BACTERIOLOGICAL ANALYSIS OF THE WATER USED IN SANITIZING EATING UTENSILS IN PANCITERIAS WITHIN TUGUEGARAO CITY

Lorena A. Javier, Ma. Katrina E. Ochoa
Melowin Mae D. Pagud, Angelica Q. Taguibao

ABSTRACT

This study aimed to determine the possibility of bacterial contamination in the water used for sanitizing eating utensils in Panciterias within Tuguegarao City. Descriptive design with the use of a questionnaire was used to evaluate the hygienic practices of the selected panciterias. Analytical Profile Index (API) kit and a total coliform count were performed to assess the possible number of bacterial pathogens present in the water. Water sample based on the availability and use of thermal sanitation was collected in the buckets of the eating utensils for bacterial culture, isolation, identification, and counting for the detection of bacterial pathogens. Results obtained from the API kit and the total coliform count was gathered and recorded for statistical analysis to correlate the samples for the presence of bacteria. Majority of the bacteria isolated from these panciterias were Aeromonas sobria which is considered to be non-pathogenic. Panciteria 5 has the highest colony count among the 7 panciterias and has a mean of 4,457 whereas Panciteria 4 has the lowest colony count with a mean of 11.5. The statistical analysis showed that there is no significant difference on the colony counts in the different panciterias since the p-value computed is less than 0.05. Panciteria 3 followed by Panciteria 4 was considered to be the cleanest and most sanitized water samples among these Panciterias. Other bacteria isolated were Aeromonas hydrophila and Enterobacter sakazakii which are non-pathogenic since the colony counts are low. Out of 7 panciterias, only 3 bacteria have been isolated and these bacteria have different colony counts.

Key words: *Thermal sanitation, hygienic practices, eating utensils, analytical profile index kit, total coliform count, culture, isolation, identification, bacterial pathogens, non-pathogenic bacteria*

INTRODUCTION

Water is a need from the preparation and cooking of pancit to washing and sanitizing eating utensils to assure the cleanliness of the panciterias and the safety of the customers. The standards of water quality preparation must be followed by all the panciterias, but those standards must not only apply to the quality of food being prepared but also to the quality of water as well. Factors affecting Water, Sanitation, and Hygiene (WASH) practices include occupation, nutrition, and energy of workers. These are the reasons why it is difficult for the workers to follow the standard protocol (Bartram, Bos, Fewtrell, Gor & Gore, 2013; WHO, 2010).

The only way to assess if there is a presence of bacteria in water is to have it tested. National Policy and Standards of WASH must be followed to ensure access to adequate sanitation, safe water, and hygiene to prevent transmission of disease especially to immunocompromised individuals. The use of complete water sanitation facilities is also important to completely eliminate the bacteria present in the water sample. People who are more likely to develop an illness are those related to inadequate water supplies, inability to maintain good hygiene, and inadequate sanitation practices. Diarrhea is the most significant disease a person may acquire if poor sanitation and hygiene is not maintained. People must have necessary information, understanding and knowledge to prevent water-related diseases and to mobilize the maintenance of those sanitation and hygienic facilities (Grossi, Klimschak, Rechenburg, Schmoll & Shinee, 2015; WHO, 2010).

Hygienic practices are very important in a panciteria because of a large number of customers daily. It is important that workers should monitor their personal hygiene to prevent cross contamination. Sanitary measures that relate particularly to utensils that come in contact with customers' mouth are crucial to the health and welfare of the customers. Utensils that are improperly washed and sanitized are carriers of bacteria and may be directly responsible for the transmission of disease. The Saliva borne disease may be transmitted by indirect contact through the medium of eating utensils (Mrak & Stewart, 2014; Marriot, 2012).

Boiling water is primarily used in sanitizing eating utensils. Pouring boiling water into eating utensils is not a reliable way to sterilize especially if the water is not placed consistently in boiling water because it is difficult to keep the water hot enough for longer periods of time. Many people try to conserve boiling water during the rinsing part of dishwashing and just hand dry dishes. This practice may lead to contamination of the water (Marriott, 2012; J. Engelkirk & P. Engelkirk, 2011).

WHO statistics shows that in 2014 about 94,000 deaths occurred due to diarrhea caused by lack of safe WASH services. People are affected by Neglected Tropical Diseases such as helminthiasis, schistosomiasis, trachoma, and lymphatic filariasis which are attributed to poor hygiene and sanitation problems (WHO, 2010).

According to the Business Permits and Licensing Office of Tuguegarao City Cagayan, as of July 7, 2018, there are a total of 66 panciterias registered in Tuguegarao City. Those include the paid and issued permits and those paid only but without permit.

Microorganisms that are present in these contaminated eating utensils may damage the reputation of the owner of the panciterias and eventually ruin his/her business. It is always safer and easier to prevent the contamination of these eating utensils by simply following the standards of food quality preparation because it is

more difficult to make these utensils safe for the customers to use again. Considering these claims, the researchers came up with an idea to develop this study to determine the bacteria present in the water that is used to sanitize the utensils.

Research Questions

This study aimed to determine the possibility of bacterial contamination in the water used for sanitizing eating utensils in Panciterias within Tuguegarao City. Specifically, the research aimed to answer the following questions:

1. What is the colony count of microorganisms present in the samples?
    a. Water sample collected in the morning
    b. Water sample collected in the afternoon
2. What are the bacterial organisms present in the samples?
3. Is there a significant difference in the colony counts (CFU) of microorganisms of water samples obtained from different panciterias?

