--------------------------|----------------------------|------|------|----|
| Food safety knowledge | 1.96 | 0.84 | 34.4 | 6.6 | 30 |
| Income status | 1.96 | 0.84 | 87.5 | 59.4 | 35 |
| Food handling training | 1.96 | 0.84 | 64.8 | 8.5 | 8 |

Therefore by comparing the sample size calculated for both specific objectives the larger sample size was taken. So the final sample size for this study was 301.

4.6 Sampling Procedures

Addis Ketema sub-city was selected because it is a congested slum area and there are dense street food vending activities. A thorough discussion was made with Addis Ketema sub-city FMHACA office staff to identify in which areas a considerable amount of street food vending activities were found. And after the discussion, four areas were selected i.e. Autobus Tera, Gojjam-Cew Berenda, Sebategna, and Meselamia. Hereafter, these locations can be replaced by SS1, SS2, SS3, and SS4 respectively. After area selection was taken place a walk-through survey was done by the researcher to count street food vendors as there is no baseline information and 417 street food vendors were counted. Having this information on hand, the calculated sample size was proportionally be allocated to the selected four areas. The study participants were selected by systematic random sampling through a random starting point after ordered them according to their sitting arrangements in every four sites starting from the major road then to the medium and small one. The sampling interval of the vendors in each selected area was determined by dividing the total number of vendors in each area by the allocated sample size. The initial vendor was selected by the lottery method

using the number found between one and the sampling interval and the subsequent participants were selected by adding a sampling interval on the initial participants.

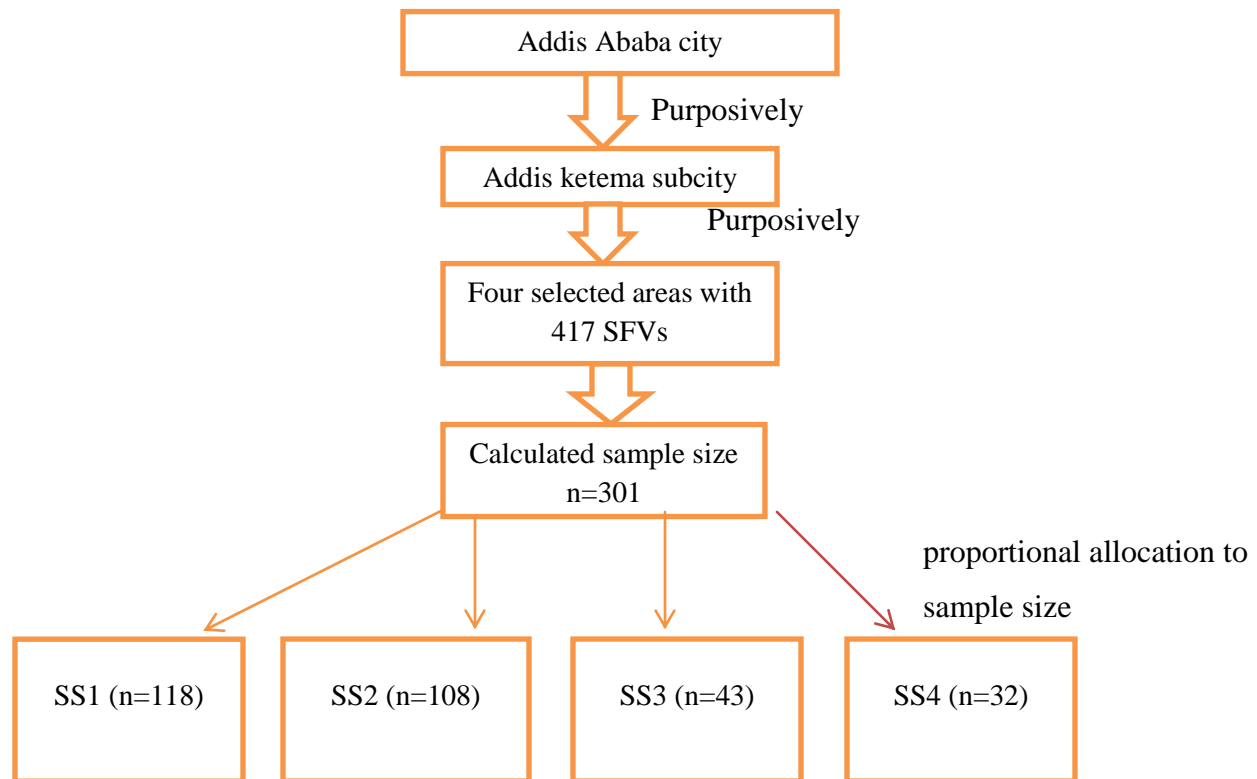


Figure 3: Sampling procedure for the assessment of hygiene and sanitary condition of street food vendors, in Addis Ketema Subcity.

4.7 Study variables

4.7.1 Dependent variables

- Vendors hygienic practice
- Bacteriological quality food samples

4.7.2 Independent variables

- Educational level, age, and gender
- Work experience
- Income status of vendors
- Adequacy of quantity water received
- Amount of money paid for water at the point of sale
- Food safety knowledge
- Environmental condition of vending sites

4.8 Data collection procedures

Data was collected using a structured interviewer-administered questionnaire, observational checklist, and lab analysis of food samples.

Questionnaire

The questionnaire was adapted from the WHO's guidelines related to the subject matter(1). Environmental health professionals and foodmicrobiologists were recruited and trained for data collection and lab analysis of samples respectively. The questionnaire was tested for clarity and validity in 5% of the sample size outside of the study area. The questionnaire was originally prepared in the English language and translated to the Amharic language. An observational checklist was also prepared to inspect the surrounding environment of the food vending unit and the practice of the vendors.

Bacteriological quality analysis

Sample collection

Concerning the bacteriological analysis of food, sixty samples of each of the selected foods were bought from the vendors in the four locations. Each food sample was collected using the vendors serving utensils and placed in sterile aluminum foil aseptically. The samples were labeled appropriately according to their sampling site, date, and code number. They were immediately placed in a cold box with lower temperatures and transported to the laboratory within one to threehours. The samples were refrigerated at 3-5⁰c and analyzed as the procedure of Ethiopian standardi.e ES ISO 48331:2015),(ES ISO 4831:2015), (ES ISO 6579:2012).

Sample processing

10g of food sample was measured and transferred to 90 ml of peptone water and homogenized by rotary shaker or in an aseptic environment which was achieved by cleaning and disinfecting by different disinfectants as well as using Bunsen burner flame. Further decimal dilutions (10⁻¹, 10⁻², 10⁻³, and 10⁻⁴) were made by taking 1 ml from the homogenized sample and adding to a sterile test tube containing 9ml sterile alkaline peptone water and mixed properly by a rotary shaker.

Aerobic mesophilic bacterial count (AMBC)

Serial dilutions were made by transferring 1ml of the previous dilution in 9ml of 0.1% peptone water. AMBC was made by incubating surface plated duplicate decimal dilutions of food samples on plate count agar (Oxid, UK) at 30°C for 72 hours. Dilutions with the total

number of colonies on a plate fewer than 300 per plate were selected and colonies were counted and calculated.

Enumeration of total coliforms:

This test of food quality assessment was analyzed with the enumeration method. Lauryl tryptose broth and brilliant green lactose bile broth were used as selective enrichment medium and confirmation medium, respectively. Three tubes of double-strength liquid selective enrichment medium were inoculated with a specified quantity of the test portion. Then the tubes were incubated at 30 °C for twenty-four hours and examined for gas formation. A similar process was applied in the confirmation medium. MPN/g is calculated from the table.

Isolation of Salmonella

Buffered peptone water inoculated at ambient temperature with test portion and incubated for 18h at 37°C during pre-enrichment of the stage of salmonella detection. Then 0.1ml of culture and 10ml of RVS broth incubated for 24 hr at 41.5°C, and 1ml of culture with 10ml of MKTT broth incubated for 24 hrs at 37°C. Following this step, XLD medium and second agar of choice are inoculated and incubated for 24hrs at 37°C. Typical colonies of Salmonella (colorless colonies with or without black centers) were picked, transferred to 5 ml nutrient broth (Oxoid), incubated at 37°C for 24 h, then streaked onto Nutrient Agar (Oxoid) for purity, and incubated at 37°C for 24 h and further characterized through a sequence of the biochemical test.

4.9 Operational Definitions

Vendor: the person who participates in street food vending activities.

Street food: ready to eat food in the form of solid or liquid that is sold on the street such as *Sambusa, Bombolino, Ambasha, Biscuit, Soup, Potato, Chips*.

Vending site: a place (location) in which street food is prepared/sold by the cluster.

Vending unit: one unit from a group of the cluster with their operators.

Hygiene practice level: a vendor who scores less than 12 points out of eighteen hygiene practice-related questions was considered as having a poor level of practice, whereas those who scored greater or equal to 12 points were considered a good level of hygienic practice.

Good environmental condition: a score of 4.5 points and above out of the seven items assessed related to surrounding of vending site.

Poor environmental condition: a score below 4.5 points out of seven items assessed related to the surrounding of vending site.

Food safety knowledge: vendors who score four points and above out of six evaluation points are considered as knowledgeable food vendors and those who score less than this point are considered as not knowledgeable.

4.10 Data Analysis Procedures

Data were entered by using EpiData version 3.1 and was cleaned before exporting to SPSS. Univariate analysis was done to describe and summarize the data using summary statistics such as mean and standard deviation. Percentages and proportions were calculated for each quantitative variable. The results of the analyses were presented in tables and charts as appropriate. About 18 questions were set to measure street food vendor's hygiene practices. A score of "1" was assigned for the correct hygiene practice whereas a score of "0" was assigned for wrong hygiene practice for each question. Then the total score was summed up and converted to 100 percent. For further analysis vendors who scored less than 12 points were considered as poor and those who scored 12 points and above considered good hygiene practice. Similarly, seven items were selected to assess the sanitary condition of vending sites and a score of 4.5 points and above classified as good environmental conditions while a score less than 4.5 points classified as a poor environmental condition. A similar trend was followed for analyzing food safety knowledge. All independent variables were fit separately into a bivariate logistic model to evaluate the degree of association with the dependent variable. Then variables that have a p-value ≤ 0.25 were exported to a multivariable logistic regression model to control for confounders. Multivariable logistic regression was performed to identify the association of independent variables with the dependent variable. The strength of association was measured using OR and 95% CI and a p-value < 0.05 considered statistically significant. Omnibus Tests and Hosmer and Lemeshow Test were used and both models have shown good fitting in the data.

4.11 Data Quality Management

The researcher provided three days training for one supervisor and four data collectors about questions and the way how to approach and treat the respondents. Data were collected by four data collectors and they were supervised by the principal investigator and co-

investigator every day concerning completeness and reliability of the data. After data collection is completed data entry was followed using EpiData software and data was cleaned before further analysis. Concerning the bacteriological quality analysis; the preparation, production, and performance testing of culture media was done according to ES ISO-11133.

4.12 Ethical Consideration

Approval was secured first from the Institutional Ethical Review Committee of Addis Ababa University, College of health science school of public health. A written letter from the school of public health was obtained and submitted to the Addis Ketema Sub City FMHACA office. Informed verbal consent was obtained from each respondent and they were also told to have the right to give-up the interview any time s/he wishes and the information was kept confidential.

4.13 Dissemination of Results

The finding of the study was disseminated to Addis Ababa University, College of health science school of public health, federal ministry of health, Addis Ababa FMHACA, Addis Ketema sub-city FMHACA office.

5. Result

A total of 301 street food vendors have participated in this study and the response rate was 95.02%. The majority of the participants were females 227(79.4%) and their mean age was 28 ± 6.756 . About 1/4th of them completed a secondary level of education whereas 160(53.2%) of them completed the primary level of education. Among 301 study participants 157(52.2%) were married and 111(36.9%) were single as it is shown in Table 1. The majority of respondents have work experience below three years 157(52%) and average working hours per day from nine to twelve hours.

Table 1: Socio-demographic characteristics of street food vendors in Addis Ketema Subcity, Addis Ababa June, 2020.

| S.no | Socio-demographic variable | | Frequency | Percent(%) |
|------|------------------------------|----------------------|-----------|------------|
| 1 | Age | Below 20 | 29 | 9.6 |
| | | 20-30 | 188 | 62.5 |
| | | 31-40 | 69 | 22.9 |
| | | 41-50 | 11 | 3.7 |
| | | Above 50 | 4 | 1.3 |
| 2 | Sex | Male | 74 | 24.6 |
| | | Female | 227 | 75.4 |
| 3 | Marital status | Single | 111 | 36.9 |
| | | Married | 157 | 52.2 |
| | | Divorced/separated | 20 | 6.6 |
| | | Widowed | 13 | 4.3 |
| 4 | Level of education | No formal education | 56 | 18.6 |
| | | Primary | 160 | 53.2 |
| | | Secondary | 77 | 25.6 |
| | | Certificate | 8 | 2.7 |
| 5 | Average monthly income | Low(<2000ETB) | 105 | 34.9 |
| | | Middle(2001-3300ETB) | 93 | 30.9 |
| | | High(>3300) | 103 | 34.2 |
| 6 | Work experience | Below 3 years | 157 | 52.2 |
| | | 3.1-6 Years | 115 | 38.2 |
| | | 6.1-9 Years | 29 | 9.6 |
| 7 | Average working hour per day | Below 4 hours | 51 | 16.9 |
| | | 4-8 hours | 64 | 21.3 |
| | | 9-12 hours | 185 | 61.5 |
| | | Above 12 hours | 1 | 0.3 |

Water supply and raw materials used by the street food vendors

From the surveyed participants, 183(60.8%) responded price as their main attribute to purchase raw materials while 15% of them choose appearance. About 207(68.8%) get water from the storage tank by paying money and 29.2% get it from home to cook food. The majority of the vendors, 271(90%) use Jerry-Can to store water at the point of sale while 26(8.6%) of them use a bucket. Concerning the types of water used to wash utensils, 217(72%) use cold water with detergents and 66(21.9%) use hot water with detergents.

