Isolation and Quantitative Analysis of *Escherichia coli* in Pancit Batil Patong in Tuguegarao City, Cagayan

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***Abstract*— Human consumption of contaminated foods is one of the most commonly implicated reason for the widespread and rising cases of food borne illnesses around the globe. Although there are already established protocol and measures to ensure food safety, there are bacteria that easily withstand and adapt the changes in the environment. *E. coli* is a gram negative bacteria that is commonly associated with several outbreaks of food borne illnesses and it is commonly found in food. Most are not pathogenic and can be easily controlled by adequate cooking at 710C. However, public health and food safety have been challenged from the existence of heat resistant *E. coli* which may pose threat to human health. This study, therefore sought to assess and characterize the *E. coli* present in Pancit Batil Patong in five Panciteria restaurants near University of Saint Louis Tuguegarao. Pancit Batil Patong is a famous local delicacy of Tuguegrao City. Using the standard microbiological identification and Vitek 2 Compact machine for confirmation, result showed that there areno *E. coli* present in the food sample, however, other bacteria such as *Staphylococcus aureus, Acinetobacter haemolyticus, Leuconostoc pseudomesenteroides, and Bacillus subtilis/ amyloliquefaciens/ atrophaeus* were identified which can greatly affect immunocompromised individuals. The absence of *E. coli* from the food samples indicates that these Panciteria restaurants have good hygiene and practiced safe handling and preparation of their foods. However, with the presence of the other bacterial isolates identified variations in the operation of these Panciteria restaurants in ensuring food may be present and worthy to be further investigated.**

Keywords*— *E. coli, pancit batil patong, microbiological identification, food safety

I. INTRODUCTION

Foods are any substance that people eat or drink in order to maintain life and growth. It is an important source of nutrients that is needed to sustain everyday life and it must be safe for consumption. But these could also put people at risk of contracting diseases as foods serve as a natural reservoir for most microorganisms especially when it is unprocessed or uncooked, and partially cooked. Fresh produce, for example, is prone to contamination and has been linked to several outbreaks of food borne disease around the globe. Meat, especially from cattle, dairy products, cheese, raw milk and eggs are considered to be the common sources of food borne diseases caused by bacterial pathogens. Ready-to-eat food and steam meals could also contract bacteria from their contaminated raw ingredients. Food contamination can easily be treated with enough heating and proper cooking but there are also external factors that can contribute again to the contamination of food; one is the unhygienic practice of the ones serving the food, the materials used for food processing, using raw ingredients, the place and the person themselves who can be infected (Akbar & Anal, 2011).

Escherichia coli, commonly abbreviated as *E. coli* is a gram-negative, rod- shaped bacterium that is normally present in the gastrointestinal tract having an important role in digestion. It is one of the commonly associated pathogens that contaminates foods causing disease. Most of the strains of *E. coli* are not pathogenic and are widely present in the surrounding, but there are six *E. coli* strains that can cause diseases, the Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC),

Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC) and Diffusely adherent *E. coli* (DAEC) (CDC, 2014) which causes Non bloody Diarrhea or Bloody Diarrhea (Akbar & Anal, 2011) , Haemolytic Uraemic Syndrome (HUS), Urinary Tract Infections, Respiratory illness and Pneumonia (Nma & Oruese, 2013; CDC, 2014). Among this strain, STEC is the most occuring due to its unique characteristic, and making it a major threat to people. This strain can change behaviors from time to time and has the ability to acquire genes from other bacteria, like *Shigella*, to become more pathogenic causing outbreaks of food borne diseases.

Shiga toxin producing *E. coli* (STEC) is also known as the Enterohemorrhagic *E. coli* (EHEC) or Verotoxin producing *E. coli* (VTEC). Studies show that STEC can grow in temperatures ranging from 7° to 50°C and can survive minimally processed vegetables (CDC, 2020). In 1982, *E. coli* O157: H7 was first identified after an outbreak of bloody diarrhea occurred due to consumption of rare cooked minced meat. This strain of *E. coli* is also the primary cause of infection in industrialized countries including the USA, Canada, and England and also in Africa, Japan and the United Kingdom. In the US, this is the major cause of HUM, causing kidney failure among children. Pathogenic *E. coli* also causes meningitis in neonates having a mortality rate of 15-40% and 50% continue their life with neurological damage. It also causes peritonitis, mastitis, septicemia and pneumonia (Gozde & Emek, 2019; Akbar & Anal, 2011).