Hypothesis

- There is no significant difference in the colony counts (CFU) of microorganisms of water samples obtained from different panciterias.

Significance of the Study

This study significantly impars knowledge to the owner and staff of the panciterias about the possible contamination of bacteria in the water used for sanitizing the utensils and served as a basis for them to improve sanitation practices since most of them neglect these practices. And upon reading this study, the people within Tuguegarao City and the tourists who want to taste the Cagayan's pancit delicacy would be enlightened on the risk-factors and water-borne diseases they may acquire when proper sanitation and cleanliness of the water used for sanitizing is not maintained.

Literature Review

National Policy and Standards of Water, Sanitation, and Hygiene

A proper sanitation practice is one of the important operations in eating and drinking establishments that require supervision and regulation. A policy for the complete removal of all visible dirt from the eating utensils to be followed by disinfection either by means of boiling water or some chlorine disinfectant was prepared by Health Department. Food Ordinance strengthened the policy by making it unlawful to operate an eating establishment unless facilities for

disinfecting and sterilizing dishes and glasses were provided (Bushong & Fletcher, 2008).

Food employees should wear complete protective work clothing including hair restraints, aprons, & gloves that are designed to effectively keep their body from contacting exposed food and food contact surfaces. Protective work clothing also protects the worker from hazards in the workplace and prevents contamination of the workplace by materials that the worker may bring into their personal clothing. Management should also insist the separation of sinks for food preparation, dishwashing, cleaning, and hand washing to prevent further contamination of the water used for sanitizing the utensils. An assessment is needed to identify the practices of the respondents to predict if WASH standards are being followed. The risky practices will be focusing on the sanitation facilities used, lack of hand-washing with antibacterial soap, unhygienic storage and collection of water, and maintenance of hygiene. Water, sanitation and hygienic facilities should be regularly maintained and operated (Bartram et al., 2013).

Policies and standards are provided but workers tend to neglect these because of the lack of facilities and knowledge on how to properly follow the protocol. Requirements for WASH provided by the National Policies include: provision of clean and adequate amount of water, provision of soap in handwashing facilities, cleaning requirements for sanitation facilities, regular inspection and maintenance of these facilities, regular maintenance and inspection of water, hygiene education for all employees, and follow-up inspections required if there is still a deficiency in following the protocol. Hygiene education is included for acquisition of knowledge and skills to adopt responsible hygiene behavior for the employees and owners (Grossi et al., 2016).

Pathogenic microorganisms found in unsterile eating utensils

Commonly isolated bacterial pathogens present in water which includes Escherichia coli O157:H7, Salmonella typhi, Shigella, Campylobacter, Vibrio cholerae, Aeromonas, Pseudomonas, and Enterobacter species (Beachet al., 2013).

The bacteria of concern outlined here have the potential to be spread through contaminated water, but it doesn't have any correlations with the presence of other commonly isolated waterborne pathogens such as coliform bacteria. Since these bacteria are not expected to be seen in water used for sanitation, there are no satisfactory microbiological indicators of their presence. Further studies are needed in order to understand the real significance and dimension of the disease caused by water contaminated with these bacteria because they are mostly isolated in food and drinking water (Boi et al., 2008).

Aeromonas hydrophila are isolated in different types of water including tap water, chlorinated water, drinking water, ground-water, and sewage-contaminated water. The wide distribution of *Aeromonas hydrophila* underlines their capacity to adapt to different environmental conditions. Members of the Aeromonas species are able to grow or survive at temperatures ranging from < 5 °C to 45 °C. Studies have shown that *Aeromonas hydrophila* is more prevalent in cleaner water compared to other species of Aeromonas. These bacteria have been isolated from chlorinated and unchlorinated water meaning they are able to withstand long periods of nutrient limitation. Concentrations of *Aeromonas hydrophila* are usually around 1,000cfu/mL and when present in large numbers, it may infect susceptible hosts including children and elderly persons. The common routes of infection are ingestion of contaminated water or food or contact of the organism if the person has open wounds. Other types of Aeromonas such as *Aeromonas sobria* and *Aeromonas caviae* grow at temperatures between 22^{o}C and 35^{o}C. Just like *Aeromonas hydrophila*, these species also can grow in water distribution system, especially in biofilms, where they may be resistant to chlorination. They are known to cause severe diarrheal disease of short duration or chronic loose stools in children, the elderly, or the immuno-compromised individuals, but Aeromonas will only cause these diseases when their presence goes beyond an infective dose for a vulnerable host (Aghdasi, Igbinosa, Igumbor, Okoh & Tom, 2012; Boi et al., 2008).

