

BACTERIAL INVESTIGATION OF RANDOMLY SELECTED STREET FOOD SAUCES SITUATED OUTSIDE A UNIVERSITY

Ashley M. Tinong, Ashley Keith A. Agustin, Jerome A. Lavadia, Shivone B. Guinaban

ABSTRACT

This research aimed to determine the presence or absence of bacteria in street food sauces of the different stalls situated outside a University. An experimental approach partnered with laboratory testing was used to investigate and assess the possible bacterial species isolated from street food sauces. The method of the study included three (sweet, sweet & spicy and vinegar) sample collections, culture, isolation and identification for the determination for the presence of bacterial pathogens. The study has shown that there was a bacterial growth upon opening and 30 minutes before closing and there is an increase in the colony count from opening of the sauce up to 30 minutes before closing of the stall as manifested in the p-value which is 0.00 which further means that there is a significant difference using 0.05 as level of significance. It was also revealed that the sweet and sweet & spicy sauces which are collected upon opening and 30 minutes before closing are usually contaminated by *Escherichia coli* (E coli) and *Klebsiella*

Key words: *Sweet, sweet & spicy and vinegar sauces, culture, isolation, bacterial identification, Escherichia coli and Klebsiella.*

INTRODUCTION

Street food vending is found worldwide with great diversity due to socio-economic and cultural factors, representing in some countries a significant proportion of the food consumed by the urban population (Lues, Rasephei, Theron and Venter, 2006). Street food trading solves major social and economic problems in developing countries like the Philippines through the provision of ready-made meals at relatively inexpensive prices and employment for teeming rural and urban populace along its value chain (Alimi 2016; Azanza, 2002). However, there have been several documented cases of food poisoning outbreaks due to street foods (Rane, 2011). According to Food and Agriculture Organization (FAO) (2005), statistics show that every year 700,000 people die from food and water borne disease in the Asia-Pacific region including the Philippines and also according to (WHO, 2010), the main health hazard associated with street foods is microbial contamination (Ghosh, Kuma & Wahi, 2007).

Additionally, other related studies made mention that microbiological status of the food has been reported to be dependent on several factors. Such factors were suggested by (Thunberg, Tran Bennett, Matthews, & Belay, 2002), in their

studies, wherein quality of raw material, handling and processing may affect food safety. Safety precautions in preparing food, microorganisms that survive the preservation and storage treatment might also serve as factors contributing to unsafe food. Other related factors also include post process contamination (Gibbs, Hogg, Meena, MeidaCarn & Teixeira, 2004; Long Adak, O'Beirne, & Gillesple, 2002).

Street vended food is very controversial from a health standpoint. The design, construction and maintenance of vending units, equipment and utensils are also important in food safety. If foods sauce were handled in a given set of utensils, one would expect to find some or all of the organisms associated with the food sauce. Equipment that are kept in the open and exposed to dust might lead to collection of air borne microorganisms (Prussin, 2015; Daniyan, 2011). The use of inappropriate materials and poor maintenance may lead to inability to effectively clean and sanitize surfaces. Illness causing bacteria can survive in many places mostly in kitchen utensils. It is supported by a study conducted by Neejrah (2007), who mentioned that food materials are also source of microorganisms and the type of microorganisms depend on the type of food sauce being handled and also the care and storage of utensils.

Thus, this study is aimed at determining the presence or absence of bacteria and the possibility of bacterial species isolated from street food sauces.

Research Questions

This study aimed to investigate the presence of bacteria in street food sauces near the vicinity of University of Saint Louis Tuguegarao.

Specifically, the research aimed to answer the following questions:

1. What is the bacterial growth isolated from the selected street food sauces during opening time and 30 minutes before store closing time in terms of ?
 - a. Qualitative bacterial growth description
 - b. Colony count (CFU) in the following medium:
 - Blood agar plate
 - McConkey agar plate
2. What are the identified food-borne bacteria present in street food sauces collected from the different stalls?
3. Is there a significant difference in the colony count of bacteria on the different culture media based on the time to which the sauces are collected?
 - a. Opening time of the store
 - b. 30 minutes before the store closes

Hypothesis

- There is no significant difference in the colony count of bacteria on the different culture media based on the time to which the sauces are collected.

Significance of the Study

This study is sufficiently beneficial to the community of Tuguegarao City near the vicinity of a university for they would become aware of the presence of bacteria in street food sauces, thus giving them precautionary measures to avoid the occurrence of bacterial infections such as diarrhea. The findings of this study also provide information and awareness among people who are fond of buying street foods and the implication to human health. Moreover, for the vendors, to be properly informed and guided especially in terms of personal hygiene, food preparation, cooking, serving and storing of street foods and street food sauces to ensure the safety of the community.

Literature Review

Code of Sanitation of 1975

The research was conducted in accordance to Presidential Decree No. 856 – Philippine Code on Sanitation of 1975, Section 14 which states that “No person or entity shall operate a food establishment for public patronage without securing a permit from the local health office.” This law also embodied all the requirements of food safety for public consumption like medical examination of food handlers and inspection of foods being sold for a possible hazard to human health. That pursuant to this law, street food vendors must also comply with the requirements in order to ensure the health safety of consumers and avoid health problems especially the community.