In the Philippines, the Department of Health (DOH) reported cases of food and water borne diseases from 2016 to 2017, one of which is bloody diarrhea. From these, 1965 of the samples were taken in the laboratory to determine what agent causes the disease. Among the top pathogens, 2.5% of *E. coli* were identified. Overall, there are a total of 3548 cases of acute bloody diarrhea from all the regions of the country, age groups from 1-4 have the numerous cases, and 45 deaths were incurred. In region II, there are 341 and 129 cases of this disease in 2016 and 2017 respectively and no deaths were incurred.

Public health and food safety have been challenged from the existence of heat resistant *E. coli* strains. The *E. coli*, AW1.7 has been said to be the most heat resistant among all the strains of this bacteria. In the 1982 outbreak of *E. coli* O157:H7, cooked ham burgers from fast food chains have been positive for this *E. coli* strain. In the study of Røssvoll et al., (2014), hamburger patties subjected to 710C cooking as recommended by the Food and Drug Administration (FDA) can't be enough to kill all strains of *E. coli* in the patty. This is because *E. coli* can penetrate inside the meat which cannot be killed when cooked inappropriately. This is congruent to the findings of Dlusskaya, McMullen & Gänzle (2011) after assessing beef meats from beef processing facilities. They have noted the extremely heat resistant *E. coli* strains, identifying AW1.7 to be the most heat stable even at 71oC which were also presented on the study of Mercer et al., (2015) of which the same strain survives in beef grilled patties with a temperature of 71oC, and O157: H7 STEC and NON-O157:H7 STEC are considered to be moderately heat resistant (Mercer et al., 2015).

Pancit Batil Patong or Pancit Tuguegarao is a popular noodle dish which Tuguegarao City, Cagayan is highly known for. It is made out of pansit miki Tuguegarao, minced carabao meat (water buffalo), bean sprouts and other vegetables topped with egg and chicharon. Batil Patong if directly translated means "beat the egg" for Batil and "placed on top" for Patong which explains the egg on top of the pancit when served. Pancit Batil Patong in a way could be susceptible to contamination as many external factors play along the process of its preparation.

According to Section 15 Rule 15b.10 of Republic Act 10611 otherwise known as the Food Safety Act of 2013, the Department of Health (DOH) shall assist the LGUs in establishing a mechanism for the issuance and enforcement of ordinances and regulations for food safety based on the national standards within their territorial jurisdiction. Furthermore, the LGUs shall be responsible for the implementation of the food safety requirements of foods produced within their areas of jurisdiction.

Prior to applying for Mayor's permit to own and operate a food establishment, the City Government assesses the kitchen area where the foods are prepared. Staff are also required to undergo medical examinations such as chest x-ray and fecalysis. The City Government also requires the use of gloves and hair nets during preparation and handling of foods. Though safety and health protocols are being done before acquiring a permit, there is still a need for microbial quality assessment for pathogens to further ensure the safety of the foods being served.

As said, *E. coli* is the most common contaminant of food. And in the contamination of food, there are many factors, including external ones that could make the contamination possible. With this, this study will focus on identifying the presence of *E. coli* on Pancit Batil Patong within Tuguegarao City. Samples are collected from the five (5) Panciteria established within the vicinity of the University of Saint Louis Tuguegarao. By the ability of *E. coli* to grow on different media and develop morphological features in gram stain and different biochemical tests, researchers must be able to identify the presence of *E. coli* from the sample. This study therefore aimed to isolate and characterize Escherichia coli, which may be present in Pancit Batil Patong sold within the vicinity of the University of Saint Louis Tuguegarao, Tuguegarao City Cagayan using determination of Colony Forming Units (CFU) and gram staining and biochemical testing of the colonies formed.