The Aeromonas and Enterobacteriaceae have many similar biochemical characteristics but are easily differentiated by oxidase test since Aeromonas are positive. *Enterobacter sakazakii* which is part of the Enterobacteriaceae family is resistant to routine sterilization methods and is the most thermotolerant among the Enterobacteriaceae. These bacteria is being found in milk powder, vegetables, rice, cheese, and various spices however, most of the attention to *Enterobacter sakazakii*-related contamination of food products has focused on powdered infant formula. It is fairly resistant to osmotic, heat, and dry stresses, which may explain, in part, its presence and survival in infant powder and similarly prepared products. The optimum temperature for growth is 39°C, it is reported to grow at less than 4°C, suggesting that this species would be able to replicate even during refrigeration. Furthermore, ES may form biofilms and thereby resist disinfectants. *Enterobacter sakazakii* can survive in powder for at least 12 months. The actual amount of ES contamination usually is low, ranging from 0.36 to 66 colony-forming units (CFU)/100 grams. Improper storage and temperature regulation may lead to an increase in bacterial load, thus facilitating outbreaks of infection. Origin of the opportunistic pathogen remains unknown. Improper handling and use of contaminated utensils may have been contributing factors in some cases. Levels of greater than 105cfu/mL are easily obtained. Detection of the organism is performed either after an enrichment procedure or by direct plating on selective plates used to detect coliforms or enteric pathogens. Presumptive colonies of coliforms are purified and identified using biochemical tests such as the API 20E biochemical identification system. The use of hot boiling water is recommended to assure the

killing of low levels of Enterobacteriaceae (Ford, Hunter, Petrosyan & Prasadarao, 2008; Bier & Miliotis, 2003).

Health effects of utilizing unsterile and unhygienic eating utensils

Most outbreaks at restaurants are due to inattention to poor sanitation practices. Problems are more likely to occur from inadequate cooking in the kitchen or cross-contamination from serving utensils. Restaurant employees tend to be paid low wages and this is the reason why most of the employees do not follow the standard protocol for sanitation practices (Bredbenner, Berning, Biggers & Quick, 2013).

Poor wages are behind a growing scarcity of employees. The reason why they don't focus much on the proper sanitation practices is that they also lack sanitation facilities. The population of the employees is lesser since the salary is not enough which makes it more difficult to practice and follow the standard protocol for sanitation. Therefore, the numbers of employees are small, and they couldn't achieve the tasks given to them. Poor sanitation is caused by the employees not having enough knowledge on how to have proper sanitation practices. Most restaurants used dishwashing waters in buckets placed on the floor. The water for washing and rinsing the utensils were rarely renewed and generally was observed to be dirty (Addiset al., 2014).

Inadequate handwashing was the first most frequently observed problem among the food-handlers. Most food-handlers reported that they always washed their hands before and after working. Only 10.5% reported that they never wash their hands. 22.1% reported that they wash their hands with water only and 76.5% wash their hands with soap and water. Hand washing without soaping, inadequate scrubbing, and washing of hands in the food preparation sinks is not properly maintained. Hands should be washed according to good hygiene practices to reduce the risk of food contamination, and water should come from a safe source, both hot and cold, and with appropriate temperature. Insufficient hand washing and sanitation will cause an increase in the bacterial count on the worker's hands since cleaning materials were not available near the sinks of the kitchen which leads to contamination of eating utensils. The hands of these of these food service employees have been shown to be a source of food and water-borne disease, mainly because of improper sanitation and hygiene. It is of utmost importance that high standards of sanitation, cleanliness and good hygiene among the employees should be maintained at all times in order to prevent infection and destroy pathogens. The second most frequently encountered problem among employees is the inadequate cleaning and sanitizing of utensils and equipment. Sanitizing of manually washed dishes was performed infrequently although it was cleaned, rinsed and air-dried in 15 kitchens. Cleaning and sanitizing of food contact surfaces were not observed in five of the kitchens. Cleaning and wiping, but not sanitizing in

10 of the kitchens was observed (Khatib & Mitwalli, 2009; Cluskey, Giampaoli & Sneed, 2007).

Unsafe water, inadequate sanitation, utilization of contaminated eating utensils, and poor hygiene may cause diarrheal diseases especially if the contaminated water is ingested. Even with only 2% of improper sanitation, the level of fecal-oral pathogens may increase and this proves that water, sanitation, and hygiene play a dominant role in transmission of disease. WHO recently estimated the global burden of disease as a direct consequence of not following the standard protocols. These diseases include diarrheal disease, typhoid fever, malnutrition, schistosomiasis, intestinal nematode infection, trachoma, and other infectious disease. Infectious diarrhea is considered to be the most common disease a person may acquire due to ingestion contaminated water, improper sanitation and hygiene, and utilization of contaminated eating utensils (Bartram et al., 2013; WHO, 2010).

Methods of sterilizing eating utensils

Utensils that are not single use should be thoroughly cleaned and sanitized before reuse. Eating utensils must be protected from contamination until it is ready to be used again. These items that are not single use should be thoroughly inspected because after using, the utensils are covered with food, grease, and other dirt that allow the bacteria to grow. To prevent further contamination, sanitizing these utensils requires two steps: Cleaning which removes the soil deposits, and sanitizing which destroy the microbes that are left on the surface of equipment. Boiling water, steam, and hot air are commonly used by the restaurants as a method for sanitizing. The item to be sanitized must first be washed before it can be properly sanitized. If boiling water is used in the third compartment of a three-compartment sink, it must be at least 77^{o}C (171^{o}F). The cleaning staff should use thermometers to measure temperatures during cleaning to make sure that the utensils and other equipment are properly sanitized (Fraser, 2009; Marriot, 2010).