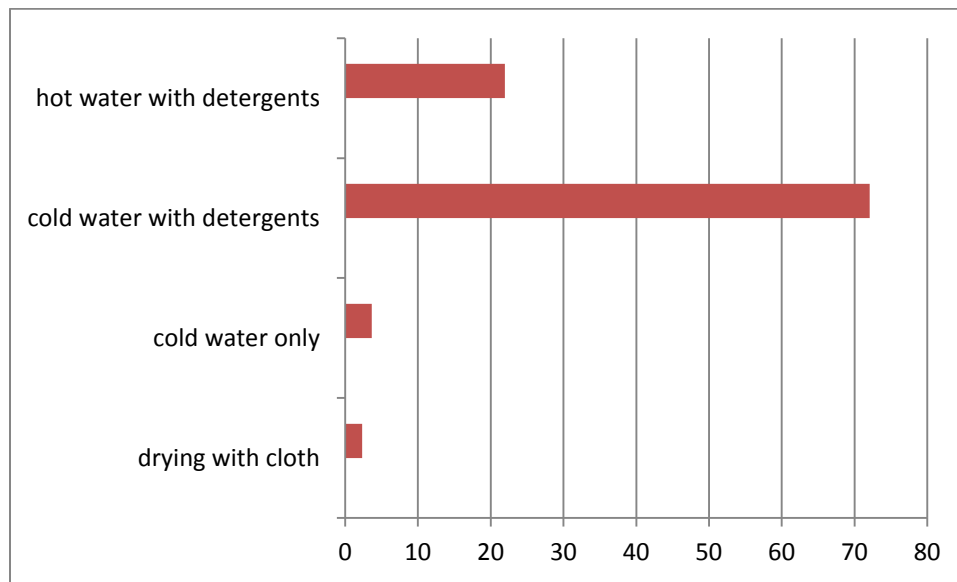


Figure 3: The types of water that street food vendors used to wash utensils in Addis Ketema sub-city, June 2020.

The level of hygiene practice by the street food vendors during different stages of food processing

Only 5% of them were taken formal training regarding food safety while 51.5% participated in such activity by observing others. Of the 301 street food vendors who participated in this study 232(77%) prepare food on the street and 62(20.6%) in the home. About 162(53.8%) of the vendors prepare food in the morning. In terms of the way food is served, 134(44.5%) of them serve food with a bare hand for their customers and 152(50.5%) by the plate. Concerning the way of transporting food 154(54.2%) reported as food is transported by human resources. The study revealed that 258(85.7%) vendors store ready to eat food openly in the street/stall. From study participants, 40.9%, 32.9%, and 16.3% reported that leftover is given to paupers, consumed by the household member, and discarded in waste containers

respectively. 192(63.8%) of the street food vendors use a plastic plate to serve while 104(34.6%) use a metal plate. About 199(66.1%) did not wash their hands before handling food and 256(85%) handle money while handling food items.

Table2: The practice of the street food vendors in different stages of food processing in Addis Ketema Subcity, June 2020.

| S.no | Variables | Response | Frequency | Percent (%) |
|------|---|-----------------------------------|-----------|-------------|
| 1 | How skill is acquired | Formal training | 15 | 5.0 |
| | | Parents | 129 | 42.9 |
| | | Observation | 155 | 51.5 |
| | | Others | 2 | .7 |
| 2 | Place of food preparation | Home | 62 | 20.6 |
| | | Stall/Street | 232 | 77.1 |
| 3 | How did you prepare food | Food is cooked during a sale | 108 | 35.9 |
| | | Food is cooked on the morning of | 162 | 53.8 |
| | | Others | 31 | 10.3 |
| 4 | How Prepared or ready-to-eat food is stored | Openly in the stalls | 258 | 85.7 |
| | | Covered utensils/warmer/coolers | 37 | 12.3 |
| | | Wheelbarrows | 5 | 1.7 |
| | | Others | 1 | .3 |
| 5 | How leftover is managed | Consumed by a member of the house | 99 | 32.9 |
| | | Stored for use next day | 19 | 6.3 |
| | | Discarded in the waste container | 49 | 16.3 |
| | | Given to paupers | 123 | 40.9 |
| | | Others | 11 | 3.7 |
| 6 | Utensils to serve food | Metal plates | 104 | 34.6 |
| | | Plastic plates | 192 | 63.8 |
| | | Cup | 5 | 1.7 |
| 7 | Washing hands before handling food | Yes | 102 | 33.9 |
| | | No | 199 | 66.1 |
| 8 | Handling money during food processing | Yes | 256 | 85.0 |
| | | No | 45 | 15 |

In addition to the above mentioned hygiene practice; half of the vendors did not separate the fresh food from ready-to-eat ones, about 28% of them sanitize utensil only three times a day, 47% of them did not wash their hand after handling raw food. Nearly 21% of the respondents prepare food even if they feel ill due to daily life pressure and about 76% did not reheat food.

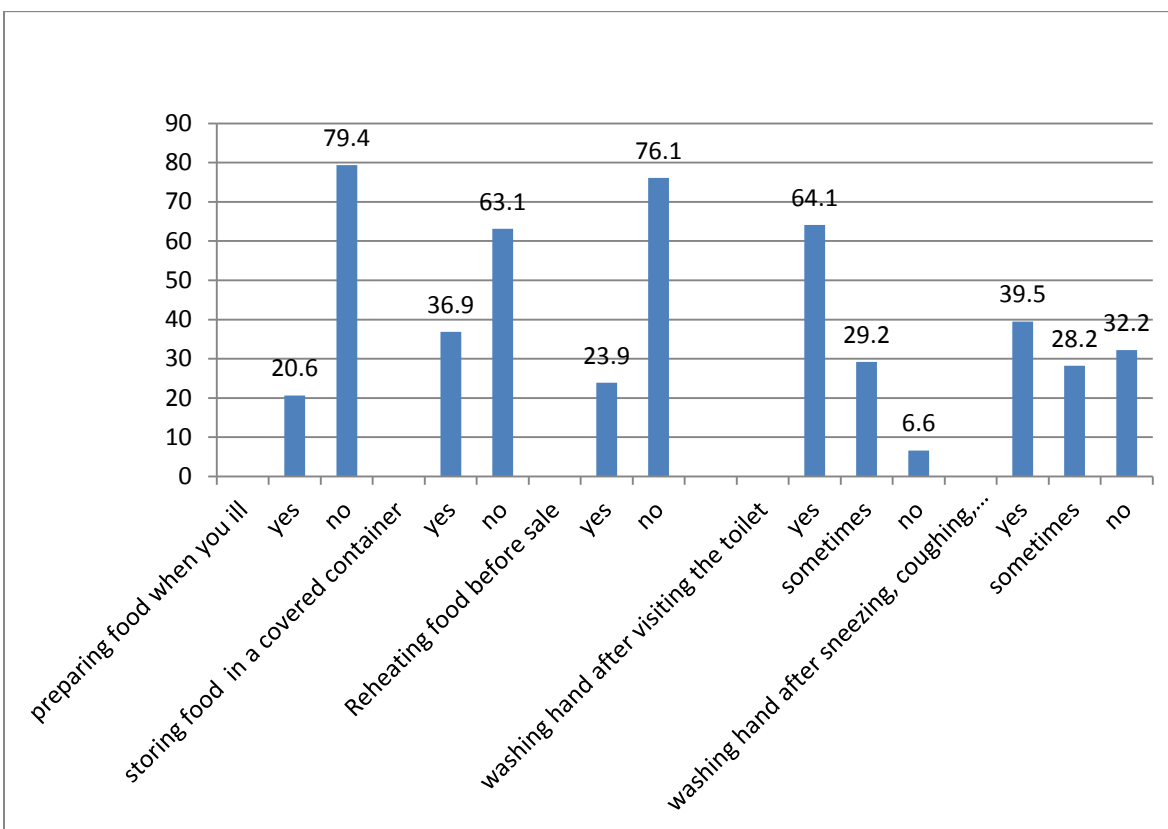


Figure 4: The hygiene practice of street food vendors in Addis Ketema Subcity, June 2020.

The environmental condition of the surrounding vending sites

Almost 86% of the study participants responded that the surrounding of the vending environment is not free from potential contaminants. Of these participants, about 76% answered dust as the potential contaminant, about 9% responded fly as the possible contaminant, 2.7% of them replied street waste and the rest of the food vendors raised the combination of these factors as the potential contaminants. The majority of them (82.7%) have access to latrine facilities by paying money either from public or private. Moreover, 213(64%) reported they encounter pests and rodents in the vending site. Furthermore, 83.1% of the participated street food vendors work in an unclean environment.

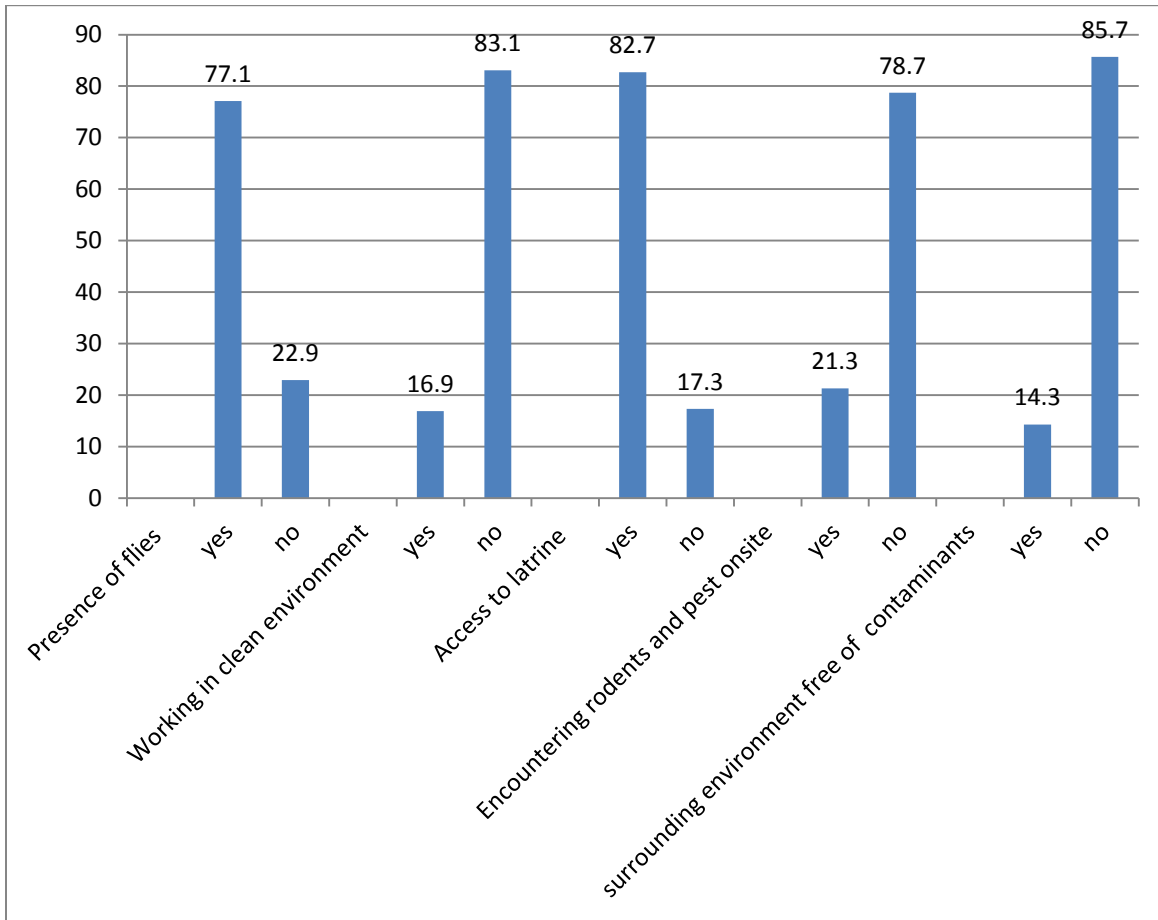


Figure 5: The environmental condition of street food vending sites in Addis Ketema Subcity, June 2020.

