Microbial Analysis of Fresh Miki Noodles Sold in Selected Stalls in Zamboanga City Public Market

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ABSTRACT

Fresh “miki” noodles are considered one of the components of Filipino families’ favorite dishes. However, food borne diseases associated with consuming fresh noodles pose a serious public health problem. This study specifically sought to microbiologically examine miki samples for presence and the number of colony counts of Salmonella sp. and Staphylococcus aureus, two common microorganisms associated with food poisoning. The samples were collected from three different stalls at different peak hours, upon the delivery of the fresh miki noodles early in the morning and in the afternoon in Zamboanga City. The 3M Petrifilms were used for the colony counting for Salmonella sp. and S. aureus. Presumptive positive for Salmonella sp. and S. aureus were further subjected to biochemical confirmation to which confirmation disks were inserted between the plate’s top film and bottom film test. Results showed that all the samples were safe from Salmonella sp. but are contaminated by S. aureus. The number of colonies has exceeded the recommended counting limit of Petrifilm Staph count plate which is 150 to 300 S. aureus colonies per 1mL of homogenate. Moreover, stall number 3 has the highest number of colony counts for S. aureus which may be accounted to the observed improper food handling. Higher colony count was observed in the afternoon than in the morning, an indication of longer duration of exposure of the fresh noodles to contaminants. These data could provide science-based decision making in the city’s policy framework on regular monitoring and proper food handling.

Keywords: food handling, Salmonella sp., Staphylococcus aureus, 3M Petrifilms, Zamboanga City

INTRODUCTION

Food is one of the basic necessities of human beings and must be free from pathogenic or disease-causing bacteria. Food is often bought from the public market and these include the fresh miki noodles which are known for its affordability and appealing taste. It can be cooked just like pansit guisado and lomi or just blanch it with hot soup like batchoy and pansit bulalo. Pancit (pansit) is a catchall term for Chinese-influenced noodle dishes in the Philippines.

Fresh miki noodles can be sold either packed or by kilo where the noodles are exposed and placed in trays in an open area that may contaminate it in some ways. Some bacteria can inhabit into the food through fecal to oral route where food and water are ingested. Improper handling is the most common mode of bacteria transmission and it is a must that food is free from any disease-causing bacteria.

Since fresh miki noodles sold in public markets are not packed, it has a high risk of temperature abuse, a situation in which microbes incubate in the food. Improper handling and over extending the product’s shelf life may also cause food to be exposed to bacteria (Corlett, 1989). According to the study of Ray (2001), the highest incidence of spoilage, especially rapid spoilage of processed food, is caused by bacteria, followed by yeast and molds.

Foods contaminated with bacteria could be a factor for common diseases of the digestive system which are essentially of two types: infections and intoxications. A bacterial infection occurs when a pathogen enters a GI (gastrointestinal tract) and multiplies. There is usually a fever, one of the body’s general responses to an infective organism in such case as the Salmonellosis caused by Salmonella sp. On the other hand, intoxication is caused by the ingestion of preformed toxin. Most intoxication, such as those caused by Staphylococcus aureus, are characterized by a very sudden appearance of a GI disturbance such as diarrhea and vomiting. Fever is less often one of the symptoms (Tortora et al., 2004).

Salmonella sp. and Staphylococcus aureus are just two of the common bacteria that cause diseases of the lower digestive system. According to the Food and Drugs Administration (FDA, 2018), these two bacteria were found present in pasta products and uncooked noodles. The Salmonella (named for their discoverer, Daniel Salmon) are gram-negative, rod-shaped bacteria that belong to the Family Enterobacteriaceae that causes inflammation of the gastrointestinal tract, moderate fever accompanied by nausea, abdominal pain, cramps and diarrhea (Trovatelli et al., 1988). It can be transmitted via the fecal to oral route with the ingestion of contaminated food.

Staphylococcus aureus is a gram-positive and is non-moving, small round shaped or non-motile cocci. According to Tortora et al. (2004), a leading cause of gastroenteritis or inflammation of the GI tract is staphylococcal food poisoning. The presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indicator of poor sanitation (Bennett & Lancette, 2001). Any food prepared in advance and not kept chilled, such as fresh noodles, are a potential source of staphylococcal food poisoning characterized by abdominal cramps, diarrhea and vomiting. S. aureus are found on the skin and transmitted via poor handling and sanitation.

There were cases of Salmonella sp. contamination in fresh miki noodles in Zamboanga City but were not published. Further, there has been no study on the microbial examination of fresh miki noodles in terms of the presence of Salmonella sp. and Staphylococcus aureus in Zamboanga City.
Thus this study was conceptualized to conduct microbial examination of fresh miki noodles sold in selected stalls in the Zamboanga City Old Public Market. Specifically, the study determined the presence and colony count of both the Salmonella sp. and Staphylococcus aureus on fresh miki noodles. The result of this study can be utilized in policy making to improve cleanliness, safety of the people and other public health issues such as proper food handling especially with those exposed to probable contaminants and to recommend regular monitoring.