II. METHODS

This study is descriptive quantitative in design. The researchers collected the sample (Pancit Batil Patong) within the vicinity of the University of Saint Louis Tuguegarao to determine and identify *E. coli*. The samples were collected in the five (5) Panciteria restaurants within the vicinity of the University of Saint Louis Tuguegarao and taken at Regional Animal Disease Diagnostic Laboratory at Carig Sur, Tuguegarao City, Cagayan where it underwent several tests for the evaluation of the presence of *E. coli*.

A. Collection and Handling of Samples

The samples were aseptically collected from the five (5) Panciteria Restaurant within the vicinity of the University of Saint Louis Tuguegarao.

Sample containers were clearly labelled and identified. The time, date, and location of the collection is recorded to ensure an organized and reliable results. Sample containers were securely sealed and placed on proper storage for safe transport in the laboratory. Samples were analyzed within 24 hours as a general rule to avoid inaccurate and imprecise results.

The samples collected were taken at Regional Animal Disease Diagnostic Laboratory at Carig Sur, Tuguegarao City, Cagayan where it underwent several tests for the evaluation of the presence of *E. coli*.

B. Isolation of E. coli from the sample

Isolation of the pathogen is performed through serial dilution method. This method is utilized to reduce a dense culture of cells to a more appropriate concentration, each dilution will reduce the concentration of bacteria by a specific amount (Lupindu, 2017). Each sample was weighed 10 grams and diluted with 90 mL of sterile distilled water and placed in stomacher or mixed through a blending apparatus. The solution was subjected to a tenfold dilution up to 10-3, to establish a higher number of colonies can be isolated. Every 1 ml of the solution were plated into Nutrient Agar plates followed by 24 hours of incubation at 37°C. After successful isolation of bacterial colonies, it is streaked onto the Eosin Methylene Blue (EMB) medium for presumptive confirmation of *E. coli* and incubated aerobically for 24 hours at 37°C. Then, the isolates were subjected to Gram staining for the preliminary confirmation of the bacteria to be followed by different biochemical techniques through Vitek 2 Compact Machine.

C. Identification of E. coli Isolates

a) Morphological Properties on Agar Plate: Observation of morphological characteristics of colonies is based on the standardized cultural characteristic of *E. coli* on Nutrient Agar. The colony is characterized by convex elevation, greyish white color, translucent-opaque structure. The bacteria are also distinguished by smooth surface on fresh isolation, rough surface on repeated subculture and mucoidal surface with capsulated strains (Tille, 2013).

b) Screening of Suspected E. coli Isolates: Suspected *E. coli* isolates from the Nutrient agar were streaked onto the eosin methylene blue (EMB) agar plate for presumptive confirmation as *E. coli*. EMB agar medium contains lactose and the dyes eosin and methylene blue that permit differentiation between enteric lactose fermenters and non-fermenter, and also the identification of the colon bacillus *E. coli*. The *E. coli* colonies are black colonies with a metallic green sheen caused by the large quantities of acid that is produced and that precipitates out the dyes onto the growth's surface (Cappuccino and Sherman, 2011). Then, the standard microbiological procedures of bacterial isolates are tested based on morphological, cultural, and biochemical characteristics.

D. Gram Staining Procedure

Gram staining is a staining procedure utilize to differentiate and classify the species of bacteria whether it is a gram-positive bacteria or gram-negative bacteria. (Bruckner, 2021). The isolates were collected from the Eosin Methylene Blue Agar and Nutrient and placed on a smear slide for the gram staining process. The crystal violet stain was added to the slide and rested for 60 seconds. After pouring off the crystal violet stain, the slide is rinsed to remove the excess stain using tap water. Then, iodine solution was added on the smear and rested for 60 seconds. The iodine solution is removed by pouring it off and the slide is rinsed using running water from a faucet. Next, decolorizer (ethanol) is poured on the slide. Adding the ethanol is stopped when the solvent has no longer color. Remove it after 5 seconds with water. Lastly, add a counterstain (basic fuchsin solution) for 60 seconds. Pour off the solution with water. Blot using a bibulous paper to withdraw the excess water.