Street Foods Sauces

Filipinos love to regale on those salivating exotic street foods found everywhere in the Philippines. There are markets, schools, church areas, and street corners everywhere and we can always see a jam of people surrounding a street food vendor because of those customers who crave for kwek- kwek, fishballs that are complemented with several sauces like sweet and sour sauce, sweet sauce, and spicy vinegar sauce (Allain, 2012; Lizotte, 2002).

Street food is an important part of Filipino culture, and those carts and stalls that often line the streets of the cities are great ways to snack like a native. Street food does exist in an endless variety. There is much diversity in the raw materials as well as in the preparation of street food sauces. More often, vendors’ stalls are usually located outdoors which are easily accessible from the street. Due to the

location where stalls are situated, it may contaminate the sauces if they are not properly covered and are exposed to flies and dust, which may harbor food borne pathogens. All those who handle food sauces, including food producers, individuals who work in markets and food service possible, to keep food safe, people who prepare food should clean hands (De Castro, 2015).

Vending Location

The conditions in which some street vendors operate are reported to be improper for the preparation and selling of street food sauces. The street food sauce is prepared either at home or at stalls, in which the stalls are located on the street side and some are in front of the school. Preparation surfaces used by some vendors have remains of foods prepared earlier that can lead to cross-contamination. Most of these street food sauces are not covered and are exposed to flies and dust, which may harbor food borne pathogens. In 70–90% of the cases, the presence of animals, insects and liquid wastes in food sauces preparation areas have been reported (Rane, 2011; Bhat & Waghray, 2004).

Food Preparation: Storage and Reheating

One of the important factors that affects food contamination and contributing to further increase in contamination is food storage temperature. Some of those factors are the preparation of food sauce long before its consumption, improper storage at ambient temperature, insufficient cooling and reheating contaminated processed food sauce, and undercooking are identified as the key factors that contribute to food poisoning outbreaks (Abid, Saiful, Shahimul and Zakiul, 2017; WHO, 2015).

Storage

Holding food sauces at high ambient temperatures for long periods of time has been reported to be a major contributor to the occurrence of food poisoning outbreaks. In preparing food, it is often held for several hours after cooking and this includes overnight holding at ambient temperatures until the foods are sold, and thus it can harbor high microbial populations (Fahmida, Frank, Paul and Sabiha, 2002).

Reheating

Time-temperature exposures during reheating must be sufficiently high or long to inactivate large quantities of infectious microorganisms that could develop during the long holding process. Some street food sauces vendors often partially or fully cook some products ahead of time, store them and then reheat them when requested by customers. However, this reheating is often inadequate to destroy bacteria that may be present as this would allow the food-borne pathogens that

germinate from spores which survived cooking or that contaminate the food after cooking, to survive and proliferate (Arzu, 2016).

Common Street Foods Usually Eaten with Sauce according to Harkins (2013)

Isaw or Grilled Chicken Intestines is a street food from the Philippines, made from chicken intestines or barbecued pig that are marinated with a mixture of soy sauce, garlic, salt, pepper, ketchup, and cooking oil. They are then either boiled, then grilled, or immediately grilled on sticks. This quick snack is often dipped in onion- or chili-infused vinegar just before it is eaten.

Fish Ball is edible, is sort of like the chicken nugget of fish, mashed up and balled up pieces of fish. These fish meatballs are primarily white or yellow in color and measure about an inch to two inches in diameter. They are made by pounding rather than grounding. A food item popular in the Philippines; in fact, its vendors who push wooden carts are regular sights in the streets.

Barbeque is a meat that has been barbecued or grilled in a highly seasoned sauce. It is prepared by thinly slicing the pork into ½ inch thick by 4 pieces. Trim the extra fat. Peel and finely chop the garlic. In a large bowl, make the marinade by combining the soy sauce and banana ketchup/ food color, black pepper, and calamansi. Add the pork pieces to the marinade. Cover the bowl with plastic wrap and refrigerate overnight. Soak the bamboo skewers in water. The 8-inch skewers are a perfect size. It is critical to soak the skewers in water, for at least an hour or so to avoid burning them while grilling the pork. It is stored at 45 degrees Fahrenheit (7 degrees Celsius) or below.

Pathogen Determination and Identification

There are basically two main forms of testing food products, one is Microbiological Quality Determination and the other is food safety, which includes analyzing and testing for food pathogens.

Pathogenic organisms are those that can cause illness in humans either by infections such as *Salmonella*, *Campylobacter* and pathogenic *E. coli* or intoxications such as *Bacillus cereus*, *Staphylococcus aureus* or *Clostridium botulinum*.

There is a range of symptoms that can occur from these organisms - from flu-like symptoms and the worst case scenario is fatality.

Wide range of food poisoning organisms includes the following:

Salmonella spp. The *Salmonella* group is probably the best-known food poisoning organism and although associated with poultry and eggs can be found in a wide variety of foods. Cases and incidence of Salmonellosis have reduced but it is still one of the major causes of UK outbreaks of food poisoning.

Listeria monocytogenes. *Listeria monocytogenes* is classed as the only pathogenic member of the *Listeria* group, however, others in the group are thought to be possible pathogens. *Listeria* is widespread in the environment and is often a post process contaminant. The incidence of Listeriosis is on the increase.

Pathogenic *E.coli.* This organism is a member of the Enterobacteriaceae which can cause virulent food poisoning; it is an indicator of fecal contamination.