The overall compliance score of hygienic practice by the street food vendors

The mean overall compliance score of hygienic practice is 8.57 ± 3.18 . For further analysis, both the hygiene practices of the vendors and the environmental conditions of vending units are categorized as good and poor. Consequently, about 82% of the vendors have poor hygiene practices, and 72% of the surveyed participants carry out their work in poor environmental conditions.

Factors associated with hygiene practice of the street food vendors

The result of bivariate analysis in the surveyed vendors, hygiene practice as the outcome variable, and other predictor variables are shown in Table 3. The result shows that adequacy of the quantity of water received, food safety knowledge, age of the vendors, Amount of payment for water at the point of sale, and environmental conditions of the vending site significantly associated with hygiene practice of the vendors.

Table 3: Bivariate analysis of the level of hygiene practice of the street vendors in Addis Ketema Sub-City, Addis Ababa, June 2020

| Variable | Response | Hygiene practice | | COR(95% CI) | P-value |
|-----------------------------------|---------------------|------------------|------------|---------------------|---------|
| | | Good (%) | Poor (%) | | |
| Sex | Male | 64(86.5) | 10(13.5%) | 1 | |
| | Female | 184(81.1%) | 43(18.9%) | .669 (.318, 1.408) | .289 |
| Educational status | No formal education | 47(83.9%) | 9(16.1%) | 1 | |
| | Primary | 141(88.1%) | 19(11.9%) | .704(.298, 1.661) | .423 |
| | Secondary | 56(72.7%) | 21(27.3%) | 1.958(.819, 4.683) | .131 |
| | Certificate | 4(66.7%) | 2(33.3%) | 2.611(.414, 16.459) | .307 |
| Marital status | Single | 89(80.2%) | 22(19.8%) | 1 | |
| | Married | 133(84.7%) | 24(15.3%) | .730(.386, 1.381) | .333 |
| | Divorced/Separated | 16(80.0%) | 4(20.0%) | 1.011(.307, 3.327) | .985 |
| | Widowed | 10(76.9%) | 3(23.1%) | 1.214(.308, 4.786) | .782 |
| Monthly income | Low | 83(79%) | 22(21%) | 1 | |
| | Middle | 82(88.2%) | 11(11.8%) | 1.140 (.579, 2.242) | .705 |
| | High | 83(80.6%) | 20(19.4%) | .599 (.270, 1.328) | .207 |
| Paying for water | Yes | 244 (82.7%) | 51 (17.3%) | .418 (.075, 2.344) | .321 |
| | No | 4 (66.7%) | 2 (33.3%) | 1 | |
| Age(years) | Below 20 | 26(89.7%) | 3(10.3%) | 3.333(0.157,70.906) | .440 |
| | 20-30 | 164(87.2%) | 24(12.8%) | .077(0.007,0.805) | .032 |
| | 31-40 | 56(81.2%) | 13(18.8%) | .049(0.005,0.488) | .010 |
| | 41-50 | 1(9.1%) | 10(90.9%) | .038(0.003,0.497) | .013 |
| | Above 50 | 1(25%) | 3(75%) | 1 | |
| Is the quantity of water that you | Yes | 41(25%) | 123(75%) | 3.472(1.742,6.921) | .000 |

| | | | | | |
|--|------------------------------|-------------|------------|---------------------|------|
| receive adequate | No | 12(8.8%) | 125(91.2%) | 1 | |
| Work experience(years) | Below 3 | 120(76.4%) | 37(23.6%) | 1.871(0.401,8.740) | .426 |
| | 3.1-6 | 101(87.8%) | 14(12.2%) | 4.162(0.945,18.338) | .059 |
| | 6.1-9 | 27(93.1%) | 2(6.9%) | 1 | |
| The environmental condition of the vending site | Poor environmental condition | 28(13.0%) | 187(87.0%) | .365(0.198, 0.674) | .001 |
| | good environmental condition | 25(29.1%) | 61(70.9%) | 1 | |
| Food safety knowledge | Not knowledgeable | 110(97.3%) | 3(2.7%) | .075(0.023,0.248) | .000 |
| | knowledgeable | 138(73.4%) | 50(26.6%) | 1 | |
| Having access to latrine facility | Yes | 206 (82.7%) | 43 (17.3%) | 1.141(.531, 2.449) | .736 |
| | No | 42 (80.8%) | 10 (19.2%) | 1 | |
| Amount of payment for water at the point of sale | | | | 1.021(.991, 1.051) | .171 |

The independent variable with p-value <0.25 was taken as initial inclusion in multivariable logistic regression analysis to determine their association with hygiene practice. According to the result of multivariable analysis, Vendors who do not have safety knowledge were 95% less likely (AOR=0.050, 95% CI (0.012, 0.219)) to have good hygienic practice as compared to those who have good knowledge about food safety. Vendors who work in the poor environmental condition of vending sites were 74% less likely (AOR=0.265, 95% CI (0.116, 0.607)) to have good hygienic practice as compared to those who work in the good environmental condition of the vending site.

Table 4: Multivariable logistic regression result of the level of hygiene practice of the street vendors in Addis Ketema Sub-City, Addis Ababa, June 2020.

| Variable | | Hygiene practice | | COR(95%CI) | AOR(95%CI) |
|--|-------------------|------------------|------------|---------------------|----------------------|
| | | Good | poor | | |
| Adequacy of the quantity of water received | Yes | 41(25%) | 123(75%) | 3.472(1.742,6.921) | .921(.382, 2.220) |
| | No | 12(8.8%) | 125(91.2%) | 1 | |
| Age (years) | Below 20 | 26(89.7%) | 3(10.3%) | 3.33(0.157,70.906) | 3.069(.048, 197.628) |
| | 20-30 | 164(87.2%) | 24(12.8%) | 0.077(0.007,0.805) | .057(.002, 1.948) |
| | 31-40 | 56(81.2%) | 13(18.8%) | 0.049(0.005,0.488) | .042(.001, 1.372) |
| | 41-50 | 1(9.1%) | 10(90.9%) | 0.038(0.003,0.497) | .027(.001, 1.101) |
| | Above 50 | 1(25%) | 3(75%) | 1 | |
| Food safety knowledge | Not knowledgeable | 110(97.3%) | 3(2.7%) | 0.075(0.023,0.248) | .050(.012, .219) *** |
| | Knowledgeable | 138(73.4%) | 50(26.6%) | 1 | |
| Environmental condition of the vending site | Poor | 28(13.0%) | 187(87.0%) | 0.365(0.198, 0.674) | .265(.116, .607) ** |
| | Good | 25(29.1%) | 61(70.9%) | 1 | |
| Amount of payment for water at the point of sale | | | | 1.021(.991, 1.051) | .987(.946, 1.029) |

*Significant < 0.05; **significant at p < 0.01; ***significant at p < 0.001.

Hosmer and Lemeshow Test (Sig.0.665)

Bacteriological quality analysis of street foods

The mean aerobic mesophilic bacteria count of SS4 was relatively higher than other sites in all food samples with the value 5.3 logCFU/g and above in three sample collection days. *Sambusa* showed the highest count in aerobic mesophilic bacteria count and total coliform count in all vending sites. Salmonella tested positive (presence) for *Sambusa* in all sites except for SS4 in the three days. In other ways, 86.7% of the sample showed an unsatisfactory level for aerobic mesophilic bacteria count and about 91.7% of the street vended food showed a satisfactory level for the total coliform count. Moreover, about 5(9%) of the sample showed presence and 55(92%) of the sample showed absence for the Salmonella test.

Table 5: The mean±SD(logCFU/g) of three days aerobic mesophilic bacteria count, total coliform, and Salmonella test of street food in Addis Ketema sub-city June 2020.

| Place of vending | Sample type | Sample size(n=60) | TAMBC | Total coliform | Positive for Salmonella |
|------------------|------------------|-------------------|-----------|----------------|-------------------------|
| SS1 | <i>Ambasha</i> | 6 | 5.24±0.39 | 1.23±0.17 | - |
| | <i>Biscuit</i> | 6 | 5.36±0.48 | 1.39±0.23 | - |
| | <i>Bombolino</i> | 6 | 4.19±0.97 | 1.38±0.15 | - |
| | <i>Sambusa</i> | 6 | 6.65±1.67 | 1.58±0.38 | 2 |
| SS2 | <i>Ambasha</i> | 3 | 5.56±1.11 | 1.47±0.32 | - |
| | <i>Biscuit</i> | 3 | 5.42±0.24 | 1.48±0.14 | - |
| | <i>Bombolino</i> | 3 | 4.05±0.79 | 1.42±0.17 | - |
| | <i>Sambusa</i> | 3 | 6.51±1.08 | 2.02±0.04 | 1 |
| SS3 | <i>Ambasha</i> | 3 | 3.99±0.70 | 1.45±0.02 | - |
| | <i>Biscuit</i> | 3 | 5.14±0.45 | 1.29±0.10 | - |
| | <i>Bombolino</i> | 3 | 3.77±0.74 | 1.18±0.20 | - |
| | <i>Sambusa</i> | 3 | 7.4±0.58 | 1.56±0.45 | 2 |
| SS4 | <i>Ambasha</i> | 3 | 5.6±1.11 | 1.46±0.23 | - |
| | <i>Biscuit</i> | 3 | 5.77±0.70 | 1.45±0.13 | - |
| | <i>Bombolino</i> | 3 | 5.31±0.68 | 1.31±0.15 | - |
| | <i>Sambusa</i> | 3 | 7.22±0.71 | 1.7±0.46 | - |

The mean count of aerobic mesophilic bacteria was highest in *Sambusa* with 6.89 log CFU/g and lowest in *Bombolino* 4.30 log CFU/g. The aerobic mesophilic bacteria count ranges from 3.21 to 6.81 log CFU/g for *Ambasha*, 4.54 to 6.56 log CFU/g for *Biscuit*, 3.05 to 5.83 log CFU/g for *Bombolino*, and 3.82 to 7.92 log CFU/g (Table 7). Similarly, the mean count of the total coliform count was highest in *Sambusa* with 1.69 log CFU/g and ranges from 1.11 to 2.05 log CFU/g.

Table 6: Mean±SD (log CFU/g) aerobic mesophilic bacteria count and total coliform counts of analyzed street food items in Addis Ketema sub-city June 2020.

| Serial no | Sample type | Number of samples | Mean of AMBC | Mean of total coliform count |
|-----------|------------------|-------------------|--------------|------------------------------|
| 1 | <i>Ambasha</i> | 15 | 5.13±0.92 | 1.29±0.41 |
| 2 | <i>Biscuit</i> | 15 | 5.41±0.48 | 1.32±0.40 |
| 3 | <i>Bombolino</i> | 15 | 4.30±0.93 | 0.99±0.63 |
| 4 | <i>Sambusa</i> | 15 | 6.89±1.19 | 1.70±0.37 |

The aerobic mesophilic bacteria count ranges from 3.35 to 7.96 log CFU/g, 3.16 to 7.46 log CFU/g, and 3.06 to 7.97 log CFU/g for SS1, SS2, and SS3 respectively. In other ways, the food sample taken from four vending sites ranges between 1.17 and 1.50 log CFU/g with total coliform count (Table 8). A Kruskal-Wallis H test was conducted to determine whether there is a significant difference between bacteriological test and a place of vending, and the result showed that there was no statistically significant difference in aerobic mesophilic bacterial count between the different vending sites, $\chi^2 = 4.089$, $p = 0.252$, with a mean rank of aerobic mesophilic bacterial count 29.46 for SS1, 30.13 for SS2, 24.71 for SS3 and 38.75 for SS4. Similarly, the Kruskal-Wallis test showed that there was no significant difference in the total coliform count among vending places, $\chi^2 = 5.044$, $p = 0.169$.

Table 7: Mean (log cfu/g) aerobic mesophilic bacteria and total coliform counts of street food with the place of vending site in Addis Ketema sub-city June 2020.

| Serial no | Sample type | Number of samples | Mean of AMBC | Mean of total coliform count |
|-----------|-------------|-------------------|--------------|------------------------------|
| 1 | SS1 | 24 | 5.36±1.30 | 1.24±0.54 |
| 2 | SS2 | 12 | 5.39±1.18 | 1.49±0.55 |
| 3 | SS3 | 12 | 5.08±1.59 | 1.17±0.60 |
| 4 | SS4 | 12 | 5.98±1.04 | 1.50±0.27 |

6. Discussion

The present study found that about 82% of the vendors have poor hygiene practices. This is the combined effect of the way how they keep their personal hygiene and their practices at different stages of food processing stages. This inturn determines street food quality. The bacteriological quality of food also showed the contamination beyond the permissible level of indicator organisms. Moreover, the study found that food safety knowledge and environmental condition of the vending site are the factors associated with the hygiene practice of the vendors.

From the survey, it was found that out of the total study participants 79.4% were females. In low-income countries, street food paves the way for employment creation for the majority of women (49). The finding of the study is comparable with studies elsewhere (5, 28, 50). 53.2% of the vendors completed primary education and about 25% of them complete secondary education. This is due to the informal nature of the business in which, anyone who is interested to generate income can involve easily in such a sector of the economy without noticing how to prepare safe food.