E. Biochemical Identification

Biochemical tests were performed to identify the unknown cultures. The identification is performed through the Vitek 2 Compact Machine. The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number.

a) Suspension Preparation: Four clean test tubes, Nutrient Agar and EMB agar with isolated organisms were prepared. Each 4 glass/test tubes were filled with 3ml 0.45% saline solution. The test tubes are then subjected to DensiCHECK to provide the density of each organism isolated from the two agars. Each agar is directly swab with sterile cotton tip to isolate the colony and placed on each designated tube. Next, the sterile cotton tip is again submerged to the test tubes and mixed to meet the standard density for each organism through the DensiCHECK. Then, the Vitek Biochemical Test Card is placed in the tube for the biochemical identification of the isolated bacteria.

b) Filling and Loading the Cards: All the cards and tubes with suspension were set in a cassette. From the PC workstation, Cassette Worksheet and record Lab ID and barcode for each card were printed. The cassette was loaded into the instrument and the Fill door was closed. Then the Start Fill button was pressed. Audible and visual indicators signal the completion of the fill process, then the cassette was transferred into the loading station and the Load door was closed. The cassette from Loading station was removed when the Indicator was seen flashing and Remove status was clicked.

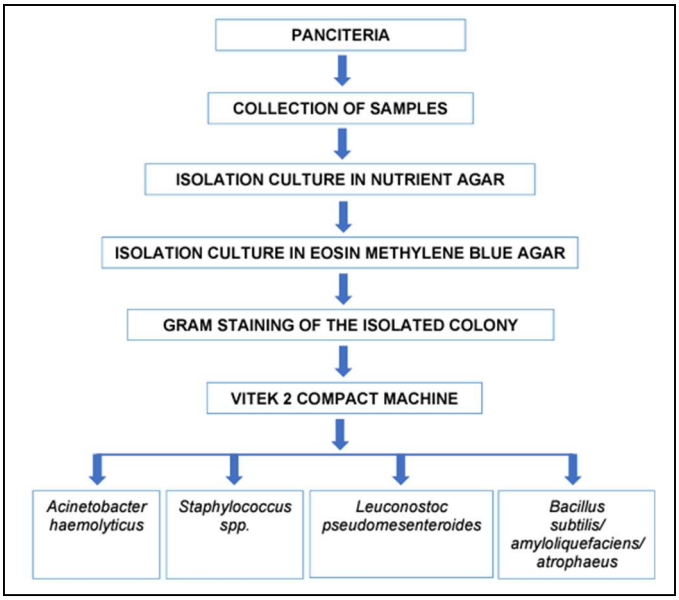

Fig. 3. Methodological framework of the study

F. Waste Disposal Management

All materials and waste incurred were treated as infectious waste and underwent an on-site decontamination prior to disposal. The disposal of samples and used culture media followed the guidelines from the Manual of Basic Practical Microbiology (2016). The procedure was accomplished with the utilization of protective equipment, with proper handling and with the guidance of the research adviser. (1) The wastes were decontaminated through autoclaving (steam sterilization) for up to 90 minutes at 2500F (1210C) as recommended by the CDC. (2) The contents were drained of the plates into the sink under running tap water. (3) After autoclaving, this waste is already safe for handling and disposal. The materials that were not disposable and can be reused were disinfected. (4) Working areas were decontaminated with a suitable disinfectant at the end of each work period.

G. Data Analysis

This study utilized descriptive statistics, specifically the measures of frequency for the measurement of the colonies. Colonies that were confirmed in the gram-staining and biochemical test are counted and reported in a tabular form. This shall represent the number of bacterial isolates confirmed in the sample tested.