Campylobacter is now the most prevalent form of food borne illness in the UK mostly associated with raw poultry and it can also be found in other food groups.

Vibrio parahaemolyticus. This is a marine organism and is generally associated with warm water fish, prawns and shellfish. Incidence is rare in the UK but it can also be found in cold waters.

Bacillus spp. This is a group of aerobic spore-forming organisms that stain gram positive or gram-variable which may survive heating and some can cause food poisoning. *Bacillus cereus* is one of these groups and your food can be tested separately for this organism.

Staphylococcus aureus. This organism could cause food poisoning and if it grows in large number can leave toxins in the product, which may survive heating. It lives on the skins of humans and animals and can easily be transferred to food products.

Clostridium perfringens. This is commonly found in organism and is a member of the Clostridia. They are anaerobic spore-forming organisms, toxin and gas-producing bacteria, which may survive heating, can be associated with meats and other products (Chris & Wuhan, 2017).

Health Hazards of Street Foods

Street food consumers in developing countries are generally more concerned about microbial hazards. Pathogens of significant public health importance such as *Salmonella*, *S. aureus*, *Listeria monocytogenes*, *Campylobacter jejuni* and *E. coli* have been isolated in some street foods in developing countries. Reports in literature show that consumers are

aware that microorganisms especially bacteria are responsible for food borne diseases but have very little knowledge about their pathogeneses. Knowledge of consumers on these microorganisms and their pathogeneses was diverse. Asiegbu reported that street food consumers in Johannesburg, South Africa were aware that certain microorganisms can cause diseases and even lead to death but knew very little about specific pathogens. Prior outbreak of food borne diseases linked to bacteria that attracted extensive mass media coverage was cited as the reason for their awareness. Samapundo also reported that over 80% of consumers interviewed in Port-au-Prince, Haiti were not aware that *Salmonella* spp., hepatitis A virus and *S. aureus* are major pathogens responsible for food related diseases outbreak (Alimi, 2016).

A survey on food safety knowledge and practices of street food vendors from a representative urban university campus in Quezon City, Philippines was done. The method used is a face-to-face interview that was conducted using a standardized survey tool containing 70 questions, which included queries on demographics and food safety knowledge and practices of street food vendors. There are topics on food safety assessment in both practices and knowledge which included: health and personal hygiene, good manufacturing procedures, food contamination, waste management, and food legislation. After the result has made, the study found that among the 54 street food vendors surveyed, establishment of food safety concepts particularly on topics that dealt with health and personal hygiene, food contamination and good manufacturing procedures. However, vendors were shown to be lack of knowledge in terms of food legislation and waste management. A significant gap between knowledge and practice on these topics was made and it was primarily attributed to the tendencies of street food vendors to compromise food safety for financial issues. Confusion in food laws was established in this test microcosm because the purveyor of food safety regulations was not the local government health unit but the business concession office of the campus administration. Providing and supplying of continuous food safety education, some financial assistance through social services affiliations, and basic water and waste management utilities were suggested to lessen the gap between knowledge and practices of safe street food vending in school campus (Azanza, 2000).

The presence of coliforms in food and water would, therefore, generally connotes faecal contamination, resulting in the risk of exposure to pathogens that cause gastrointestinal diseases, such as diarrhea and typhoid fever (Nekere, 2011).

This finding was supported by the study made in Calcutta, India wherein *Escherichia coli*, chiefly an indicator of faecal contamination, was detected in 55 percent of the samples tested. Although not all serotypes of *E. coli* are pathogenic in nature, some are enteropathogenic.

According to Mensah in 2002, of the 511 street food items examined in Accra, 69.7% contained mesophilic bacteria, 5.5% contained *Bacillus cereus*, 31.9% contained *S. aureus* and 33.7% contained Enterobacteriaceae (Mensah, 2005).

Research Paradigm

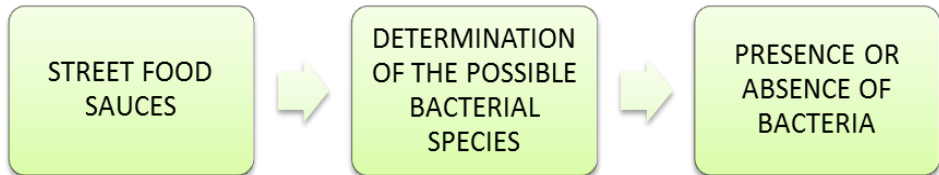


Figure 1. *Research Paradigm.*

The illustration shown above represents the concept of the whole study. This includes the whole process that was involved in the conduct of the study from gathering of sample sauces, determination of the possible bacterial species and presence or absence of bacteria in street food sauces.

METHODS

Research Design

An experimental research was utilized in this study since it describes the microbiological quality of street foods utilizing laboratory procedure.

Locale of the Study

The study was conducted within the vicinity of a university in Tuguegarao City particularly those small street food stalls. Food stall 1 is located in front of the college main gate of the university. Food stall 2 is located along Arellano Street, and food stall 3 is located along Lecaros Extension.

Sampling Design

Convenience sampling was used to identify three food stalls where sample sauces were collected and subjected to laboratory experimentation.