Heat sterilization with the use of boiling water is the most practical, efficient, inexpensive, and most frequently used method of sterilization to sanitize utensils that may be contaminated by bacteria. Boiling water at the correct temperature and for sufficient time has a very broad disinfecting effect. It is non-toxic if it is not applied to living tissue, and it does not possess any harm to the environment, compared to many chemical agents. Unless the items to be decontaminated cannot withstand the temperature associated with boiling water disinfection, it should be subjected first to a thorough cleaning process. Washer sterilizer/disinfectors use boiling water but because of the decreasing temperature of the boiling water, the water may be contaminated with bacteria due to improper sterilizing techniques. Thus, thermal death is reached in less time when higher temperatures are used; lower temperatures are effective when a longer contact time is used. As the temperature is increased, microorganisms will be killed at a faster rate. Water sterilization by boiling is preferred over any method of chemical disinfection. This

method is safe because disease-causing microorganisms cannot survive a constant heat. The boiling temperature and the time to reach the temperature are sufficient to kill all pathogenic organisms. The vegetative forms of most pathogens are quite easily destroyed by boiling for 30 minutes. Thus, utensils used in panciterias may be disinfected by boiling for 30 minutes. Because the temperature at which water boils is lower at higher altitudes, water should always be boiled for longer times at high altitudes. Boiling is not always effective, however, because a heat-resistant bacterium that may be present often survives boiling. Also, because thermophiles thrive at high temperatures, boiling is not an effective means of killing them (J. Engelkirk & P. Engelkirk, 2011; Stein, 2008).

Because enteric pathogens are killed within seconds by boiling water and are killed rapidly at temperatures >60°C, the traditional advice to boil water for 10 minutes to ensure safe water is excessive. Because the time required to heat water from a temperature of 55°C to a boil works toward disinfection, any water that is brought to a boil should be adequately disinfected. Boiling water for 1 min or keeping water covered and then allowing it to cool slowly after boiling can add an extra margin of safety. The boiling point decreases with increasing altitude, but this is not significant when compared to the time required to achieve thermal death at these temperatures (Mrak et al., 2014).

Water temperatures above 160° F (70° C) kill all pathogens within 30 minutes and above 185° F (85° C) within a few minutes. So, the time it takes for the water to reach the boiling point (212° F or 100° C) from 160° F (70° C), all pathogens will be killed, even at high altitude. Immersing eating utensils into water heated to 82^{o}C or higher is another way to sterilize eating utensils, but this is not a reliable method because it is difficult to keep the water hot enough for longer periods of time unless water quality monitoring is observed. Boiling water can sanitize eating utensils but, in some cases, spores may survive more than an hour of boiling temperatures. The time needed to sterilize the eating utensils depends on the temperature and volume of the water. If the eating utensils and other equipment are sterilized at a lower temperature, the temperature of the water must be constantly kept for longer hours. Examples of times and temperatures used for sterilization are 15 minutes of heat at 85°C (l85°F), or 20 minutes at 82°C (l80°F). The volume of water and how fast it is flowing can determine how long it takes for the item being sterilized to reach the right temperature. Boiling water is readily available and is not toxic (Mrak et al., 2014; Curtis, 2013; Marriot, 2010).

Research Paradigm

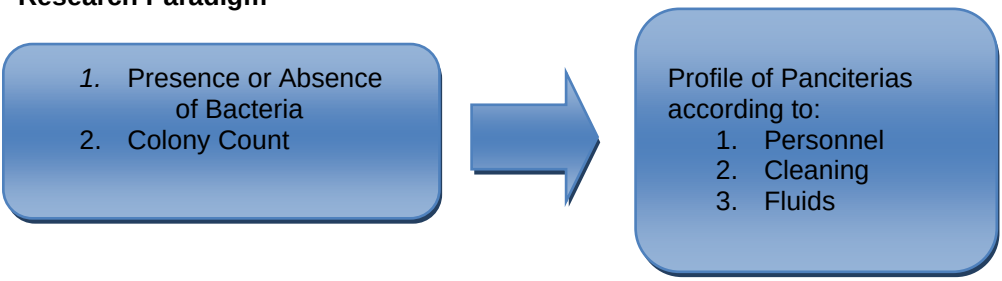

Figure 1.0 *Research Simulacrum*

The figure above shows the whole concept of the study in which, the researchers used an interview to obtain basic information using the Interagency Manufacturer Quality Questionnaire on good sterilization practices by the Médecins Sans Frontières (2013) which is based on the profile of panciterias according to Personnel, Cleaning, and Fluids. Analytical Profile Index Kit and total coliform count were used to detect if there is a presence or absence of bacteria in the water and for the computation of the colony count.

METHODS

Research Design

The researchers used the descriptive quantitative method applying bacteriological tests to determine microorganisms present in the water samples from different panciterias. Analytical Profile Index (API) kit and total coliform count were the two major methods performed in this study.

Locale of Study

The study was conducted in Tuguegarao City. Specifically, bacterial colony count was performed at the Department of Agriculture Regional Office II.

Samples of the Study

Of the 66 panciterias, only 7 owners gave their consent.

Data Gathering Procedure

1. Collection of sample and preservation

The procedure followed by the researchers was based on the standard protocol developed by Doris, Maupin, McGlynn, Peggy and Shayegani (2009).

1.1. The water samples were collected from the 7 panciterias in Tuguegarao City.
1.2. A 100mL volume of water from each panciteria was taken in sterile containers.
1.3. Two water samples were collected every after 5-6hours in different containers to confirm the presence and identify the bacteria. The times of collection were 11 am and 4 pm.
1.4. The containers were air tighten with stopper and were wrapped by tape to restrict contact of air with water samples.
1.5. The samples were transported to the laboratory in an isolated foam box.