H. Ethical Considerations

This study underwent the evaluation of the Board of Ethics of this university, Dean of the School of Health and Allied Sciences, Vice President for the Academics and President of the University. All protocols in conducting this study were subjected for the approval by Ethics Committee of the university. Further, the researchers ensured to protect and store all the data collected and regard it as confidential. Lastly, the research underwent ethical review and clearance from the University Research Ethics Board

III. Results

TABLE I. Number of Colonies and Morphological Properties of Isolates from Nutrient Agar

Panciteria	Sample No.	No. of Colonies	Characteristics	
			Color	*Texture*
A	1	1	White	Mucoid
B	1	1	Grayish White	Mucoid
C	1	1	White	Mucoid
D	1	N/A	N/A	N/A
E	1	1	Grayish color	Dry

Table 1 illustrates the number of bacterial colonies isolated from the different samples. The Panciteria A, B, C and E established one bacterial colony each on the Nutrient agar medium. For the Panciteria A and Panciteria C, the bacterial colony has the same characteristics with an appearance of whitish color and mucoidal texture. For the Panciteria B it exhibits a bacterial colony characterized by grayish white color with mucoidal texture. Then, the Panciteria E produced a bacterial colony distinguished through its dry appearance and grayish color. Lastly, only the Panciteria D didn't harbor any bacterial colony on the first medium. The (-) symbol indicates that there are no bacterial isolates for the other samples.

TABLE II. Number of Colonies and Morphological Properties of Isolates from Eosin Methylene Blue Agar

Panciteria	Sample No.	No. of Colonies	Characteristics	
			Color	*Texture*
A	1	1	N/A	N/A
B	1	1	Colorless	Mucoid
C	1	1	N/A	N/A

Results in this regard are presented in this Table 2, which represents the number of bacterial isolates from the Eosin Methylene Blue Agar. Here, the colonies isolated on Nutrient Agar that have the general characteristic of *E. coli* are streaked on the EMB agar for the presumptive identification of *E. coli*. Only Panciteria B provided a bacterial isolate which exhibits a colorless appearance with mucoidal texture. The (-) symbol indicates that there are no bacterial isolates for the other samples.

TABLE III. Biochemical Characteristics of Pathogenic Bacteria Identified in the Food Samples

Panciteria	Colony No.	Characteristics		Bacteria Identified
		Gram Staining	*Lactose Fermentation*	
A	1	Gram Positive	Cocci in clusters	*Staphylococcus spp.*
B	1	Gram	Cocci in chain	*Acinetobacter haemolyticus*
C	1	Negative	Cocci in clusters	*Leuconostoc pseudomesenteroides*

Panciteria	Colony No.	Characteristics		Bacteria Identified
		Gram Staining	*Lactose Fermentation*	
E	1	Gram Positive	Bacilli in chain	*Bacillus*

The bacterial colony isolated from Panciteria A and Panciteria C, showed cells exhibiting gram-positive stain with a shape of cocci in clusters which is consistent with the bacteria species of *Staphylococcus* (with 98% probability) and Leuconostoc pseudomesenteroides (with 87% probability). As for the bacterial colony isolated from the Panciteria E, it exhibits a gram-positive stain in the shape of bacilli in chain which is consistent with the bacteria specie of *Bacillus subtilis/ amyloliquefaciens/ atrophaeus* (with 92% probability). Lastly, the sample from Panciteria B showed cells displaying gram-negative stains in the shape of cocci in chains which is consistent with the bacteria *Acinetobacter haemolyticus* (with 92% probability).

IV. Discussion

The study isolated and characterized the bacterial colonies of the samples from the five (5) Pancit Batil Patong Panciteria within the vicinity of University of Saint Louis Tuguegarao, Tuguegarao City Cagayan. The attempt on the isolation and quantitative analysis of the *E. coli* was the major objective of the present study, however, the presence of chosen bacteria was not confirmed.

Studies showed that the bacterial genera of Escherichia can grow in temperatures ranging from 7°C to 50°C and can survive minimally processed vegetables (CDC, 2020; Stanley, 2016). Whereas, according to the Food Safety Education (2011) the average cooking temperature ranges from 63°C to 74°C, hence the growth of possible colonies in both agar is perhaps inhibited on the cooking process of the sample. Since *E. coli* can easily be controlled at specified temperature, this may suggest that the Pancit Batil Patong served in the five Panciteria restaurant are adequately cooked and they practice safe food handling.