About 68% of the vendors can get water from the storage tank by paying money both for water vendors and those who carry it. This is higher than that is reported by Nureddin (5) in Nigeria, which is about 57.6%, and concerning the types of water used in utensil washing about 72% of the vendors used cold water with detergents. Muyanja et al (8) also revealed that 76.9% of street food vendors used soap and cold water to clean cups and plates. Using only cold water facilitates cross-contamination among utensils and cooked food due to oily and impure rinse water.

This specific study found that about 45% of the vendors serve food with a bare hand for their customers. This finding is comparable with the study done by Chukuezil that about 47% handled food with bare hands (29). Touching food manually could lead to contamination and the entrance pathogenic microbes on foods if the street food vendors did not clean their hands appropriately. It is recommended to use tidy tongs, forks, spoons, and not reusable gloves during handling food (51). Moreover, it was found that about 258 (85.7%) of the vendors store ready to eat food openly in the street. The study conducted in Shijiazhuang city, China revealed that 66% of them exposed food to dust, fumes, and flies (9).

About 199(66.1%) of the street food vendors did not wash their hands prior to handling food items and 85% of the study participants handle money during food processing.

This is greater (29) than that is reported by the study conducted in Owerri, Nigeria which is 62% and lower than of Florianopolis, Brazil(95%) (19). These variations might be due to socio-demographic factors such as educational status and food handling training among street food vendors. Handling money while serving food may introduce contaminants through hand contact with the food because the frequency of touching money by different people is high. Barro et al found pathogenic microbes on the piece of money and the risk is increased when the vendors serve food with bare hands (21).

The study found that about 45.2% of the street food vendors in Addis Ketema sub-city reported as they dispose of waste in the waste container, about 35.5% on the road, about 9.6% in drainage/gutter, and 6.6% of them dump on the open area which is comparable with the study conducted in Nigeria. Nureddin et al found less than 40% used the waste bin and the rest of the wastewas dumped on the street, major road, and drainage channel (5).

About 18% of the vendors had a good level of hygiene practice and on the reverse to this 82% of them had a poor level of hygiene practice. This result is lower than that of the study conducted in Dessie town which was (53%) and a study conducted in Gondar (58%) in Ethiopia (22). This is due to the poorhygiene profile of the participants, food safety knowledge and the congested settlement of the sub-city as a result of big markets and bus station with poor infrastructure at the street food vending sites. Moreover, during data collection it was observed that about 48% of the vendors did not cover their hair while handling food, about 63% of them did not store food in covered containers, 76% of the participants did not reheat food before a sale, half of them wore hand jewelry during food processing and about 42% the vendors had long nails.

According tothe result of multivariable logistic regression,vendors who have poor knowledge about safety were 95% less likely (AOR=0.050, 95% CI (0.012, 0.219) to have good hygienic practice as compared to those who have good knowledge about food safety. This is because anyone who has knowledge concerning food safety can easily understand the consequence behind the factor. Muyanja and sezgin found that food safety knowledge is a risk factor for foodborne illness (2, 8). Different studies found a similar result which states that hygiene practice is directly related to the status of food safety knowledge (27, 52, 53). However, eventhough good knowledge and hygiene practice is associated due to a shortage of water and lack of infrastructure knowledge does not change to practice. This means the unavailability of these facilities hinders their commitment to hygiene practice (28).

Vendors who work in the poor environmental condition of vending sites were 74% less likely (AOR=0.265,95% CI (0.116, 0.607) to have good hygienic practice as compared to those who work in the good environmental condition of the vending site. This is because poor environmental sanitation in the place where foods are vended can contribute to poor food storage and transport conditions. Lack of waste disposal infrastructures paves the way to the accumulation of waste at food vending sites. This facilitates a favorable environment for the pest population which in turn increased the risk of food contamination and the spread of foodborne infections (24, 54). Furthermore, FAO strongly recommends a vending place should be located far away from any source of contamination such as wastewater, dust, rubbish, and animals (34).

Bacteriological quality analysis of street food

The aerobic mesophilic bacterial (AMBC) counts range from 3.77 to 7.4 logCFU/g. The study conducted in Jimma found that the mean count of AMB ranges from 4.0 to 6.6 log CFU/g. The high AMBC shows the low-quality standard of street vended foods (15). The study conducted in Hawassa also reported that the total aerobic count ranges from 5.3 to 6.83 log CFU/g. Such differences are majorly attributed to variation in food contents, preparation methods, personal hygiene, food handling, and serving mechanism of vendors(16). Another study conducted in Nagpur city, India also reported between 4 and 6.6 log CFU/g (40) while Derbew et al reported aerobic mesophilic count between 5.04 and 5.56 log CFU/g among the sampled food items (45).

According to the Microbiological quality guide for ready-to-eat foods, food samples with mean AMB ≥ 4 log CFU/g belonged to an “unsatisfactory level” (55). About 86.7% of the sample showed an unsatisfactory level for total aerobic mesophilic bacteria count. This finding is almost comparable with the study conducted in the Democratic Republic of Congo which is 90.5% were found to be the unsatisfactory quality with total aerobic plate count analysis (44) and higher than that of a study done in Gondar which is reported as 65.5% of them are found above the recommended limits (17).

The mean count of total coliform in the present study is between 1.18 to 2.02 logCFU/g. The guideline sets the limit for total coliform < 2 log CFU/g as satisfactory and ≥ 2 log CFU/g as unsatisfactory (55). The cause of coliform to occur in food is fecally contaminated water or food after preparation. This can be linked to street-vended foods as a result of improper processing, inadequate heating, using unclean water during

preparation, and from contaminated surfaces such as “chopping boards, knives, and serving wares” (56). The finding is lower than the study conducted by Atkur Titarmare which was in the range of a log of 3-4 in almost all the samples (40) and another study that dictates the level of coliforms between 2.48 to 3.81 log CFU/g (45). These variations may be due to the kinds and number of food samples, food contents, environmental sanitation and personal hygiene of the vendors and the extent of exposure of the foods to the different foreign substances.

The present study found that about 9% of the sample indicated a positive test for *Salmonella*. The finding is greater than the study conducted in Gondar which revealed that any food sample did not show a positive result for the *Salmonella* test (45) and less than the study conducted by Nemo et al and Tesfaye et al which is reported as 13.13% and 12.9% samples were positive for *Salmonella* respectively (15, 46). In principle, *Salmonella* species should not be found in ready-to-consumed food (55). Because the consumption of foods that contain pathogenic micro-organisms may result in food-borne diseases. On the other side, the presence of this pathogen in the food is an indicator of poor food preparation and handling practices (57).

The mean count of aerobic mesophilic bacteria was highest in *Sambusa* with 6.89 log CFU/g and lowest in *Bombolino* 4.30 log CFU/g. Tesfaye W. et al revealed that *Sambusa* was highly contaminated with bacteria. The reason attributed to such a result is that *Sambusa* is handled excessively during transport, storage and sale (15). The aerobic mesophilic bacteria ranges from 3.21 to 6.81 log CFU/g for *Ambasha*, 4.54 to 6.56 log CFU/g for *Biscuit*, 3.05 to 5.83 log CFU/g for *Bombolino*, and 3.82 to 7.92 log CFU/g for *Sambusa*. Most processed foods are considered harmful if they have large populations of aerobic mesophilic microorganisms, even if the organisms are non-pathogens (58). The study done by Nemo revealed that aerobic mesophilic bacteria count was found with 6.6 log CFU/g, 5.1 log CFU/g and 4.0 log CFU/g in *Firfir*, *Bread*, and *Sambusa* respectively (46). The mean count of total coliform count also was highest in *Sambusa* with 1.69 log CFU/g and ranges from 1.11 to 2.05 log CFU/g. From the survey, it is assessed that the number of vendors that prepare *Bombolino* is the lowest (21%) among the other food items and on the other hand *Sambusa* had the highest number of customers.

The other practice that is observed during the study was some customers pick the food by choosing for themselves. Additionally from the observational checklist, it was found that about 93.3% of the vendors handle money while serving food and about 73% did not store food in a covered container. This exposes food to different contaminants

like flies and dust. About 70% of vendors did not wear an apron. None of them had handwashing and soap at their vending unit from which the sample was taken. These practices may be contributory factors for the test beyond the recommended limits. In general factors such as lack of water supply at the vending sites, inappropriate transportation and storage of food, handling money during food processing, infrequent handwashing, not taking the required food safety training and shortage of sanitary and proper waste disposal facilities could pave the way to contamination of food and this, in turn, increase public health problems. Assuring public health safety measures in street food vending activities is better than clinical treatment of foodborne illnesses (8, 28).

Concerning the place of vending, there was no great difference in the test carried out. The result of the ANOVA equivalent nonparametric test (Krus-Wallis test) showed there are no significant differences in vending sites on bacteriological tests, i.e aerobic mesophilic bacteria count with p-value=0.252 and total coliform, with p-value =0.169. This means location wise there is no significant difference in the food quality analysis test. This is because of the similarity in the status of the vendors and infrastructure among sampling locations.

7. Limitations of the study

Concerning the bacteriological quality analysis, it would have been better if the sampling conduct includes a swab test for the utensil that vendor's use and other extra microbiological tests to see the extent or level of contamination of street food. The other thing is the case of the Covid 19 pandemic, in which it may overestimate the hygiene practice of the street food vendor from the usual time.

8. Conclusion

The level of hygiene practice by the street food vendors during different stages of food processing and the environmental condition of the surrounding vending site was poor. That is about 82% of the vendors who had poor hygiene practice and 72% of the surveyed participants' carried out their work in poor environmental conditions. Moreover, the bacteriological quality analysis of food also shows the result beyond a recommended limit for the tests conducted. This contamination of the foods is associated with the poor personal hygiene of the vendors, poor environmental condition of vending places, inappropriate handling and processing of food and improper storage as well as exposure of food to flies and dust.

The hygiene practice is determined by food safety knowledge and the environmental condition of the vending site. Therefore educating street food vendors about food hygiene and providing basic infrastructures may help to improve the hygiene practice of the food vendors and reduces loads of foodborne illnesses.

9. Recommendations

To tackle the problems that arise from consuming street food which inturn results in foodborne diseases the following recommendations are forwarded;

To FMHACA and health bureau

- ✓ The institutions like food, medicine, and health care administration and control authority, health office, and other concerned bodies should give training to the vendors on food handling and safety practices that increase their food safety knowledge.
- ✓ FMHACA branch offices which are found at the Woreda level should conduct an inspection on street food vendors which is targeted at supporting, regulating, and sustaining the sector without compromising food safety to exploit the benefits of street-vended food.

To Addis Ketema Sub-City

- ✓ Sub-city administration should give attention and provide the necessary infrastructure to the vendors in order to improve the safety of street-vended foods.

To street food vendors

- ✓ The street food vendors should practice proper personal hygiene and environmental sanitation to minimize the contamination level of food.
- ✓ They should apply WHO recommendation rules like five keys to safer food.

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Annex I: Participant information sheet

My name is _____ I am working as an enumerator for the study conducted in this street food vending by Biruk Tesema who is studying for his Master's degree at Addis Ababa University and I would like to ask you to give your full attention to elaborate about the general information of the study and study participant

The study title: Assessing the hygiene and sanitary condition of street food vending in Addis Ketema Sub-City, Addis Ababa.

Purpose of the study: The aim of the questionnaire is to collect pertinent information on the hygiene of street food vendors and sanitary conditions of vending sites on the streets of this Sub-City. The result that will come out of this study will be used by the government and concerned bodies to base their rational decision to develop appropriate strategies to combat this problem.

Procedure and duration: I will be assessing the hygienic practice of the vendors starting from the raw material you use, the way you prepare, handle and store food; your personal hygiene, the way they manage utensils and your waste management practice by using questionnaires and observational checklist. The interview question requires a maximum of 20 minutes to complete.

Risks and benefits: The research is intended to benefit the vendors indirectly by identifying and reporting the challenges that they face as a whole to the concerned bodies. The research will introduce no risk to the participant.

Confidentiality: The information we will collect from you will completely be kept confidential and will be disclosed to any third person other than the people participating in this study.

Rights: participating in this study is voluntarism based and you can quit the study any time you want. You will have no penalty if you did not express a desire to participate. I, however, do hope that you will participate in the study since the data that will come from you will be important for us.

For any question that you need to ask us, you can use the contact address hereunder.

Address

Tel :+251921100333

Email:bure.tese@yahoo.com

Annex II: Consent form

Code No.....