2. Preparation of Culture Media

The procedure followed by the researchers was based on the standard protocol developed by Doris, Maupin, McGlynn, Peggy, & Shayegani (2009).

2.1. Blood Agar Plate (BAP)

2.1.1. 500mL of TSA was prepared in a flask according to the instructions given on the label of the dehydrated powder. The media were heated and fully dissolved with no powder on the walls of the vessel before autoclaving.
2.1.2. The media were autoclaved at 121 ºC for 20 minutes.
2.1.3. The media were made to be cooled in water bath at 60 ºC.
2.1.4. 5% sterile and defibrinated sheep blood was added.
2.1.5. 20 mL of media were dispensed into 15x100mm petri dishes. The media were allowed to solidify and condensation to dry.
2.1.6. The plates were placed in sterile plastic bags and stored at 4 ºC until use.

2.2. MacConkey (MAC) agar

2.2.1. MAC was prepared according to manufacturer's instructions.
2.2.2. The media were sterilized by autoclaving at 121 ºC for 15 minutes.
2.2.3. The media were made to be cooled in water bath at 50 ºC.
2.2.4. 20 mL of medium were dispensed into 15x100 mm petri dishes. The media could solidify and condensation to dry.
2.2.5. The plates were placed in sterile plastic bags and stored at 4 ºC until use.

2.3. Plate Count Agar (PCA)

1. 23.5 grams was suspended in 1000mL distilled water.
2. The PCA was heated to boiling to dissolve the medium completely.
3. The media were sterilized by autoclaving at 121 ºC for 15 minutes.
4. The media were made to be cooled at 45-50 ºC.
5. The media were mixed well and poured into sterile petri plates.

3. Inoculation of Samples and Incubation

The procedure followed by the researchers was based on the protocol of the Department of Agriculture Region II.

3.1. All samples were thoroughly mixed by rapidly making about 25 complete up and down movements.
3.2. Each plate was marked with the sample number.
3.3. 14 samples were inoculated using an applicator stick into 14 blood agar media for isolation of bacteria.
3.4. 14 samples were inoculated using an applicator stick into 14 MacConkey media for isolation of bacteria.
3.5. 56 plates of plate count agar were utilized. Duplicate plates were prepared for each volume.

3.6. Dilution Preparation

3.6.1. 1ml of undiluted water sample was transferred to 99mL Peptone Water diluent which served as the 10-2 dilution to be used for plating.
3.6.2. The plates were labeled with 100, 10-1, 10-2, 10-3 dilutions.
3.6.3. 6.3 1 mL and 0.1 mL of the undiluted water sample were transferred into plates marked as 100 and 10-1.
3.6.4. 6.4 1 mL and 0.1 mL of the 10-2 diluted water sample were transferred into plates marked as 10-2 and 10-3.
3.6.5. 6.5 Approximately 10-12 mL liquefied PCA maintained at 44 to 46 ºC was poured to each plate within 10-20 minutes of original dilution.
3.6.6. 6.6 Sample dilutions and agar medium were immediately mixed thoroughly and uniformly by alternate rotation and back forth motion of plates on flat surface.
3.6.7. Invert petri dishes and incubate promptly for 48 ± 2h at 35ºC.

4. Colony Count

The procedure followed by the researchers was based on the protocol of the Department of Agriculture Region II.

4.1. All colony-forming units (CFU), including those of pinpoint size on selected plates was counted. The dilutions used and total numbers of colonies counted were recorded in the record sheet.

4.2. Plates from all dilutions with no colonies were reported as less than one (<1) divided by the corresponding largest samples volume used.

4.3. When number of colonies exceeded 300, the result reported was “too numerous to count” (TNTC).

4.4. Calculation

Compute bacterial count per milliliter by following equation:

$$\text{CFU/mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish,mL}}$$

or for successive dilutions:

Weighted mean count, CFU/ml= sum of all colonies

((1.0 x n1) + (0.1 x n2) + …..) x (d)

Where:

n1- number of plates in first dilution counted

n2- number of plates in second dilution counted

d- dilution from which the first counts were obtained

4.5. Reporting of Results

4.5.1. When colonies on duplicate plates and or consecutive dilutions are counted and results are averaged before being recorded, the values obtained were rounded off to two significant figures only when converting to CFU.

4.5.2. For more than 3 digits CFU, the value was rounded off to two significant figures. If the third digit is 6 or above, it was rounded off to the digit above (e.g. 456= 450); if 4 or below, it was rounded off to the digit below (e.g. 454= 450). If the third digit is 5, it was rounded off to the digit below if the first 2 digits form an even number (e.g. 445= 440); it was rounded off to the digit above if the first 2 digits form an odd number (e.g. 455= 460).

5. Analytical Profile Index 20ESystem

The procedure followed by the researchers was based on the protocol of the Department of Agriculture Region II.

5.1. Preparation of the strip

5.1.1. An incubation box (tray and lid) was prepared and about 5ml of distilled water was distributed into the honey comb wells of the tray to create a humid atmosphere.
5.1.2. The strain reference was recorded on the elongated flap of the tray.
5.1.3. The strip was removed from its packaging
5.1.4. The strip was placed in the incubation box.

5.2. Preparation of the inoculums

5.2.1. A tube containing 5ml of sterile distilled water without additives was prepared and used.
5.2.2. A single well isolated colony from an isolation plate was removed using a pipette.
5.2.3. It was carefully emulsified to achieve a homogenous bacterial suspension.