Since *E. coli* is a bacteria identified to be the common contaminants of food and that it can most be found anywhere, the tendency of having such bacteria in the food sample is highly possible but the fact that there are existing and effective measures to control food contamination may possibly prevent its occurrence. In the 1982 outbreak of *E. coli* O157:H7, cooked ham burgers from fast food chains have been positive for this *E. coli* strain. In the study of Røssvoll et al., (2014), hamburger patties subjected to 71°C cooking as per recommended by Food and Drug Administration (FDA) is not enough to kill all strains of *E. coli* in the patty. This is because *E. coli* can penetrate inside the meat which cannot be killed when cooked inappropriately. This is congruent to the findings of Dlusskaya, McMullen and Gänzle (2011) after assessing beef meats from beef processing facility. They have noted the extremely heat resistant *E. coli* strains which were also presented on the study of Mercer et al., (2015) of which the same strain survives in beef grilled patties with a temperature of 71°C. With these, it can be justified that the absence of *E. coli* in the Pancit Batil Patong from the five different Panciteria restaurant in Tuguegarao denotes that the food is properly cooked with adequate temperature, enough to kill all *E. coli* strains.

In addition, total coliform bacteria and *E. coli* counts have long been used as indicators of poor sanitary conditions and fecal contamination in food. The presence of *E. coli* in food may indicate fecal contamination, poor hygienic conditions, and the existence of enteric pathogens. For instance, the presence of *E. coli* in fruits and vegetables is very important in assessing inadequate hygiene. The absence of *E. coli* in the food may suggest that the preventive measures for food safety and risk management controls at all stages of the food product continuum are well implemented and followed. This also means that proper hygiene and controls were incorporated into all processes, from agricultural production to final preparations and serving (WHO, 2018). Since there is no *E. coli* isolated from the food sample collected from the five Panciteria of interest, this indicates that the Panciteria restaurant near the University of Saint Louis Tuguegarao have a good hygiene and effective measures in food handling and preparation.

While it is true that all of the five Panciteria restaurant didn't contain *E. coli*, a total of four (4) bacterial isolates were identified, three (3) of which were isolates from the Nutrient agar and one (1) were from the Eosin Methylene Blue Agar. During the investigation, the findings revealed the presence of bacterial genera characterized as Staphylococcus spp with a probability of 98% isolated in Panciteria A; *Acinetobacter haemolyticus* with a probability of 92% in Panciteria B; *Leuconostoc pseudomesenteroides* with a probability of 87% in Panciteria C; and *Bacillus subtilis/ amyloliquefaciens/ atrophaeus* with a probability of 92% in Panciteria E. Since the bacteria that were isolated in Panciteria A, B, C, and E varied in specie, meaning they are not consistent to other Panciteria, there is a tendency that the occurrence of this bacteria may be due to a certain instance which should be considered and assessed on a case-to-case basis. Contrarily, only Panciteria D didn't harbor any bacteria which denotes the possibility that they're careful in ensuring food cleanliness and may be an indication that there are existing variations on the way they handle and prepare their foods compared with the other Panciteria restaurants.

Among the isolated bacteria, the most clinically significant is the bacterial genera of Staphylococcus, which is a pathogenic gram-positive bacterium that can produced toxins leading to Staph food poisoning in humans (Marino et al., 2010). *Staphylococcus spp.* can be found on hair, nasal passage, throat, and skin of more than 50% of healthy individuals and the surrounding environment (chopping boards, surfaces, and utensils). These bacteria can be transmitted from person to person, to any object or even to the food itself if it is not properly prepared. Considering these factors, cross contamination might have occurred when the person is infected, or any object used in the food preparation is contaminated with *Staphyloccocus*. Other bacteria isolated are *Acinetobacter haemolyticus* which is a gram-negative bacteria mostly present in the microbiota of healthy human skin and once isolated from the the feces of children experiencing bloody diarrhea (de Amorim & dos Santos Nascimento, 2017). Since there are only limited reports and studies regarding this bacteria, the presence of this in the food sample from panciteria B may suggest that the person who cook and serves the food may harbor this bacterium and accidentally contaminates the food; while *Leuconostoc pseudomesenteroides* is a gram positive bacteria that can be