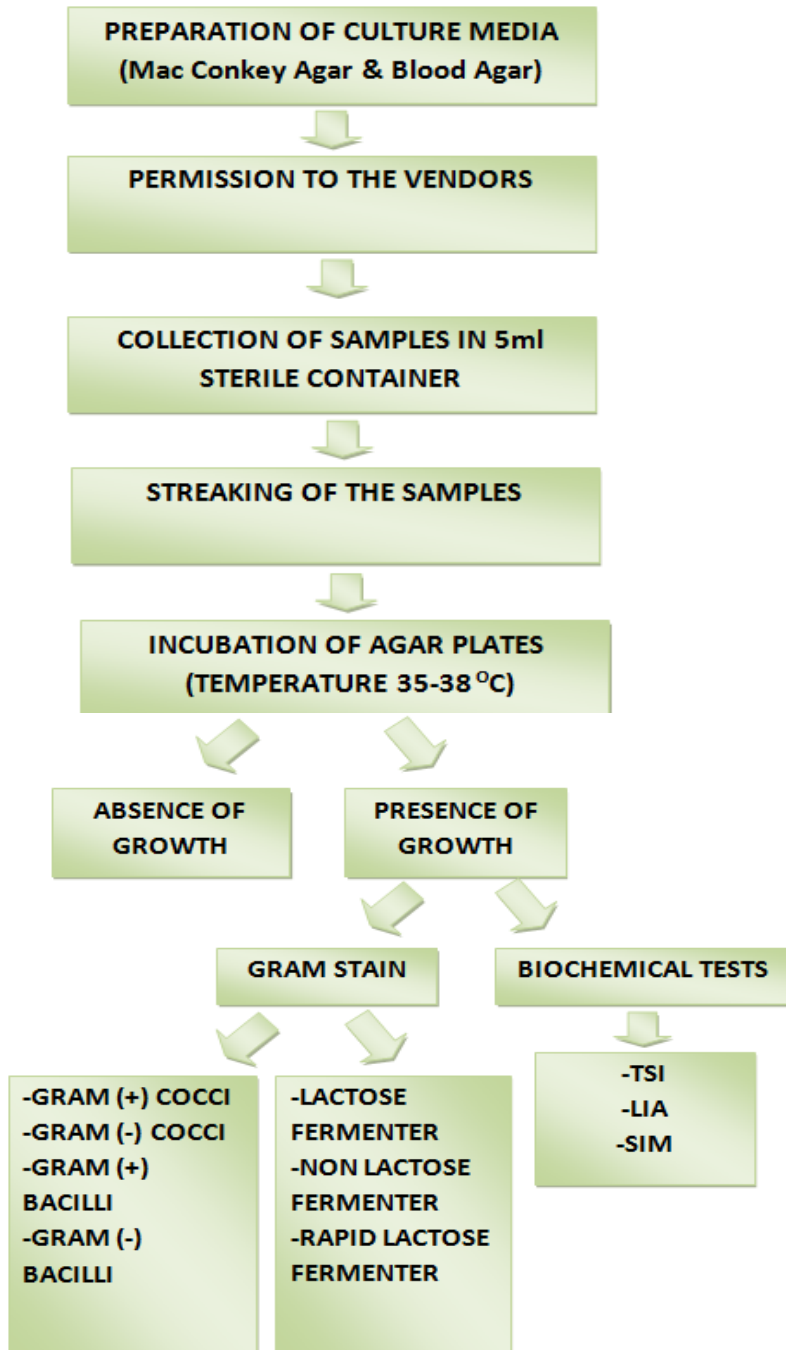


Figure 2. Methodological Framework

Data Gathering Procedure

1. Submission of Permission / Request Letter

The researchers sought the permission of the street food vendors to conduct the study with the assurance that the results will be treated with utmost confidentiality.

2. Sample collection

Samples included commonly consumed street food sauces like hot sweet, sweet and vinegar. Three samples for each kind were collected and the same were collected before the opening of the stall and 30 minutes before the closing of the stall. Samples gathered were separately put in 5ml sterile plastic container. Through proper handling, the researchers used gloves to prevent contamination and after that, the researchers placed it on an ice box then immediately transported and processed at the university's medical laboratory to ensure accuracy of the result.

3. Preparation of Culture Media

The procedure followed by the researchers was based on the standard protocol developed by HiMedia Laboratories (2015).

3.1. Blood Agar Base (Infusion Agar)

- 3.1.1. Forty (40) grams of blood agar base were suspended in 1000 mL Erlenmeyer flask with distilled water.
- 3.1.2. Dissolved the medium completely.
- 3.1.3. The Erlenmeyer flask with medium was sterilized by autoclaving at 15 lbs pressure (121OC) for 15 minutes.
- 3.1.4. The sterilized Erlenmeyer flask was cooled to 45-50OC. And 5% v/v sterilized defibrinated blood was added to the medium.
- 3.1.5. The medium with defibrinated blood was mixed well and poured into sterile Petri plates.

3.2. MacConkey Agar

- 3.2.1. 49.53 grams of MacConkey agar were suspended in 1000 mL Erlenmeyer flask with distilled water.
- 3.2.2. Dissolved the medium completely.
- 3.2.3. The Erlenmeyer flask with medium was sterilized by autoclaving at 15 lbs pressure (121OC) for 15 minutes.
- 3.2.4. The sterilized Erlenmeyer flask was cooled to 45-50OC.