5.3. Inoculation of the strip

5.3.1. With the same pipette, the bacterial suspension was distributed into the tubes of the strip.
5.3.2. For the CIT, VP, and GEL tests, both tube and cupule were filled.
5.3.3. For other tests, only the tubes were filled.
5.3.4. For the tests ADH, LDC, ODC, H2S and URE, anaerobiosis by overlaying with mineral oil was created.
5.3.5. The kits were closed and incubated at 36 ºC ± 2 ºC for 18-24 hours.

5.4. After the incubation period, the strip was read by referring to the Reading Table.
5.5. Interpretation is obtained in numerical profile.

Data Analysis

The researchers used one-way Analysis of Variance (ANOVA) as a statistical tool to compare the colony count results of the seven (7) panciterias within Tuguegarao City using 0.05 level of significance and least significant difference for comparative analysis.

Waste Management Disposal

The entire process of waste disposal was performed at Department of Agriculture Regional Office II. The plates used were sterilized using an autoclave for half an hour at around 121˚C. The media which solidifies on cooling was poured out in the sink and was flushed with water while the plates were hot. The plates were brushed with water and detergents and were rinsed first with tap water followed by distilled water. The plates were placed in tray for drying. Inoculating loops were heated in the Bunsen burner before returning. Containers with sample waters were disposed in the biological wastes.

Ethical Considerations

The results obtained from this study were confidential among the researchers and the owner of the panciteria including the name of the panciteria and the individuals involved to avoid future conflict. Prior to the experimentation, the researchers sought permission from the Associate Dean, Academic Dean, University Research Ethics Board, University Administration, and University President. The researchers were given an ethical clearance # 51521.

The researchers further asked for the permission from the panciteria owners in Tuguegarao City for the collection of water samples from their container bucket. Only pancterias whose owners gave their consent were involved in this research study.

The researchers sought assistance from the Department of Agriculture Regional Office II through the help of a licensed medical technologist.

RESULTS

Table 1. *Colony Count of Microorganisms present in Water Samples of the different Panciterias*

Panciteria	Colony Forming Unit (CFU) Count per gram (mL)		CFU Count Mean
	AM Sample	PM Sample	
P1	1194	1036	1115
P2	869	860	864.5
P3	7	10	8.5
P4	9	14	11.5
P5	4495	4419	4457
P6	2284	2248	2266
P7	1514	1608	1561

The table shows that majority of the samples from the different panciterias have significant number of microorganisms that formed except P3 and P4.

Table 2. *Bacteria identified in Water Samples of the Different Panciterias*

PANCITERIA	*Aeromonas sobria*		Aeromonas hydrophilia		Enterobacter sakazakaii	
	AM	PM	AM	PM	AM	PM
P1	+	+	-	-	-	-
P2	+	+	-	-	-	-
P3	+	+	-	-	-	-
P4	+	+	-	-	-	-
P5	-	-	+	+	-	-
P6	-	+	-	-	-	+
P7	+	+	-	-	-	-

Table 2 shows eleven samples of panciterias have Aeromonas sobria while Aeromonas hydrophila only have two and only one sample have Enterobacter sakazakaii.

Table 3. *Test of Significant Difference in the Colony Count of Microorganisms between the Samples Collected in the Morning and Afternoon*

	t-value	**p-value**	**Decision**
Samples collected in the Morning vs samples collected in the afternoon	0.858	0.424	Accept Ho

The table shows that there is no significant difference in the colony count regardless of time of collection of water sample.

DISCUSSION

The interval time for samples collected from the selected 7 panciterias is 5-6 hours, one in the morning and one in the afternoon. A total of 14 samples were inoculated on the Blood Agar Plate and McConkey Agar Plate at the same time for bacterial identification using the API kit. Plate Count Agar Plate is used in counting the colonies per sample. According to Bier and Miliotis, the detection of the organism is performed either after an enrichment procedure or by direct plating on selective plates used to detect coliforms or enteric pathogens. Presumptive colonies of coliforms are purified and identified using biochemical tests such as the API 20E biochemical identification system. The use of hot boiling water is recommended to assure the killing of low levels of Enterobacteriaceae. This claim is supported by the Department of Agricultural Laboratory. The normal colony count seen in water is between 25-250cfu/mL. Plates with more than 250 colonies record the counts as Too Numerous To Count (TNTC) and this indicates large number of

bacteria but may not be pathogenic to humans unless the water is ingested (Aghdasi et al., 2012).

The bacteria isolated from these panciterias include *Aeromonas hydrophila* and *Aeromonas sobria* which are usually seen in drinking water. Also the *Enterobacter sakazakii*, which is seen in milk and it is rarely seen in water. Since majority of these bacteria isolated from the different panciterias are greater than 250 cfu, there is a significant difference between the colony count identified to that of the standard non-pathogenic bacterial count which is less than 25cfu/mL (Aghdasi et al., 2012; Boi et al., 2008).

The concentrations of *Aeromonas hydrophila* are usually around 1,000cfu/mL and when present in large numbers, it may infect susceptible hosts including children and elderly persons. Aeromonas species and other gram-negative bacteria including Enterobacter, Escherichia coli, Salmonella, and Shigella require more than 1000 colonies/100mL of water for it to be considered pathogenic. Only one panciteria has no colonies meaning, the water used from this panciteria is non-pathogenic and is safe to use (Boi et al., 2008).