I (the respondent), the undersigned, am told that the researcher is going to conduct a survey on hygiene and sanitary condition of street food vendors in Addis Ketema Sub-City, Addis Ababa, and s/he acquainted with me the first time s/he meets. I am told that the result of the study will be used by both the government and concerned bodies to commence the strategies to alleviate the problem. I am also told that the research will benefit street food vendors in general including me, the respondent and that will not inflict any harm to me. Besides, I am briefed that I will be interviewed for not more than 20 minutes. Moreover, I have notified involvement in this study is entirely interest-based and that I can quit from the study any time I want. Likewise, I am enlightened that I will not be subjected to any form of punishment following my failure to participate in the study. In the same way, I am explained that the information collected from me will not by any means be disclosed to any people other than those participating in the study unless obtained permission from me. Equally, I am told that I can ask them the question I found difficult or any type otherwise. Could I have your permission to continue?

a) Agree.....b) disagree.....

if disagree, skip to the next respondent by writing the reason for his/her refusal.....

Date of interview.....time started.....time completed.....

Result of interview :1 completed 2:respondent not available 3:refused 4:incomplete

Checked by supervisor: name signature.....date.....

For any convenience and problem, you can contact the principal investigator.

Phone 0921100333 email: bure.tese@yahoo.com

Annex III: Questionnaire

Date of interview _____ Code number (ID) _____

Name of the interviewer: _____

Place _____

Part One: Socio-Demographic Characteristics Of Food Vendors

| S. no | Questions | Response | Go to |
|-------|--|--|-------|
| 101 | Age of the vendors | Specify_____ | |
| 102 | Sex of the vendors | 1. Male | |
| | | 2. Female | |
| 103 | What is your marital status? | 1. Single 2. Married 3. Divorced/separated 4. Widowed | |
| 104 | What is your highest level of education? | 1. Illiterate 2. Primary 3. Secondary 4. Certificate 5. Diploma 6. University/college | |
| 105 | What is your average monthly income in (Eth Birr) | Specify_____ | |
| 106 | What is your work experience in participating in these activities? | Specify_____ | |
| 107 | What is your average working hour per day? | Specify_____ | |

Part Two: Questions Concerning Raw Materials

| | | | |
|-----|--|--|--|
| 201 | From where you purchase raw materials? | 1. Formal Retailers 2. Wholesale Stores 3. Informal Market 4. Other Specify | |
|-----|--|--|--|

| | | | |
|---|--|---|--------------------|
| 202 | What is your most important attribute in the purchase of raw materials /ingredients? | <ol style="list-style-type: none"> 1. Appearance 2. Brand 3. Price 4. Expiration date | |
| Part Three Questions Concerning Water Supply | | | |
| 301 | Where did you get water to cook food? | <ol style="list-style-type: none"> 1. From home 2. From stores 3. Other (specify) _____ | |
| 302 | What type of water did you use to prepare food? | <ol style="list-style-type: none"> 1. Spring water 2. Boiled or filtered water 3. Tapwater 4. Other (specify) _____ | |
| 303 | Where did you store water to prepare food | <ol style="list-style-type: none"> 1. Jerry can 2. Bucket 3. Other (specify) _____ | |
| 304 | What type of water did you use to wash utensils? | <ol style="list-style-type: none"> 1. Coldwater only 2. Coldwater with detergents 3. Hot water with detergents 4. Drying with cloth | |
| 305 | How often did you change the water used for clean utensils? | <ol style="list-style-type: none"> 1. After each phase of washing 2. Once a day 3. Twice a day 4. At the end of the day | |
| 306 | Do you pay for water? | <ol style="list-style-type: none"> 1. Yes 2. No | If no,(skip to q.) |
| 307 | How much do you pay a month? | Specify _____ | |
| 308 | Is the quantity of water that you receive (from your main source of water) adequate? | <ol style="list-style-type: none"> 1. Yes 2. No | |

Part Four: Preparation and Processing

| | | | |
|------------|--|---|--|
| 401 | When should you wash your hands? | <ol style="list-style-type: none"> 1. After visiting the toilet 2. Before preparing food 3. Both 4. Neither 5. Other(specify) 6. Don't Know | |
| 402 | Where did you prepare food? | <ol style="list-style-type: none"> 1. Home 2. Stall/street 3. Both | |
| 403 | How did you prepare food? | <ol style="list-style-type: none"> 1. Food is cooked during a sale 2. Food is cooked on the morning of the sale 3. Others(specify)_____ | |
| 404 | Is cleaning or disinfecting performed during production that may contaminate food? | <ol style="list-style-type: none"> 1. Yes 2. No | |
| 405 | What kind of product serve? (multiple answers are possible) | <ol style="list-style-type: none"> 1. Bonbolino 2. Sambussa 3. Chips 4. Soup 5. Potato 6. Ambasha 7. Biscuit 8. Others s(specify)_____ | |
| 406 | How did you Serve food? | <ol style="list-style-type: none"> 1. Food is served with fork/spoon 2. Food is served with bare hands 3. Food is served into cup/plate 4. Food is served into paper/leaves | |
| 407 | Did you re-use oil for frying several times? | <ol style="list-style-type: none"> 1. Yes 2. No | |

Part Five: Transportation, Handling And Storage Of Prepared Food

| | | | |
|------------|-----------------------------|--|--|
| 501 | How did you transport food? | <ol style="list-style-type: none"> 1. Car 2. Bus 3. Motorcycle 4. Cart of sale | |
|------------|-----------------------------|--|--|

| | | | |
|--|--|---|--|
| | | 5. Other specify_____ | |
| 502 | How did you handle Prepared or ready-to-eat foods, | 1. With pertinent utensils 2. Manually (with a bare hand) 3. others specify_____ | |
| 503 | Are fresh foods separated from ready-to-eat ones? | 1. Yes 2. No | |
| 504 | Did you use the preservation system at different stages to store food? | 1. Yes 2. No | |
| 505 | How did you store Prepared or ready-to-eat foods? | 1. Openly in the stalls 2. Wheelbarrows 3. Coveredutensils/warmers/coolers 4. others specify_____ | |
| 506 | How did you manage leftovers? | 1. Consumed by a member of the household 2. Stored for use next day 3. Discarded in the waste container 4. others specify_____ | |
| Part Six : Vending Unit, Equipment, And Utensil | | | |
| 601 | Did you use Separate utensils for each food preparation(raw or cooked and ready-to-eat)? | 1. Yes 2. No | |
| 602 | In which utensil did you serve food? | 1. Metal plates 2. Plastic plates 3. Cup 4. Other (specify)_____ | |
| 603 | What did you use to pack the food items while selling? | 1. A plastic bag 2. A paper bag 3. A newspaper 4. Others,specify_____ | |
| Part Seven: Cleaning And Sanitizing | | | |
| 701 | What do you use for cleaning up the utensils after the customers use them at this site? | 1. Running water and soap 2. Running water only 3. Water in a container and soap 4. Water in a container only | |

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| | | 5. Not applicable since my food items are take away | |
| 702 | How many times did you sanitize utensils at the point of sale? | 1. Once a day 2. Twice a day 3. Three times a day 4. Many times during work time | |
| 703 | Which products did you use to 'sanitize' at the point of sale? | 1. Chlorine solution 2. Alcohol 3. All-purpose cleaner product 4. Saponaceous 5. Detergent 6. Other | |
| Part Eight: Food Handlers | | | |
| 801 | How did you acquire food preparation skills? | 1. Formal training 2. Parents 3. Observation 4. Others, specify..... | |
| 802 | Do you have access to fresh running water at this site? | 1. Yes 2. No | |
| 803 | Do you always wash your hands before handling food items at this site? | 1. Yes 2. No | |
| 804 | Do you always wash your hands after handling raw food items at this site? | 1. Yes 2. No | If yes go to 805, if no skip |
| 805 | If yes, what do you use while washing your hands? | 1. Soap and running water 2. Running water only 3. Water in a container 4. Water in a container and soap 5. Others, specify..... | |
| 806 | What products did you use for supplementary hand-washing? | 1. Alcohol 2. Alcohol gel for hands 3. Other | |
| 807 | Do you always wear protective clothing | 1. Yes | |

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| | (apron) while preparing or handling food items at this site? | 2. No | |
| 808 | Did you prepare food when you ill? | 1. Yes 2. No | |
| 809 | Did you wash your hand after visiting the toilet? | 1. Yes 2. Sometimes 3. No | |
| 810 | Did you wash your hand after sneezing, coughing, smoking? | 1. Yes 2. Sometimes 3. No | |
| Part Nine: Waste Management Practice | | | |
| 901 | How do you dispose of any waste that may be generated from your work at this site? | 1. Open area dumping 2. Waste bin 3. On the street/road 4. Drainage/Gutter 5. Bush 6. Others, specify_____ | |
| 902 | Do you encounter pests and rodents on-site? | 1. Yes 2. No | If yes go 903 if no skip |
| 903 | How are these pests controlled? | Specify_____ | |
| 904 | Is the surrounding environment free of potential contaminants? | 1. Yes 2. No | If no go to 905, if yes skip it |
| 905 | If no, what are they? | Specify_____ | |
| 906 | Do you have access to a toilet facility at this site? | 1. Yes 2. No | If yes go 907, if no skip |
| 907 | Who is the owner of the facility | 1. Public 2. Private 3. Others specify_____ | |
| 908 | If yes, what type of toilet facility do you have? | 1. Ventilated improved Latrine 2. Water Flush latrine 3. Other(specify)_____ | |

መለያኮድ _____

የመረጃ ስብሰባ ባቢያንም : _____

ክፍል አንድ: ማህበራዊ እና ስነ-ሕዝባዊ ሁኔታዎችን በተመለከተ

| ተ.ቁ | ጥያቄዎች | አማራጭ መልሶች | እለፍ |
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| 101 | እድሜዎ ስንት ነው? | ጥቀስ _____ | |
| 102 | የምግብ ሻጭ/ጩጩታ | 1. ወንድ | |
| | | 2. ሴት | |
| 103 | የጋብቻ ሁኔታዎ ምን ይመስላል? | 1. ያላገባ 2. ያገባ 3. አግብቶ የፈታ 4. በሞት የተለየ 5. ሌላ ካለ የጠቀስ----- | |
| 104 | የትምህርት ደረጃዎ ስንት ነው? | 1. አልተማርኩም 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4. ሰርተፍኬት 5. ዲፕሎም 6. መጀመሪያ ድግሪ | |
| 105 | አማካይ የወር ገቢዎ ስንት ነው? | ጥቀስ _____ | |
| 106 | ለምን ያህል ጊዜ ነው በዚህ ስራ ላይ የተሳተፉት? | ጥቀስ _____ | 60 6 |
| | | — | 31.8 |
| 107 | ይህን ስራ በቀን ምን ያህል ሰዓት ይሰራሉ? | ጥቀስ _____ | |

ክፍል 2 : ለምግብ ዝግጅት የሚያገለግሉ ጥሬ እቃዎችን በተመለከተ የተዘጋጁ ጥያቄዎች

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| 201 | ጥሬ እቃዎችን ከየት ነው የሚገዙት? | 1. በችርቻሮ ከሚሸጡ ህጋዊ ነገዶች 2. በጅምላ ከሚሸጡ ህጋዊ ነገዶች 3. ህጋዊ ካልሆነ ነገዶች 4. ሌላ ካለ..... | |
| 202 | ጥሬ እቃዎችን ለመግዛት ዋነኛ መለኪያዎች ምን ይሁኑ? | 1. መልክ 2. ብራንድ 3. ዋጋ 4. የአገልግሎት ጊዜ | |

ክፍል 3 : የውሃ አቅርቦትን በተመለከተ የተዘጋጁ ጥያቄዎች

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| 301 | ምግብ ለማብሰል የምትጠቀሙበት ወሃ ከየት ነው የምታገኙት? | 1. ከቤት 2. ከማጠራቀሚያ 3. ሌላ ካለ _____ | |
| 302 | ምግብ ለማዘጋጀት ምን ዓይነት ወሃ ነው የምትጠቀሙት? | 1. የምንጭ ወሃ 2. የፈላ / የተጣራ ወሃ | |

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| | | 3. የቧንቧውሃ 4. ሌላካለ _____ | |
| 303 | ለመግብዝግጅት የምትጠቀሙት ወሃ ምን ዓይነት ነው የምትታጠሩት? | 1. ጄሪካን 2. ባልዲ 3. ሌላካለ _____ | |
| 304 | እቃዎችን ለማጠቃለያ እይነት ወሃ ነው የምትጠቀሙት? | 1. ቀዝቃዛውሃ ብቻ 2. ቀዝቃዛውሃና ሳሙና 3. ሙቅ ወሃና ሳሙና 4. በጨርቅ መወልወል | |
| 305 | በምን ያህል ጊዜ ነው ለእቃዎች ጠቢቅ የሚሆነው ነው የምትቀይሩት? | 1. ከእያንዳንዱ አጥበት በኋላ 2. በቀን አንዴ 3. በቀን ሁለቴ 4. ስራዎችን ሲጨርሱ | |
| 306 | ለውሃት ከፍላጎት? | 1. አዎ 2. አይ | አይከሆንዝለል |
| 307 | ምን ያህል? | ጥቀስ _____ | |
| 308 | በቂ የውሃ አቅርቦት አለ? | 1. አዎ 2. አይ | |