3.2.5. The medium was mixed well and poured into sterile Petri plates.

4. Culturing of Bacteria and Colony Count

The procedure followed by the researchers was based on the standard protocol developed by Essential Microbiology of Hogg (2005).

In performing bacterial culture for the samples collected from the different food stalls, the following procedures were undertaken by the researchers:

- 4.1. The collected samples from the different food stalls were inoculated into MacConkey and Blood agar plates.
- 4.2. The inoculated plates were incubated at 35-37 °C for 18-24 hours.
- 4.3. After 18-24 hours, the bacterial growths were checked.
- 4.4. A bacterial colony counter was utilized to identify the number of colonies present in the agar plates.
- 4.5. Once colony forming units (CFU) were determined, the value of CFU/m³ was enumerated.

5. Bacterial Identification

The following procedures/steps were used in determining the characteristics of bacteria that grew on the plates to know the possible bacterial species.

5.1. Gram Stain

- 5.1.1. A thin smear from the culture was made. The smear was fixed by flooding with 95% methanol and allowed to air dry.
- 5.1.2. The fixed smear was overlaid with crystal violet for 30 seconds to 1 minute and rinsed with distilled water.
- 5.1.3. The smear was then flooded with Gram's iodine.
- 5.1.4. After 1 minute, the smear was rinsed with distilled water.
- 5.1.5. Acetone or ethyl alcohol was added drop by drop to the smear to decolorize the colors left after the addition of Crystal Violet and Gram's Iodine.
- 5.1.6. The smear was immediately rinsed with distilled water.
- 5.1.7. Safranin O was then added on the smear by flooding it.
- 5.1.8. After 30 seconds, the smear was rinsed with distilled water then the slide was allowed to drain and was dried.
- 5.1.9. The smear was examined under the oil immersion objective to view the characteristic reaction, morphology, white blood cells and other important structures.
- 5.1.10. Interpretation:

- 5.1.10.1.1. Gram positive organism stain blue-purple.
- 5.1.10.1.2. Gram negative organism stain pink-red.

5.2. Biochemical Tests

This test was used to determine the bacterial physiology that differs from one species to another. The procedure followed by the researchers was based on the standard protocol developed by Patricia (2013).

5.3. Triple Sugar Iron Agar

- 5.3.1. The TSI medium was warmed to room temperature before use.
- 5.3.2. A pure culture of the organism was obtained using an inoculation needle and was tested. Well-isolated colonies were selected for testing.
- 5.3.3. Inoculation was done by stabbing the bottom of the tube. The surface of the slant was streaked using a needle.
- 5.3.4. Tubes was incubated aerobically at 35-37C for 18-48 hours.
- 5.3.5. Reaction in the medium was examined.(Check appendix K).

5.4. Lysine Iron Agar (LIA)

- 5.4.1. The LIA medium was warmed to room temperature before use.
- 5.4.2. A pure culture of the organism was obtained using an inoculation needle and was tested. Well-isolated colonies were selected for testing.
- 5.4.3. Inoculation was done by stabbing the bottom of the tube. The surface of the slant was streaked using a needle.
- 5.4.4. Tubes was incubated aerobically at 35-37C for 18-48 hours.
- 5.4.5. Reaction in the medium was examined.
 - 5.4.6. Positive decarboxylation (butt), negative deamination (slant).
 - 5.4.7. Negative decarboxylation (butt), positive deamination (slant).

5.5. Sulfide, Indole, Motility (SIM)

- 5.5.1. The SIM medium was warmed to room temperature before use.
- 5.5.2. A pure culture of the organism was obtained using an inoculation needle and was tested. Well-isolated colonies were selected for testing.
- 5.5.3. Inoculation was done by stabbing 1/2 the bottom of the tube.
- 5.5.4. Tubes was incubated aerobically at 35-37C for 18-48 hours.

- 5.5.5. Reaction in the medium was examined and observed for the development of a pink to red color.
- 5.5.6. Sulfide
 - 5.5.6.1. Positive H₂S test is denoted by a blackening of the medium along the line of inoculation.
 - 5.5.6.2. Negative H₂S test is denoted by the absence of blackening.
- 5.5.7. Indole
 - 5.5.7.1. Positive test for indole is denoted when a pink to red color band is formed at the top of the medium after addition of Kovacs Reagent.
 - 5.5.7.2. Negative indole test has a yellow color after addition of Kovacs Reagent.
- 5.5.8. Motility
 - 5.5.8.1. Positive motility test is indicated by a diffuse zone of growth flaring from the line of inoculation.
 - 5.5.8.2. Negative motility test is indicated by growth confined to the stab line.

Data Analysis

Paired sample T-test was used to determine significant difference on the bacterial growth based on the time to which the samples were collected.

Waste Management Disposal

As per indicated in the Standard Operating Procedures, any authorized persons using infectious materials in the course of conducting laboratory testing and experimentation is mandated to dispose properly all what have been used. The samples used in this study were sealed in a bio-hazard plastic and were placed in an autoclave, sterilized to a high pressure with a saturated steam of 121°C (249 °F) for around 15–20 minutes depending on the size of the load and the contents. And the same were collected for proper disposal. The researchers also made sure to disinfect the different laboratory equipment such as glassware and the tools used in collecting samples. Gloves, masks and other contaminated materials were discarded.