The item to be sanitized must first be washed before it can be properly sanitized. If boiling water is used in the third compartment of a three-compartment sink, it must be at least 77ºC (171ºF). Most of the panciterias have the same practices in sanitizing their eating utensils. The Panciteria 5 has the poorest sanitation practice among the 7 panciterias and the Panciteria 3 is the most sanitized panciteria. A proper sanitation practice is one of the important operations in eating and drinking establishments that require supervision and regulation (Bushong et al., 2008; Fraser, 2009).

Water sterilization by boiling is preferred over any method of chemical disinfection. This method is safe because disease-causing microorganisms cannot survive a constant heat. The boiling temperature and the time to reach the temperature are sufficient to kill all pathogenic organisms. The vegetative forms of most pathogens are quite easily destroyed by boiling for 30 minutes. Thus, utensils used in panciterias may be disinfected by boiling for 30 minutes. Water temperatures above 160° F (70° C) kill all pathogens within 30 minutes and above 185° F (85° C) within a few minutes. So the time it takes for the water to reach the boiling point (212° F or 100° C) from 160° F (70° C), all pathogens will be killed, even at high altitude. The time needed to sterilize the eating utensils depends on the temperature and volume of the water. If the eating utensils and other equipment are sterilized at a lower temperature, the temperature of the water must be constantly kept for longer hours. Examples of times and temperatures used for sterilization are 15 minutes of heat at 85°C (l85°F), or 20 minutes at 82°C (l80°F) (Curtis, 2013; Engelkirk & P. Engelkirk, 2011; Marriot, 2010).

CONCLUSION

This research study concludes that the water used to sanitize the eating utensils in panciterias within Tuguegarao City is contaminated by bacteria namely *Aeromonas sobria*, *Aeromonas hydrophila* and *Enterobacter sakazakii*.

RECOMMENDATIONS

By the end of the research investigation, the researchers identified some problems and came up with the following recommendations.

1. The researchers should call other panciteria owners to participate in the research study.
2. To analyze not only the water used in sterilizing panciterias but also the eating and cooking utensils through swabbing.
3. The panciteria owners should perform the following:
    3.1 Use of boiling water instead of hot water.
    3.2 Sterilization of eating utensils every after use.

REFERENCES

Strunz, E. C., Addiss, D. G., Stocks, M. E., Ogden, S., Utzinger, J., & Freeman, M. C. (2014). Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. *PLoS medicine*, *11*(3).

Aghdasi F., Igbinosa I., Igumbor E., Okoh, A., & Tom, M. (2012). Emerging Aeromonas Infections and their Significance in Public Health. *Scientific World Journal*, 2012; 2012: 625023. doi: 10.1100/2012/625023.

Ahdal, M., Alhamlan, F., & Qahtani, A. (2015). Recommended Advanced Techniques for Waterborne Pathogen Detection in Developing Countries. *J Infect DevCtries*, 9(2), 128-135. doi: 10.3855/jidc.6101.

Bartram, J., Bos, R., Fewtrell, L., Gore, F., & Gor, F. (2013). Water, Sanitation, and Hygiene. *Environmental Burden of Disease Series, No. 15.*

Balows, A., Rhoden, D., Smith, P., & Tomfohrde, K. (2008). API System: a Multitube Micromethod for Identification of Enterobacteriaceae. *Journal of Applied Microbiology*. *24(3): 449-452.*

Beach, M., Blackburn, B., Calderon, R., Chen, N., Craun, G., Hill, V., Levy, D., & Yoder, J. (2013). Surveillance for Waterborne Disease Outbreaks Associated with Recreational Water from https://www.cdc.gov/healthywater/surveillance/rec-water-surveillance-reports.html.

Bier, J. & Miliotis, M. (2003). International Handbook of Foodborne Pathogens. New York, Basel: Marcel Dekker, Inc. Retrieved on August 21, 2003 from https://eddymercado.files.wordpress.com/2012/11/manual-internacional-de-contaminacic3b3n-patogc3a9nica-de-alimentos.pdf

Bloomfield, T., Cogan, T., & Humphrey, T. (2011). The Effectiveness of Hygiene Procedures for Prevention of Cross-Contamination from Chicken Carcases

in the Domestic Kitchen. Letters in Applied Microbiology, doi: 0.1046/j.1472-765X.2011.00656.x.

Boi, P., Bruscolini, F., Citterio B., Manti A., Papa S., Pianetti A., Sabatini L., & Rocchi L. (2008). Determination of Viability of *Aeromonas hydrophila* in Increasing Concentrations of Sodium Chloride at Different Temperatures by Flow Cytometry and Plate Count Technique. Retrieved on July 18, 2008 from https://www.sciencedirect.com/science/article/pii/S0168160508004017

Boisson, S., Clasen, T., Desai, D., Gupta, R., McLaughlin, C., Nayaar, N., & Shah, N. (2008). Microbiological Effectiveness and Cost of Disinfecting Water by Boiling in Semi-Urban India. *The American Journal of Tropical Medicine and Hygiene*, 79(3), 407-413. doi: https://doi.org/10.4269/ajtmh.2008.79.407).