ክፍል 4 : ምግብ ዝግጅትን በተመለከተ የተዘጋጁት ጥያቄዎች

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| 401 | መቼ መቼ ነው አጃች ሁን የምትታጠቡት? | 1. ከመጸዳጃ በኋላ 2. ምግብ ከማዘጋጀት በፊት 3. በሁለቱም ጊዜ 4. በጭራሽ 5. ሌላካለ 6. አላውቅም | |
| 402 | የትነው ምግብ የምትዘጋጁት ? | 1. ቤት 2. መንገድ ላይ 3. በሁለቱም | |
| 403 | ምግብ እንዴት ነው የሚያዘጋጁት? | 1. እየሸጥኩ አዘጋጃለሁ 2. ጥዋት ላይ ነው የማዘጋጀው 3. ሌላካለ _____ | |
| 404 | በምግብ ዝግጅት መካከል ምግብን ሊበክሉ የሚችሉ ነገሮች ይጻፉ? | 1. አዎ 2. አይ | |
| 405 | ምን እይነት ምግብ ስንው የምትሰሩት? (በዙመም ረጥይቻ ላል) | 1. በንቢሊኖ 2. ሳንቡሳ | |

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| | | <ol style="list-style-type: none"> 3. ቼፕስ 4. ሾርባ 5. ድንች 6. አምባሻ 7. ብስኩት 8. ሌላካለ_____ | |
| 406 | ምግብ እንዴት ነው የምታቀርቡት? | <ol style="list-style-type: none"> 1. በሹካ/በማንኪያ 2. በእጅ ነው የሚበላው 3. በኩባያ / በዝርግሳህን 4. በወረቀት / በቅጠል | |
| 407 | ምግብ ለመጥበስ ዘይት ለብዙ ጊዜ በተደጋጋሚ ይጠቀማሉ? | <ol style="list-style-type: none"> 1. አዎ 2. አይ | |
| ክፍል 5: የተዘጋጁ ምግቦችን አጓጓዝ፣ እያዘና አቀማግጥን በተመለከተ የተዘጋጁ ጥያቄዎች | | | |
| 501 | ምግብ እንዴት ነው የምታጓጓዙት? | <ol style="list-style-type: none"> 1. በሚኒባስ 2. በባስ 3. በሞተር ሳይክል 4. በጋሪ 5. ሌላካለ_____ | |
| 502 | የተዘጋጁት ምግቦችን እንዴት ነው የምትይዙት? | <ol style="list-style-type: none"> 1. በንጹህ እቃ 2. በእጅ 3. ሌላካለ _____ | |
| 503 | ጥሬና የበሰሉ ምግቦች ተለያይተው ነው የሚቀመጡት? | <ol style="list-style-type: none"> 1. አዎ 2. አይ | |
| 504 | በተለያዩ ደረጃዎች ምግብ እንዳይበላሽ የማቆያ ዘዴዎችን ጠቀማላቹ? | <ol style="list-style-type: none"> 1. አዎ 2. አይ | |
| 505 | የተዘጋጁት ምግቦችን የትኩረት ነው የምታከማችሁት? | <ol style="list-style-type: none"> 1. በመደብር ውስጥ ክፍት በማስቀመጥ 2. በእጅ ጋሪ ላይ 3. በተከደ ነጻ እቃዎች 4. ፍሪጅ ውስጥ 5. ቁምሳጥን ውስጥ 6. ሌላካለ_____ | |
| 506 | የተረፉ ምግቦችን እንዴት ነው የምታደርጉት? | <ol style="list-style-type: none"> 1. በቤተሰብ አባል ይበላል 2. ለሚቀጥለው ቀን ይቀመጣል 3. በቆሻሻ መጣያ ውስጥ ይጣላል 4. ሌላካለ_____ | |

ክፍል 6: የሽያጭ አካባቢ ንጹህነትና በተመለከተ የተዘጋጁ ጥያቄዎች

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| 601 | ምግብ ስታዘጋጁ ለየብቻ ለጥሬ ልብ ለሰጠ ምግብ ምን ዓይነት ጥያቄዎችን እቃት ጠቀማላቹ ? | <ol style="list-style-type: none"> 1. አዎ 2. አይ | |
| 602 | በየትኛው እቃነው ምግብ የምታቀርቡት? | <ol style="list-style-type: none"> 1. በብረት ሳህን 2. በፕላስቲክ ሳህን 3. ኩባያ 4. ሌላ ካለ _____ | |
| 603 | ምግብ ጠቅል ለውለመሽ ጥምን አይነት ጠቅላያነው የሚጠቀሙት? | <ol style="list-style-type: none"> 1. ፕላስቲክ 2. ወረቀት 3. ጋዜጣ 4. ሌላ ካለ _____ | |

ክፍል 7 : በሽያጭ ቦታ የእቃዎች ጽዳትን በተመለከተ የተዘጋጁ ጥያቄዎች

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| 701 | ተገልጋዮች ከተጠቀሙ በኋላ እቃዎችን ለማጠቃለያ ምን ዓይነት ውጤት ጠቀሙት? | <ol style="list-style-type: none"> 1. ወሃና ሳሙና 2. ውሃ ብቻ 3. ቴክኒካዊ ስለሆነ አልጠቀምም 4. ሌላ ካለ _____ | |
| 702 | በሽያጭ ቦታ ስንት ጊዜ እቃዎችን የሚያጥቡት? | <ol style="list-style-type: none"> 1. በቀን አንድ 2. በቀን ሁለት 3. በቀን ሶስት 4. ብዙ ጊዜ | |
| 703 | እቃ ለማጠቃለያ የትኛውን ማጽጃነው የሚጠቀሙት? | <ol style="list-style-type: none"> 1. ክሎሪን 2. አልኮል 3. ሁሉን አቀፍ ማጽጃ ምርቶች 4. ሳሙና 5. ማጽጃ 6. ሌላ ካለ----- | |

ክፍል 8: የምግብ አዘጋጅን በተመለከተ የተዘጋጁ ጥያቄዎች

| | | | |
|------------|----------------------------------|---|--|
| 801 | የምግብ ዝግጅት ሙያ እንዴት አገኙ? | <ol style="list-style-type: none"> 1. በስልጠና 2. ከቤተሰብ 3. ሌሎችን በማየት 4. ሌላ ካለ----- | |
| 802 | እዚህ ቦታ የማይቆራረጥ ውሃ ታገኛላችሁ? | <ol style="list-style-type: none"> 1. አዎ 2. አይ | |
| 803 | ዘወትር ምግብ ከማዘጋጀት ያልፈተኑ ለምን ይታጠባሉ? | <ol style="list-style-type: none"> 1. አዎ | |

| | | | |
|---|---|--|------------------------------|
| | | 2. አይ | |
| 804 | ዘወትር ያልበሰሉ ምግቦች ከነኩ በኋላ አጅዎን ይታጠባሉ? | 1. አዎ 2. አይ | አዎ ከሆነ ወደ 805 ሂዱ፣ አይ ከሆነ ዝለል |
| 805 | አዎ ከሆነ አጅዎን ለመታጠብ ምን ድንኳን ወይንም የሚጠቀሙት? | 1. የቧንቧ ውሃና ሳሙና 2. የቧንቧ ውሃ ብቻ 3. የማጠራቀሚያ ውሃ 4. የማጠራቀሚያ ውሃና ሳሙና 5. ሌላ ካለ----- | |
| 806 | ከዚህ በተጨማሪ አጅዎን ለመታጠብ ምን አይነት ምርቶችን ወይንም የሚጠቀሙት? | 1. አልኮል 2. የአጅማ ጽጃ አልኮል 3. ሌላ ካለ----- | |
| 807 | ሁል ጊዜ ምግብ ሲያዘጋጁ/ሲያቀርቡ ሽርጥ ይለብሳሉ? | 1. አዎ 2. አይ | |
| 808 | ከታማ ሙሙ ምግብ ያዘጋጁሉ? | 1. አዎ 2. አይ | |
| 809 | ከመጸዳጃ በኋላ አጅዎን ይታጠባሉ? | 1. አዎ 2. አንዳንዴ 3. አይ | |
| 810 | ካስነጠስ ምክሳል ምክጫሉ በኋላ አጅዎን ይታጠባሉ? | 1. አዎ 2. አንዳንዴ 3. አይ | |
| ክፍል 9: ቆሻሻ አያ ዝስርዓትን በተመለከተ የተዘጋጁት ጥያቄዎች | | | |
| 901 | በስራ አካባቢ ያለውን ጭንቀትን ቆሻሻ ለማድረግ እንዴት ወይንም የሚያስወግዱት? | 1. በክፍት ቦታ 2. በቆሻሻ ማጠራቀሚያ 3. በመንገድ ላይ 4. በፍላጎት ቆሻሻ ማስወገጃ 5. በዛፍ ስር 6. ሌላ ካለ _____ | |
| 902 | እዚህ በታተሞችና አይጦች አገጥሞ አቸሁ ያውቃል? | 1. አዎ 2. አይ | አዎ ከሆነ ወደ 903 ሂዱ፣ አይ ከሆነ ዝለል |
| 903 | አዎ ከሆነ እንዴት ወይንም የሚቆጣጠሩት? | ግለጽ _____ | |
| 904 | ስራ ቢታላቅ ካባቢ ውምግብ ሲለብኩ ከሚችሉ ነገሮች ነጻነት? | 1. አዎ 2. አይ | አይ ከሆነ ወደ 905 ሂዱ፣ |

| | | | |
|-----|----------------------|---|-----------------------------------|
| | | | አዎከሆነዝለል |
| 905 | አይከሆነምንድንናቸው? | ግለጽ _____ | |
| 906 | እዚህአካባቢመጻዳጃቤትታገኛላቸው? | 1. አዎ 2. አይ | አዎከሆነወደ907 ሂድ፣ አይከሆነ 2ቱንዝለል |
| 907 | ባለቤትነቱየማንነው? | 1. የህዝብ 2. የግለሰብ 3. ሌላካለ | |
| 908 | አዎከሆነምንአይነትመጻዳጃነው? | 1. ቪኦሎይን 2. በውሃግፊትየሚሰራ 3. ሌላካለ_____ | |

አካባቢውን በመቃኘት የሚሞላ መጠይቅ

1. ሁልጊዜም ግብረሰብ ሆኖ ማጋጃ/ ሲያቀርቡ ጸጉር ይገኛሉ?

- (i) አዎ (ii) አይ

2. ምግብ ሲያዘጋጁ/ ሲያቀርቡ በመሀል ብርይ ይዛሉ?

- (i) አዎ (ii) አይ

3. ምግብ እንዴት ነው የሚሸጡት ?

1. ክዳን ካለው ዝርግት ሪ/ሳህን
2. ክዳን ከሌለው ዝርግት ሪ/ሳህን
3. ሌላ ካለ _____

የምግብ ሻጮች ልምድ

ምግብ በተከደ ነገር ስቀመጫው ስጥተቀምጧል ወይ?

1. አዎ 2. አይ

ምግብ ለዝንቦች ተጋልጧል ወይ?

1. አዎ 2. አይ

ምግብ ከመሸጡ በፊት ይሞቃል ወይ?

1. አዎ 2. አይ

የምግብ ሻጮች የእጅ ጥላታ ይገኛል ወይ?

1. አዎ 2. አይ

የምግብ ሻጮች ጥፍራቸውን አሳድገዋል ወይ?

1. አዎ 2. አይ

የምግብ ሻጮች ሽርጥ አድርገዋል ወይ?

1. አዎ 2. አይ

የምግብ ሻጮች ምግብ ሲሸጡ ራስ ታልበትን ፋሻቸው ይነፋሉ ወይ?

1. አዎ 2. አይ

አካባቢን ጽህፍ ማለኪያዎች

የምግብ ሻጮች በንጹህ ቦታ ላይ የሚሰሩት ወይ?

1. አዎ 2. አይ

የቆሻሻ ማጠራቀሚያ አለ ወይ?

1. አዎ 2. አይ

የቆሻሻ መጣያ ስፍራ አለ ወይ?

1. አዎ 2. አይ

የእጅ መታጠቢያ አለ ወይ?

1. አዎ 2. አይ

ሳሙና አለ ወይ?

1. አዎ 2. አይ

ዝንቦች አሉ ወይ ?

1. አዎ 2. አይ

አይጥ/በረሮ አለ ወይ?

1. አዎ 2. አይ

Annex V: Laboratory procedures

Microbiological Methods for Analysis of Foods

1. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms: Colony count at 30 °C by the pour plate technique (ES ISO 48331:2015)

1.1 preparation of initial suspension (for cereal by-products)

- Mix dry powders well in their container before weighing out the test sample.
- Add 1 part test sample to 9 parts of peptone salt solution and mix.
- Before homogenization leave to stand for 20 min to 30 min at 18 °C to 27°C
- If the viscosity of the suspension increases so that it becomes too thick or viscous to mix well or pipette, add a further equal volume of peptone salt solution to produce a 1 to 20 initial suspension.
- Mix according to the product with either a peristaltic homogenizer for 1 mn, or in a rotary blender.
- In the enumeration, calculation take into account additional dilution made.
- Homogenize and make a 1 in 2 dilution.