Ethical Considerations

The researchers asked permission for ethical clearance from the Associate Dean, Dean of School of Education, Arts, Sciences and Health, Vice- President for Academics, University President and University Research Ethics Board. This research study was given ethical clearance number 51514. A letter was addressed to the City Health Office for the permission to collect samples. A request letter to collect samples of sauces was also given to the street vendors.

The researchers sought permission from the Laboratory in-charge in requesting for the materials used to conduct the study. The researchers asked guidance from a licensed Medical Technologist in the collection of samples and performing the test for the quality assurance of the results.

RESULTS

Table 1.1. *Qualitative Bacterial Growth Identification on the Samples Collected.*

Street food Sauces	Stall 1		Stall 2		Stall 3	
	Opening	30 minutes before Closing	Opening	30 minutes before Closing	Opening	30 minutes before Closing
Sweet	G	G	G	G	G	G
Sweet & Spicy	G	G	G	G	G	G
Vinegar	NG	NG	NG	NG	NG	NG

Legend: (G) = Growth; (NG) = No Growth

Table 1.1 shows positive growth in the samples of sweet and sweet and spicy sauces among all respondents during opening time and 30 minutes before closing time of the stores.

Table 1.2. *Mean Colony Count of Bacterial Growth in the different Culture Media of Sauces Collected*

Culture Medium Used	Type of Sauce	Colony Count of Sample taken during Store Opening Time (CFU)	Colony Count of Samples take 30 minutes before Store Closing Time (CFU)
Blood Agar Plate	Sweet Sauce	231.5556	321.4444
	Sweet and Spicy Sauce	228.1667	313.6667
McConkey Agar Plate	Sweet Sauce	243.5556	340.2222
	Sweet and Spicy Sauce	248.1667	334.8333

Table 1.2 shows significant number of colonies formed in the samples on both types of culture media used.

Table 2.1. Morphological Characteristics of Bacterial Growth based on Time of Collection of Individual Samples

Type of Sauce	Time of Collection	Description of Result	Bacterial Classification as to Gram Stain
Sweet	Opening	Pink colonies, Lactose fermenter	Gram (-) bacilli
	30 minutes before closing	Pink colonies, Lactose fermenter	Gram (-) bacilli
Sweet & Spicy	Opening	Pink colonies, Lactose fermenter	Gram (-) bacilli
	30 minutes before closing	Pink colonies, Lactose fermenter	Gram (-) bacilli

It can be gleaned from the table above that gram-negative bacteria are present in the sauces tested.

Table 2.3. Biochemical Test for Bacterial Identification on Samples collected during Opening Time of Stores

Samples		Growth on Mac Conkey					
		Biochemical Tests					
Trial 1,2 and 3		S	I	M	LIA	TSI	Result
1	Stall Sweet sauce	-	-	+	k/k	A/AG	Escherichia coli
	Sweet and Spicy sauce	-	-	-	k/k	A/AG	Klebsiella spp.
2	Stall Sweet sauce	-	-	+	k/k	A/AG	Escherichia coli
	Sweet and Spicy sauce	-	-	-	k/k	A/AG	Klebsiella spp.
3	Stall Sweet sauce	-	-	-	k/k	A/AG	Klebsiella spp.

The table reveals that *Escherichia coli* and *Klebsiella* bacteria are present in the different samples of sauces during the opening time of the stalls.

Table 2.4. Biochemical Test for Bacterial Identification on Samples collected 30 minutes before Closing Time of Stores

Samples		Growth on Mac Conkey					
		Biochemical Tests					
Trial 1,2 and 3		S	I	M	LIA	TSI	Result
Stall 1	Sweet	-	-	+	k/k	A/AG	Escherichia coli

	sauce						
	Sweet and Spicy sauce	-	-	-	k/k	A/AG	Klebsiella spp.
Stall 2	Sweet sauce	-	-	+	k/k	A/AG	Escherichia coli
	Sweet and Spicy sauce	-	-	-	k/k	A/AG	Klebsiella spp.
Stall 3	Sweet sauce	-	-	-	k/k	A/AG	Klebsiella spp.

The table reveals that *Escherichia coli* and *Klebsiella* bacteria are present in the different samples of sauces 30minutes before closing time of the stalls.

Table 3. Test of Significant Difference in the Colony Count of Different Samples of Sauces taken during Opening time and 30 minutes before Closing Time of Store

Category	t-value	p-value	Decision
Sweet Sauce in Blood Agar Plate	-20.364	.000	Reject Ho
Sweet and Spicy Sauce in Blood Agar Plate	-14.469	.000	Reject Ho
Sweet Sauce in McConkey Agar Plate	-27.161	.000	Reject Ho
Sweet and Spicy Sauce in McConkey Agar Plate	-18.133	.000	Reject Ho

The table above presents that there is a significant increase in the number of bacterial colonies formed from the samples of sweet and sweet and spicy sauces based on the time of collection of the samples. It can also be noted that the significant increase in colony count is observed on the two types of culture media used.

DISCUSSION

The research study aimed to determine the presence or absence of bacteria of the street food sauces of the different stalls situated outside a University. To achieve the objective of the study, culturing of the samples, colony count, gram staining and biochemical test were conducted. The biochemical test was utilized to identify the type of bacteria present.