Bredbenner,C., Berning, J., Biggers, J., & Quick, V. (2013). Food Safety in Home Kitchens: A Synthesis of the Literature. *International Journal of Environmental Research and Public Health. 2013 Sep*; *10(9): 4060–4085.* Doi: 10.3390/ijerph10094060.

Bushong, R. & Fletcher, A. (2008) Administrative Problems in Securing Proper Bactericidal Treatment of Eating and Drinking Utensils. *American Journal of Public Health,* 30(3), 653-656.

Butler, D., Gavan, T., & Lobregat, C. (2007). Reproducibility of the analytab (API 20E) system. *PubMed.*

Clasen, T., Miller, L., & Rosa, G. (2010). Microbiological Effectiveness of Disinfecting Water by Boiling in Rural Guatemala. *The American Journal of Tropical Medicine and Hygiene*, 82(3), 473–477. doi: https://dx.doi.org/10.4269%2Fajtmh.2010.09-0320.

Cluskey, M., Giampaoli, J., & Sneed, J. (2013). Developing a Practical Audit Tool for Assessing Employee Food-Handling Practices. *The Journal of Child Nutrition and Management*, 26(2).

Curtis, R. (2013). The Backpacker's Field Manual: A Comprehensive Guide to Mastering Backcountry Skills. United States: Three River Press. Retrieved on September 3, 2013 from https://www.amazon.com/Backpackers-Field-Manual-Revised-Updated/dp/1400053099

Davis, G., & George, S. (2011). Rapid Identification of Enterobacteriaceae by Using Noncommercial Micro-Tests in Conjunction with API 20E Profile Data. *Journal of Clinical Microbiology, Oct. 2011, p. 399-403.*

Demir, F., Kivanc, M., & Yilmaz, M. (2011). The Occurrence of Aeromonas in Drinking Water, Tap Water and the Porsuk River. *Brazilian Journal of Microbiology*, doi: http://dx.doi.org/10.1590/S1517-83822011000100016.

Eisenberg, J. N., Porco, T., & Scott, J. C. (2007). Integrating Disease Control Strategies: Balancing Water Sanitation and Hygiene Interventions to Reduce Diarrheal Disease Burden. *American Journal of Public Health*, 97(5), 846-852. doi:10.2105/ajph.2006.086207.

Engelkirk, J. & Engelkirk, P. (2011). Burton's Microbiology for the Health Sciences. Philadelphia, Pa: Lippincott Williams &Wilkins..

Ford, H., Hunter, C., Petrosyan, M., & Prasadarao, N. (2008). Enterobacter sakazakii: An Emerging Pathogen in Infants and Neonates. *Surgical Infections, Volume 9, Number 5, 2008*. Doi: 10.1089/sur.2008.006.

Fraser, A. (2009). Cleaning and Sanitizing. North Carolina: North Carolina A & T State University. Retrieved on August 18, 2009 from http://www.foodsafetysite.com/resources/pdfs/EnglishServSafe/ENGSection11Cleaning.pdf

Grossi, V., Klimschak E., Rechenburg, A., Shinee, E., & Schmoll, O. (2016). The Situation of Water, Sanitation, and Hygiene in Schools in the pan-European Region. *World Health Organization Regional Office for Europe (UNECE).*

Khatib, I. & Mitwalli, S. (2009). Food sanitation practices in restaurants of Ramallah and Al-Bireh district of Palestine. *Eastern Mediterranean Health Journal*, 15(4), 951.

Holmes, B., Lapage, S., & Willcox, W. (2009). Identification of Enterobacteriaceae by the API 20E system. *Journal of Clinical Pathology*; 31(1): 22-30.

Jorgensen, H., Pfaller, A., Carroll, K., Funke, G., Landry, L., Richter, S., & Warnock, W. (2015). *Manual of Clinical Microbiology, 11th Edition. Vol. 1.*

Mallman, W. (2010). A Critical Study of Various Types of Detergents and Disinfectants for Used in Dishwashing. *American Journal of Public Health.*

Marriot, N. (2010). *Sanitizing Methods*. United States of America: Chapman and Hall. Retrieved on October 6, 2010 from https://link.springer.com/chapter/10.1007/978-1-4615-6045-6_6

Marriot, N. (2012). Principles of Food Sanitation. United States of America: Springer International Publishing AG. Retrieved on July 16, 2012 from https://www.springer.com/cn/book/9781441920386

Mrak, E., & Stewart, G. (2014). Advances in Food Research Volume II. New York: Academic Press Inc. Retrieved on June 13, 2014 from https://www.elsevier.com/books/advances-in-food-research/mrak/978-0-12-016402-8

Shayegani, A., Medhi, G., Maupin, K., Peggy, A., McGlynn, D., & Doris, L., (2009). Evaluation of the API 20E System for Identification of Non-fermentative Gram-Negative Bacteria. New York: *APH Publication.*

Siringan, M. (2007). Bacteriological Examination of Water. *Journal of Public Health*

Standard Methods for the Examination of Water and Wastewater 22nd Edition (2012). *American Public Health Association, American Water Works Association, Water Environment Federation.*

Stein, M. (2008). A Manual for Self-Reliance, Sustainability, and Surviving the Long Emergency. United States of America: Green Publishing. Retrieved on December 2, 2008 from https://www.amazon.com/When-Technology-Fails-Self-Reliance-Sustainability/dp/1933392452

World Health Organization Geneva (2010). Guidelines for Drinking-Water Quality. Hongkong: *World Health Organizations Library Publication*.