1.2 Further Decimal Dilution

- Transfer, by means of pipette, 1 ml of the initial suspension with an uncertainty of measurement of $\pm 5\%$, into a tube containing 9ml of sterile diluent at the appropriate temperature.
- For optimal precision, do not introduce the pipette more than 1 cm into the initial suspension.
- Mix thoroughly, preferably by using a mechanical stirrer for 5s to 10s, to obtain a 10^{-2} dilution.
- If necessary repeat these operation using the 10^{-2} and further dilution by using at each dilution a new sterile pipette to obtain 10^{-3} , 10^{-4} , etc., dilution until the appropriate number of microorganism has been obtained.

The time lapse between the end of the preparation of the initial suspension and the instant when the inoculum comes into contact with the culture medium shall not exceed 45 min while limiting to 30 min the lapsed time between the preparation of the initial suspension and the beginning of the preparation of the following decimal dilution, unless otherwise specified in the specific international standard.

1.3 Inoculation and incubation

1.3.1 Take two sterile Petri dishes. Transfer to each dish, by means of a sterile pipette, 1 ml of the test sample if liquid, or 1 ml of the initial suspension (10^{-1} dilution) in the case of other products.

If plates from more than one dilution are prepared, this may be reduced to one dish.

1.3.2 Take one other sterile Petri dish. Use another sterile pipette to dispense 1 ml of the 10⁻¹ dilution (liquid product) or 1 ml of the 10⁻² dilution (other products).

1.3.3 If necessary, repeat the procedure with the further dilutions, using a new sterile pipette for each decimal dilution.

1.3.4 If appropriate and possible, select only the critical dilutions steps (at least two consecutive

decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 10 and 300 colonies per plate.

1.3.5 Pour about 12 ml to 15 ml of the plate count agar at 44 °C to 47 °C into each Petri dish.

The time elapsed between the end of the preparation of the initial suspension (or of the 10⁻¹ dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 45 min.

1.3.6 Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface.

1.3.7 After complete solidification, and only in the case where it is suspected that the product under examination contains microorganisms whose colonies overgrow the surface of the medium, pour about 4 ml of the overlay medium or plate count agar at 44 °C to 47 °C on to the surface of the inoculated medium. Allow to solidify as specified in the above step.

1.3.8 Invert the prepared plates and place them in the incubator at (30 ± 1) °C. Incubate for (72 ± 3) h.

1.4 Counting of colonies

1.4.1 After the specified incubation period, retain the plates with, if possible, fewer than 300 colonies. Count the colonies on the plates, using the colony-counting equipment if necessary.

Examine the dishes under subdued light. It is important that pinpoint colonies be included in the count; however, it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, in order to distinguish colonies from foreign matter.

1.4.2 Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the

dish and calculate the corresponding number of the entire dish. If more than one-quarter is overgrown by spreading colonies, discard the count.

1.5 Method Of Calculation

Calculate the number of N of the microorganism present in the test sample as a weighted mean from two successive dilution using equation

$$N = \frac{\sum C}{V \cdot d}$$

Where $\sum C$ is the sum of the colonies counted on the two dishes retained from two successive dilution, at least one of which contains a minimum of 10 colonies;

V is the volume of the inoculum placed in each dish, in millilitres

D is the dilution corresponding to the first dilution retained.

2 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms – Most probable number technique (ES ISO 4831:2015)

2.1 preparation of initial suspension - Prepare as directed under 1.1

2.2 Dilutions- Prepare as directed under 1.2

2.3 Inoculation and incubation

2.3.1 It is usual that there is a combination of three tubes for each dilution series. However, for some products and/or each time that results of greater accuracy are required, it may be necessary to inoculate series consisting of more than three tubes (e.g. five tubes). For these cases, for the calculation of the MPN see the relevant tables included in ISO 7218.

2.3.2 Take three tubes of double-strength selective enrichment medium. Using a sterile pipette transfer to each of these tubes 10 ml of the test sample if liquid, or 10 ml of the initial suspension in the case of other products.

2.3.3 Then take three tubes of single-strength selective enrichment medium. Using a fresh sterile pipette, transfer to each of these tubes 1 ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products.

2.3.4 For each of the further dilutions, continue as described in 2.3.3. Use a fresh sterile pipette for each dilution. Carefully mix the inoculum and the medium.

2.3.5 Leave the tubes of double-strength medium 2.3.2 in the incubator set at 30 °C or 37 °C (as agreed) for 24 h ± 2 h.

2.3.6 Leave the tubes of single-strength medium (2.3.3 and 2.3.4) in the incubator set at 30 °C or 37 °C (as agreed) for 24 h ± 2 h or, if neither gas formation nor opacity preventing of the detection of gas formation is observed at this stage, continue incubation for another 24 h ± 2 h.

2.4 Confirmation

2.4.1 From each of the incubated tubes from 2.3.5, inoculate with a loop a tube of confirmation medium. Incubate in the incubator set at 30 °C or 37 °C (as agreed) for 24 h ± 2 h or, if gas formation is not observed at this stage, continue incubation for another 24 h ± 2 h.

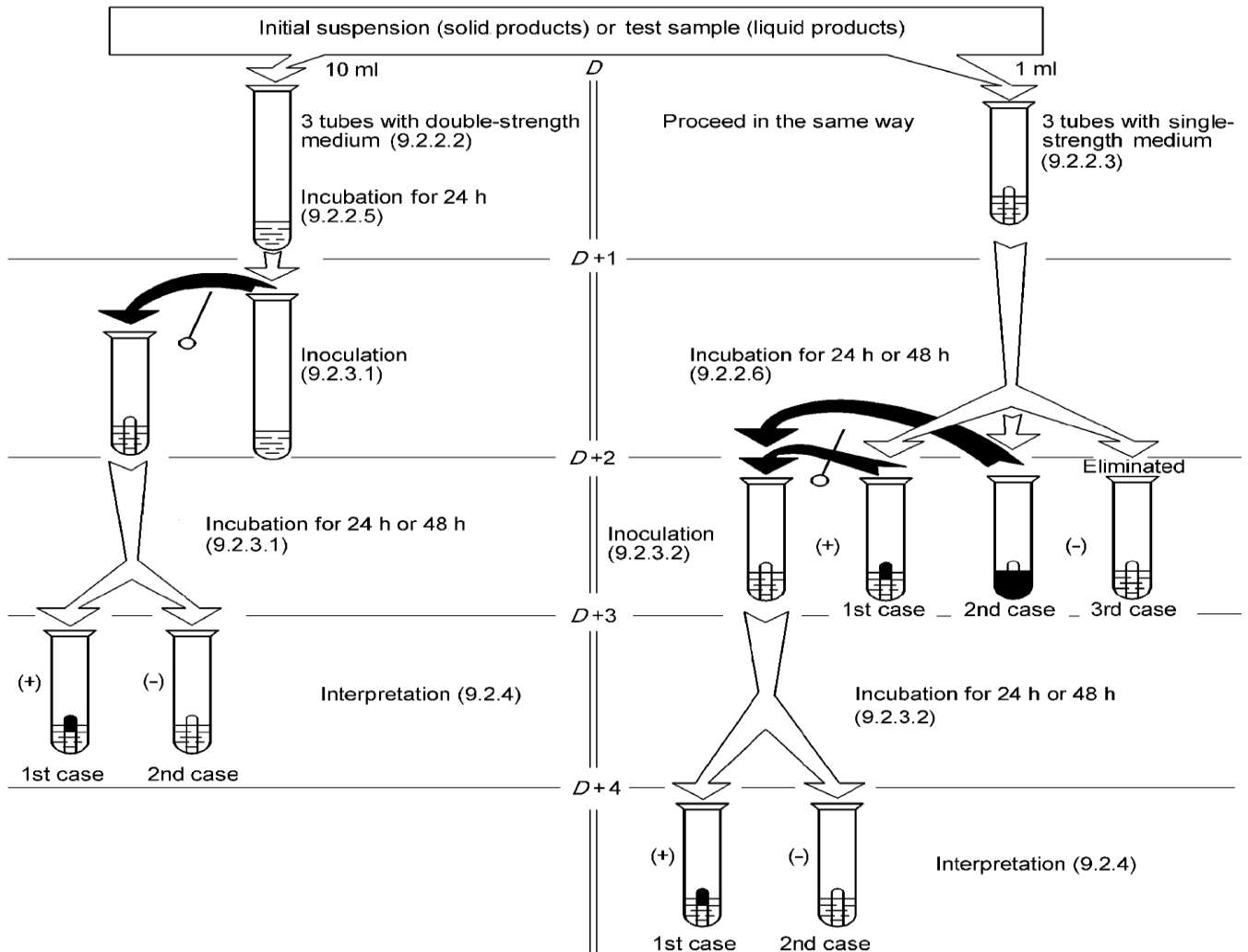
2.4.2 Carry out the same procedure as described in 2.4.1 for the incubated tubes from 2.3.6 showing gas formation, or opacity preventing the detection of gas formation, when either of these features is first observed (i.e. after 24 h ± 2 h or after 48 h ± 2 h).

2.5 Interpretation

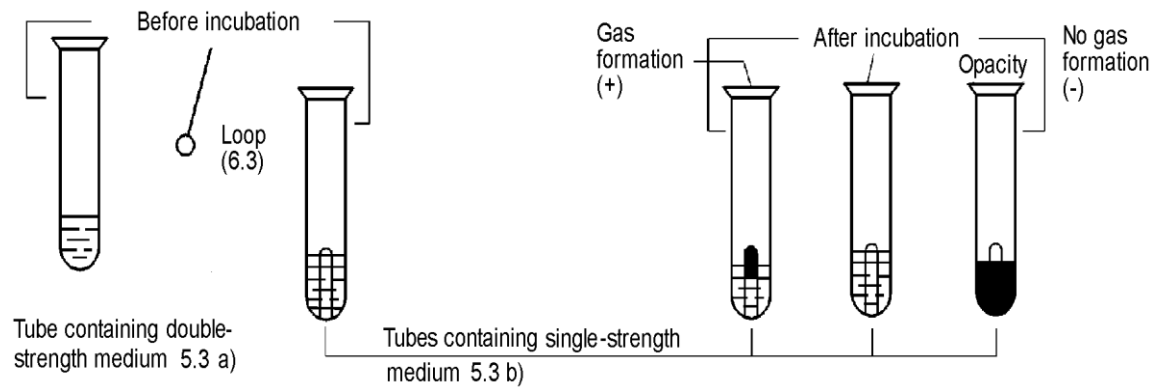
For each dilution, count the total number of tubes in which gas formation is observed in 2.4 (positive tubes) after 24 h ± 2 h and (if used) 48 h ± 2 h.

2.6 Calculation and expression of results

Calculate the most probable number from the number of positive tubes at each dilution.



Details of enumeration stage



Details of confirming stage

3. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. (ES ISO 6579:2012)

3.1 Test portion, initial suspension and dilutions

3.1.1 General

For preparation of the initial suspension, in the general case use as diluent the pre-enrichment medium (buffered peptone water).

If the specified mass of test portion is other than 25 g, use the necessary quantity of pre-enrichment medium to yield a 1/10 dilution. To reduce the examination workload when more than one 25 g test portion from a specified lot of food has to be examined, and when evidence is available that compositing (pooling the test portions) does not affect the result for that particular food, the test portions may be composited. For example, if 10 test portions of 25 g are to be examined, combine the 10 units to form a composite test portion of 250 g and add 2.25 l of pre-enrichment broth.

Alternatively, the 0.1 ml (in 10 ml of RVS broth) and 1 ml (in 10 ml of MKTTn broth) portions of the pre-enrichment broth from the 10 separate test portions (see 3.3.1) may be composited for enrichment in 100 ml of selective enrichment media.

3.2 Non-selective pre-enrichment

Incubate the initial suspension at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $18\text{ h} \pm 2\text{ h}$.

3.3 Selective enrichment

3.3.1 Transfer 0,1 ml of the culture obtained in 3.2 to a tube containing 10 ml of the RVS broth; transfer 1 ml of the culture obtained in 3.2 to a tube containing 10 ml of MKTTn broth .

3.3.2 Incubate the inoculated RVS broth (3.3.1) at $41.5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$ and the inoculated MKTTn broth at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$. Care should be taken that the maximum allowed incubation temperature ($42.5\text{ }^{\circ}\text{C}$) is not exceeded.

3.4 Plating out and identification

3.4.1 After incubation for 24 h \pm 3 h, using the culture obtained in the RVS broth (3.3.2), inoculate by means of a loop the surface of one large-size Petri dish containing the first selective plating-out medium (XLD agar), so that well-isolated colonies will be obtained.

In the absence of large dishes, use two small dishes one after the other, using the same loop.

Proceed in the same way with the second selective plating-out medium using a sterile loop and Petri dishes as above.

3.4.2 After incubation for 24 h \pm 3 h, using the culture obtained in the MKTTn broth (3.3.2), repeat the procedure described in 9.4.1 with the two selective plating-out media.

3.4.3 Invert the dishes (3.4.1 and 3.4.2) so that the bottom is uppermost, and place them in the incubator set at 37 °C for the first plating-out medium. The manufacturer's instructions shall be followed for the second plating-out medium.