Street food sauces; namely: sweet, sweet & spicy and vinegar were cultured in a culture media (Blood agar plate and Mac Conkey agar plate). The result showed that there was an observed growth of microorganism in sweet and sweet & spicy while no growth for vinegar sauces upon opening and 30 minutes before closing of the stalls. Gram stain shows the characteristics of gram negative bacteria and lactose fermenter. Also, statistical analysis showed that there is a significant difference on the colony count from the time of collection in the sweet and sweet & spicy. This means that there was an increase in the colony count of sweet and sweet & spicy from opening up to 30 minutes before closing of the store as manifested in the p-value which is 0.00 using 0.05 as level of significance (De Castro, 2015; Eufemio et al., 2015).

Biochemical test revealed that sweet and sweet & spicy sauces which were collected upon opening and 30 minutes before closing are usually contaminated with *Escherichia coli*, *Klebsiella* which is known to be two of the several types of bacteria that normally inhabit the intestine of humans and animals (Davis, 2006); thus, may cause health problems especially in the digestive system.

With all the findings of this research, these may be used as an eye-opener for the customers to be more careful and cautious in eating street foods with sauces and a waking call for street vendors to prepare healthily and properly their sauces.

CONCLUSION

This research study concluded that street food sauces like sweet and sweet & spicy in different street food sauce preparations are mostly contaminated with *Escherichia coli* and *Klebsiella* as manifested in the biochemical test. It also concluded that even prior the opening of the sauces, as revealed in this study, the presence of bacteria is already evident. Contributing factors for street food sauces contamination may be the poor environmental sanitation, poor food handling, preparation and storage.

RECOMMENDATIONS

Based on the result of this research study, the following recommendations are given:

- To get sample from all stalls outside of a University.
- To identify the presence of other pathogen.
- The Local Government Units (LGU) should consider the following:
 - Improve sanitary policies and guidelines in the operation of food stalls.
 - Establishment of adequate facilities and utility services for street vendors to fully attain food safety and monitor regular sanitation of

the environment and food handling practices to maintain food safety and promote disease prevention.

- The extension services of the School of Education Arts Sciences and Health of the university on food safety and disease prevention should be done to increase awareness of consumers.

REFERENCES

- Alimi, B. A. (2016). Risk factors in street food practices in developing countries: A review. *Food Science and Human Wellness*, 5(3), 141-148. Retrieved on March 26, 2017 from <http://www.sciencedirect.com/science/article/pii/S2213453016300441>
- Azanza M.P., Gatchalian C.F., Ortega M.P. (2000). Food safety knowledge and practices of streetfood vendors in a Philippines university campus. *Int J Food Sci Nutr*, 51(4):235-246. Retrieved on March 28, 2017 from <https://www.ncbi.nlm.nih.gov/pubmed/11027035>
- Bhat, R. V., & Waghray, K. (2000). Profile of Street Foods Sold in Asian Countries. *World Rev Nutr Diet*, 86, 53-99. Retrieved on March 28, 2017 from <https://www.karger.com/Article/Pdf/59731>
- Center for disease control and prevention (2011). Healthcare-associated Infections Retrieved on March 28, 2017 from <https://www.cdc.gov/hai/organisms/gram-negative-bacteria.htm>
- Chakravarty, I. & Canet, I. (2004). *Street food in Calcutta*. Retrieved on March 28, 2017 from <http://www.fao.org/docrep/w3699t/w3699t06.htm>
- Barcelon, E. G., Collado, D. M. R., Eustaquio, S. A., Luna, M. N. H., Santos, K. J. C., Sombrano, M. C. S., & Villaceran, D. P. (2015). Consumer Perception and Microbiological Analysis on Safety of Street Food Dipping Sauces. *Asian Journal of Agriculture and Food Sciences*, 3(1). Retrieved on April 29, 2017 from <https://www.ajouronline.com/index.php/AJAFS/article/viewFile/2355/1265>
- Bryan, F. L., Teufel, P., Roohi, S., Qadar, F., Riaz, S., & Malik, Z. U. R. (1992). Hazards and critical control points of food preparation and storage in homes in a village and a town in Pakistan. *Journal of food protection*, 55(9), 714-721. Retrieved on March 29, 2017 from <http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-55.9.714?code=fopr-site>
- Daniyan, S. Y., Abalaka, M. E., Momoh, J. A., & Adabara, N. U. (2011). Microbiological and physiochemical assessment of street vended soyabean cheese sold in Minna, Nigeria. *International Journal of Biomedical and Advance Research*, 2(1), 25-31. Retrieved on March 28, 2017 from <http://www.ss-journals.com/index.php/ijbar/article/view/56/56>
- Davis C.P. (1996). Normal Flora. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 6. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK7617/> Retrieved on April 29, 2017