found in vegetables that are not well cooked and in food that might be near its expiration or were already spoiled (Sade, 2011). This bacterium is commonly associated with food spoilage which may suggest that the presence of this bacteria in the food sample from panciteria C may be due to expired or nearly spoiled ingredients; and lastly, *Bacillus subtilis/amyloliquefaciens/atrophaeus* which produces endospores (Hirooka, 2014), and is found mainly in soil which served as its natural reservoir (Tuazon, 2017). Cross-contamination might have occurred when the food is in contact with a soil harboring these bacteria. Contrary to *Staphylococcus*, these bacteria are of less significance since they are infrequently found in nature, and there have been very few cases reported related to them. Also, it has been said that these bacteria can rarely infect healthy people but may be harmful to those who are severely ill or immunocompromised individuals (Serra & Earl, 2014).

Food harboring bacteria is an impeding problem that causes food borne illnesses in many countries including the Philippines, and this may reflect the sanitary conditions, hygiene and food handling practices of every food establishment and the public in general. This may be a challenge to the people and to the concerned agency to ensure food safety as food is a primary and basic need for humans, hence, it must always be clean and safe for consumption.

V. CONLUSION

The study confirmed that there are no *E. coli* strains present in Pancit Batil Patong collected from the five Panciteria restaurants of interest. The absence of *E. coli* from the food samples indicates that these Panciteria restaurants have good hygiene and practiced safe handling and preparation of their foods.

While it's true that these Panciteria restaurants didn't harbor *E. coli*, other bacteria such as *Staphylococcus spp., Acinetobacter haemolyticus, Leuconostoc pseudomesenteroides,* and *Bacillus subtilis/ amyloliquefaciens/ atrophaeus* were isolated. Contrarily, only one Panciteria didn't harbor any bacteria which may be an indication that they have better practices in safe food handling and preparation compared to other Panciteria. This may also suggest that there might be variations in the operation of these Panciteria restaurants in ensuring food safety which may have influenced the result of the study.

VI. RECCOMENDATIONS

Based on the findings and conclusion presented, the researchers recommend conducting a similar study that covers a wider scope of area which comprises restaurants that produce Pancit Batil Patong. Future researchers should also include the testing of soaked utensils such as spoons and forks that may harbor pathogenic microorganisms, especially if the water used in the utensils is not properly replaced every day. The determination of other possible areas of contamination within the restaurant is highly recommended such as table surfaces, and chopping boards and knives used to sliced Onions and Calamansi that brings additional flavor to the food. Hence, these recommendations are significant in order to determine other possible bacteria that may possess risk in causing human diseases.

Since there is no *E. coli* isolated from the food samples from the Panciterias of interest, this may suggest that these Panciterias have practiced good and safe practices in the preparation of their foods. However, the fact that other Panciterias except Panciteria D harbor bacteria, this strongly implies that there are differences in the way these Panciteria restaurants practiced food safety which should be considered on a case-to-case basis. The possibility to rule out a variation in their practices may help further researchers and concerned individuals to create more effective food safety measures which can be applicable for all Panciterias. The study will help the City Officials, Department of Health (DOH) and Food and Drugs Administration (FDA) to formulate guidelines that will improve the overall quality of food handling and preparation for the prevention of possible future outbreaks.

One of the colony isolates identified in the study is the bacterial genera of *Staphylococcus* with an unknown species. Hence, future studies should include identification of bacterial strain which is necessary for accurate disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Lastly, further studies must take into consideration the performance of antimicrobial sensitivity on identified bacterial isolates. This laboratory process will ascertain possible drug resistance in identified pathogens to ensure susceptibility to drugs of choice for particular infections.

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