3.4.4 After incubation for 24 h \pm 3 h, examine the plates (3.4.3) for the presence of typical colonies of *Salmonella* and atypical colonies that may be *Salmonella* (see Note). Mark their position on the bottom of the dish.

Typical colonies of *Salmonella* grown on XLD agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator.

NOTE *Salmonella* H₂S negative variants (e.g. *S. Paratyphi* A) grown on XLD agar are pink with a darker pink centre.

Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening.

Incubate the second selective solid medium at the appropriate temperature and examine after the appropriate time to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

3.5 Confirmation

3.5.1 General

If shown to be reliable, commercially available identification kits for the biochemical examination of *Salmonella* may be used. The use of identification kits concerns the biochemical confirmation of colonies. These kits should be used following the manufacturer's instructions.

NOTE The recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance may vary somewhat, not only from serovar to

serovar, but also from batch to batch of the selective culture medium used.

3.5.2 Selection of colonies for confirmation

- For confirmation, take from each dish (two small-sized dishes or one large-sized dish) of each selective medium (see 3.4) at least one colony considered to be typical or suspect and a further four colonies if the first is negative.
- It is recommended that at least five colonies be identified in the case of epidemiological studies. If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.
- Streak the selected colonies onto the surface of pre-dried nutrient agar plates, in a manner which will allow well-isolated colonies to develop. Incubate the inoculated plates (3.4.3) at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$.
- Use pure cultures for biochemical and serological confirmation.

3.5.3 Biochemical confirmation

3.5.3.1 General

By means of an inoculating wire, inoculate the media specified in 3.5.3.2 to 3.5.3.7 with each of the cultures obtained from the colonies selected in 3.5.2.

3.5.3.2 TSI agar

Streak the agar slant surface and stab the butt. Incubate at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$.

Interpret the changes in the medium as follows.

a) Butt

- yellow glucose positive (glucose used)
- red or unchanged glucose negative (glucose not used)
- black formation of hydrogen sulfide
- bubbles or cracks gas formation from glucose

b) Slant surface

- yellow lactose and/or sucrose positive (lactose and/or sucrose used)
- red or unchanged lactose and sucrose negative (neither lactose nor sucrose used)
- Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) formation of hydrogen sulfide (blackening of the agar) (3.5.3.8).
- When a lactose-positive *Salmonella* is isolated, the TSI slant is yellow. Thus, preliminary confirmation of *Salmonella* cultures shall not be based on the results of the TSI agar test only (see 3.5.3).

3.5.3.3 Urea agar

- Streak the agar slant surface. Incubate at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$ and examine at

intervals. If the reaction is positive, splitting of urea liberates ammonia, which changes the colour of phenol red to rose-pink and later to deep cerise. The reaction is often apparent after 2 h to 4 h.

3.5.3.4 L-Lysine decarboxylation medium

- Inoculate just below the surface of the liquid medium. Incubate at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$.
- Turbidity and a purple colour after incubation indicates a positive reaction. A yellow colour indicates a negative reaction.

3.5.3.5 Detection of β -galactosidase

- Suspend a loopful of the suspected colony in a tube containing 0,25 ml of the saline solution.
- Add 1 drop of toluene and shake the tube. Put the tube in a water bath set at $37\text{ }^{\circ}\text{C}$ and leave for several minutes (approximately 5 min). Add 0,25 ml of the reagent for detection of β -galactosidase and mix.
- Replace the tube in the water bath set at $37\text{ }^{\circ}\text{C}$ and leave for $24\text{ h} \pm 3\text{ h}$, examining the tube at intervals.
- A yellow colour indicates a positive reaction. The reaction is often apparent after 20 min.
- If prepared paper discs are used, follow the manufacturer's instructions.

3.5.3.6 Medium for Voges-Proskauer (VP) reaction

- Suspend a loopful of the suspected colony in a sterile tube containing 3 ml of the VP medium.
- Incubate at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$.
- After incubation, add two drops of the creatine solution, three drops of the ethanolic solution of 1-naphthol and then two drops of the potassium hydroxide solution; shake after the addition of each reagent.
- The formation of a pink to bright red colour within 15 min indicates a positive reaction.

3.5.3.7 Medium for indole reaction

Inoculate a tube containing 5 ml of the tryptone/tryptophan medium with the suspected colony.

Incubate at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$. After incubation, add 1 ml of the Kovacs reagent.

The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

3.5.3.8 Interpretation of the biochemical tests

Salmonella generally show the reactions given in Table 1.

3.5.4 Serological confirmation and serotyping

3.5.4.1 General

The detection of the presence of *Salmonella* O-, Vi- and H-antigens is tested by slide agglutination with the appropriate sera, from pure colonies (9.5.2) and after auto-agglutinable strains have been eliminated. Use the antisera according to the producer's instructions if different from the description below.

3.5.4.2 Elimination of auto-agglutinable strains

Place one drop of the saline solution onto a carefully cleaned glass slide. Disperse in the drop, by means of a loop, part of the colony to be tested, in order to obtain a homogeneous and turbid suspension.

NOTE It is also possible to disperse part of the colony to be tested in a drop of water, and then to mix this solution with one drop of saline solution.

Rock the slide gently for 30 s to 60 s. Observe the result against a dark background, preferably with the aid of a magnifying glass.

If the bacteria have clumped into more or less distinct units, the strain is considered auto-agglutinable, and shall not be submitted to the following tests as the detection of the antigens is not feasible.

Table 1 — Interpretation of biochemical tests

| Test ^a (9.5.3.2 to 9.5.3.7) | <i>Salmonella</i> strain | | | | | | | | |
|--|--------------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------|
| | <i>S. Typhi</i> | | <i>S. Paratyphi A</i> | | <i>S. Paratyphi B</i> | | <i>S. Paratyphi C</i> | | Other str |
| | Reaction | % ^b | Reaction | % ^b | Reaction | % ^c | Reaction | % ^c | |
| TSI acid from glucose | + | 100 | + | 100 | + | | + | | + |
| TSI gas from glucose | - ^d | 0 | + | 100 | + | | + | | + |
| TSI acid from lactose | - | 2 | - | 100 | - | | - | | - |
| TSI acid from sucrose | - | 0 | - | 0 | - | | - | | - |
| TSI hydrogen sulfide produced | + | 97 | - | 10 | + | | + | | + |
| Urea hydrolysis | - | 0 | - | 0 | - | | - | | - |
| Lysine decarboxylation | + | 98 | - | 0 | + | | + | | + |
| β -Galactosidase reaction | - | 0 | - | 0 | - | | - | | - |
| Voges-Proskauer reaction | - | 0 | - | 0 | - | | - | | - |
| Production of indole | - | 0 | - | 0 | - | | - | | - |

From reference [5].

^b These percentages indicate that not all isolates of *Salmonella* serotype show the reactions marked $\square\square$ or -. These percentages may vary between and within serotypes of food poisoning serotypes from different locations.

^c The percentages are not known from available literature.

^d *Salmonella* Typhi is anaerogenic.

^e The *Salmonella enterica* subspecies *arizonae* gives a positive or negative lactose

reaction but is always α -galactosidase positive. For the study of these strains it may be useful to carry out complementary tests.

3.5.4.3 Examination for O-antigens

Using one non-autoagglutinating pure colony, proceed according to 3.5.4.2, using one drop of the anti-O serum instead of the saline solution. If agglutination occurs, the reaction is considered positive. Use the poly- and monovalent sera one after the other.

3.5.4.4 Examination for Vi-antigens

Proceed according to 3.5.4.2, but using one drop of the anti-Vi serum instead of the saline solution. If agglutination occurs, the reaction is considered positive.

3.5.4.5 Examination for H-antigens

Inoculate the semi-solid nutrient agar with a pure non-auto-agglutinable colony. Incubate the medium at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24\text{ h} \pm 3\text{ h}$.

Use this culture for examination for the H-antigens, proceeding according to 3.5.4.2, but using one drop of the anti-H serum instead of the saline solution. If agglutination occurs, the reaction is considered positive.

3.5.5 Interpretation of biochemical and serological reactions

Table 2 gives the interpretation of the confirmatory tests (3.5.3 and 3.5.4) carried out on the colonies used (3.5.2).

| Biochemical reactions | Auto-agglutination | Serological reactions | Interpretation |
|-----------------------|--------------------|-------------------------------|--|
| Typical | No | O-, Vi- or H-antigen positive | Strains considered to be <i>Salmonella</i> |
| Typical | No | All reactions negative | May be <i>Salmonella</i> |
| Typical | Yes | Not tested (see 9.5.4.2) | |
| No typical reactions | No/Yes | O-, Vi- or H-antigen positive | |
| No typical reactions | No/Yes | All reactions negative | Not considered to be <i>Salmonella</i> |

3.5.6 Definitive confirmation

Strains which are considered to be *Salmonella*, or which may be *Salmonella* (see Table 2), shall be sent to a recognized *Salmonella* reference centre for definitive typing.

This dispatch shall be accompanied by all possible information concerning the strain(s) and whether it is an outbreak or in food.

3.6 Expression of results

In accordance with the results of the interpretation, indicate the presence or absence of *Salmonella* in a test portion of $x\text{ g}$ or $x\text{ ml}$ of product.

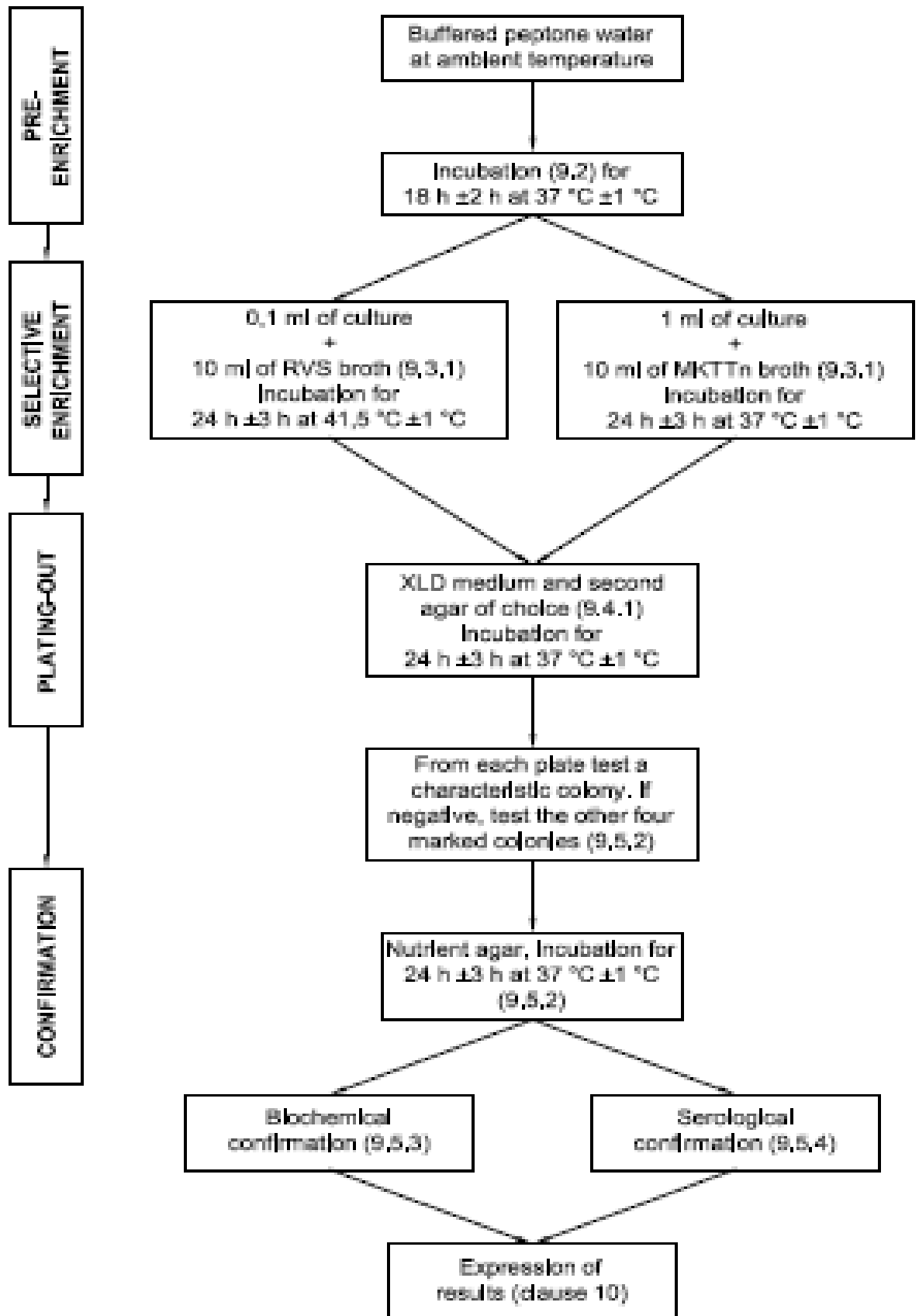


Diagram of the procedure