- Dilbaghi, N., & Sharma, S. (2007). Food spoilage, food infections and intoxications caused by microorganisms and methods for their detection. Retrieved on March 29, 2017 from <https://pdfs.semanticscholar.org/bf19/79600620a165f83e3e27433c6121531aeb9f.pdf>
- Hassan, M. Z., Islam, M. S., Salauddin, M., Zafr, A. H. A., & Alam, S. (2017). Food Safety Knowledge, Attitudes and Practices of Chotpoti Vendors in Dhaka, Bangladesh. *Journal of Enam Medical College*, 7(2), 69-76. Retrieved on March 29, 2017 from https://www.researchgate.net/publication/317341753_Food_Safety_Knowledge_Attitudes_and_Practices_of_Chotpoti_Vendors_in_Dhaka_Bangladesh
- Harkins, D. (2013). *What Is Isaw?* Retrieved on March 26, 2017 from <http://www.wisegeek.com/what-is-isaw.htm#didyouknowout>
- Hogg, S. (2005). *Essential microbiology*. John Wiley & Sons Ltd: South Sussex, England. Retrieved on April 28, 2017 from http://www.grsmu.by/files/file/university/cafedry/microbiologii-virysologii-immynologii/files/essential_microbiology.pdf
- Iliades, C. (2011). *The Most Common Causes of Food Poisoning*. Retrieved on March 29, 2017 from <https://www.everydayhealth.com/digestive-health/the-most-common-causes-of-food-poisoning.aspx>
- Lisa, A. A. (2016). *Identification of microbiological contamination of ready to eat food vended in streets of different institutions in Dhaka city, Bangladesh* (Doctoral dissertation, East West University). Retrieved on March 28, 2017 from http://dspace.ewubd.edu/bitstream/handle/123456789/2210/Arzu_Arafin_Lisa.pdf?sequence=1&isAllowed=y
- Lizotte, C. (2002). *Wanna Know About Filipino Street Food? It's All Here!* Retrieved on March 26, 2017 from <https://www.continentscondiments.com/exotic-filipino-street-food-philippines>
- Long, S. M., Adak, G. K., O'Brien, S. J., & Gillespie, I. A. (2002). General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992-2000. *Communicable Disease and Public Health*, 5(2), 101-105. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/12166293>
- Lues, J. F., Rasephei, M. R., Venter, P., & Theron, M. M. (2006). Assessing food safety and associated food handling practices in street food vending. *International Journal of Environmental Health Research*, 16(5), 319-328. Retrieved on March 29, 2017 from <https://www.ncbi.nlm.nih.gov/pubmed/16990173>
- Mensah, N. (2005). *Informal Food Distribution Sector in Africa (Street Foods): Importance and challenges*. Retrieved on March 29, 2017 from <http://www.fao.org/docrep/meeting/010/a0215e/A0215E26.htm>
- Ofofu, A. H., Dzignbede, B. A., Agidi, J. E. L., Adjei, J., & Koranteng, A. (2014). A study into microbial quality of ready to eat foods sold in the Sunyani

Municipality of Ghana. *Global Journal Biology Agriculture Health & Sciences*, 3(3), 84-91. Retrieved on March 26, 2017 from <http://gifre.org/library/upload/volume/84-91-Microbial-vol-3-3-14-gjbahs.pdf>

- Nkere, C. K., Ibe, N. I., & Iroegbu, C. U. (2011). Bacteriological quality of foods and water sold by vendors and in restaurants in Nsukka, Enugu State, Nigeria: a comparative study of three microbiological methods. *Journal of health, population, and nutrition*, 29(6), 560–566. Retrieved April 8, 2017 from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3259718/>
- Patricia, T. (2013). Bailey & Scott's Diagnostic Microbiology Thirteenth Edition. Retrieved on April 29, 2017 from <https://www.dropbox.com/s/0q6rqodr6vug0cs/Bailey%20Scott%27s%20Diagnostic%20Microbiology%2013th%20Ed.pdf?dl=0>
- Prussin, A. J., 2nd, & Marr, L. C. (2015). Sources of airborne microorganisms in the built environment. *Microbiome*, 3, 78. Retrieved on April 8, 2017 from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4688924/>
- Rane S. (2011). Street vended food in developing world: hazard analyses. *Indian journal of microbiology*, 51(1), 100–106. Retrieved on April 26, 2017 from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3209856/#CR27>.
- Shamiksha, S. (2014) Importance of Biochemical Tests of Bacteria. Retrieved on April 26, 2017 from <http://www.yourarticlelibrary.com/experiments/importance-of-biochemical-tests-of-bacteria/26624/>
- Snyder, O. (2013). Assuring Retail Food Excellence Worldwide Through Industry Self-Control. Retrieved on March 27, 2017 from <http://www.hi-tm.com>
- Tambekar, D. H., Jaiswal, V. J., Dhanorkar, D. V., Gulhane, P. B., & Dudhane, M. N. (2009). Microbial quality and safety of street vended fruit juices: a case study of Amravati city. *Internet Journal of Food Safety*, 10(7), 72-76.
- Thunberg, R. L., Tran, T. T., Bennett, R. W., Matthews, R. N., & Belay, N. (2002). Microbial evaluation of selected fresh produce obtained at retail markets. *Journal of food protection*, 65(4), 677-682. Retrieved on April 6, 2017 from <http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-65.4.677?code=fopr-site>
- Winarno, F. G., & Allain, A. (1991). Street foods in developing countries: lessons from Asia. *Food, nutrition and agriculture*, 1(1), 11-18. Retrieved on March 26, 2017 from <http://www.fao.org/docrep/u3550t/u3550t08.htm>
- World Health Organization (2015). Food Safety: What you should know. Retrieved on March 28, 2017 from http://www.searo.who.int/entity/world_health_day/2015/whd-what-you-should-know/en
- World Health Organization (2010). Bulletin of the World Health Organization 7: 80. Retrieved on March 27, 2017 from www.foodsafetymatters.gov.au