METHODS

Fresh miki noodles were taken at the Zamboanga City Old Public Market observing peak hour which is around ten to one (10:00am – 1:00 pm) and upon the delivery of the noodles early in the morning (Figure 1).

![Figure 1. Research samples at the study site.](image)

The Zamboanga City Old Public Market was purposively selected on the basis of food handling of fresh miki noodles. The microbial experiment was conducted at the Microbiology Room of the Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University. All apparatuses were autoclaved to ensure that there were no cross contaminants in the samples collected.

To ensure consistency, the samples were taken right from the market and to attain more representative samples, fresh miki were taken from three (3) different stalls in the said market with consideration to the time of delivery early in the morning around 5:00am – 6:00am and at peak hours when most people buy noodles around 10:00am – 1:00pm. Plastic bags which served as the container of the fresh miki were labelled for uniformity and to avoid cross contaminations. Microbial examinations were done three (3) times during for three (3) consecutive months which served as replicates.

Microbial test procedures for Salmonella sp. and Staphylococcus aureus were completely the same since only 3M Petrifilms were used for both. The Petrifilms were used for the colony counts of Salmonella sp. and Staphylococcus aureus. The presence of colonies for both microorganisms indicates their presence in the fresh miki.

For the Salmonella Detection and Colony count, the fresh miki taken from three (3) different stalls in Zamboanga Old Public Market were subjected to microbial examination. Samples were no longer transferred to any other containers. Twenty-five (25) grams of fresh miki noodles were weighed and 3M Salmonella Enrichment Supplement was also weighed aseptically with appropriate amount.
The 3M Salmonella Enrichment Supplement was added to the prepared and autoclaved 3M Salmonella Enrichment Base (constitutes 37 grams of Salmonella Base) and one (1) liter of purified water. Blended fresh miki noodles were placed in a sample bag and Salmonella Enrichment Supplement and Base were also added. The enriched sample was then homogenized and incubated at 42°C for 24 hours. Salmonella sp. enrichment base is composed of Nutrient mix and selective mix and was used with Salmonella sp. enrichment supplement for the selective enrichment of Salmonella sp. in food products. It is associated with the use of Salmonella sp. express plates which is composed of Polyester film, Polystyrene foam, gelling agent, paper, adhesive and indicators (3M_Petrifilm_Salm_Express_Interpretation_Guide.pdf).

Two (2) mL of sterile diluent (distilled water) was placed onto the center of the bottom of the Petrifilm which were then covered and pressed with a spreader to distribute the diluent evenly. Hydrated plates were stored at room temperature away from light for eight (8) hours to allow the gel to form (3M Food Safety, 2013).

A sterile 10 μL loop was used to withdraw a loop-full of sample and was streaked from the top to the bottom of the hydrated plates to obtain isolated colonies. A gentle sweeping motion with even pressure was applied when covering the plates to remove air bubbles. The plates were incubated at 42°C for 24 hours. Using a marker, isolated presumptive positive Salmonella colonies were circled from the plate top film. Presumptive positive colonies on plate appeared as red to dark red colony with yellow zone (3M Food Safety, 2013).

Confirmation disk was inserted between the plate’s top film and bottom film to avoid entrapping of air bubbles. The plates with confirmation disk were incubated again at 42°C for five (5) hours. Encircled colonies from the plate top film were observed (3M Food Safety, 2013). For the Staphylococcus aureus detection and Colony Count, twenty-five (25) grams of blended fresh miki noodles were weighed and placed into the sample bag. Two hundred twenty-five (225) mL of buffered phosphate dilution water were also added to the bag and were homogenized. It is a sample-ready culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for Staphylococcus aureus. Red-violet colonies on the plate are S. aureus. (Murray-Brown Laboratories Inc., 2019).

One (1) mL diluted sample was placed onto the center of the film using a sterilized pipette. The top film was rolled down onto the sample to avoid entrapping bubbles and to prevent the sample from getting off into the film. A spreader was used to distributed inoculum over the circular area (Murray-Brown Laboratories, Inc., 2019).

Plates were incubated at 37°C for 24 hours. Colonies of red to violet were re-incubated at 37°C for three (3) hours for confirmatory test. The pink zones present in every red to violet colonies were counted as Staphylococcus aureus (Murray-Brown Laboratories, Inc., 2019).

For Salmonella sp., colonies were counted after incubation of the Petrifilm with confirmation disk at 42°C for 6 hours. Non-Salmonella colonies on plate were observed as (1) a red colony with no yellow zone and no gas bubbles; (2) red colony with magenta zone and (3) blue-green colony with yellow zone and is associated with gas bubbles (3M Food Safety, 2013).

For Staphylococcus aureus, colonies were counted after incubation of the Petrifilm at 37°C for 24 hours. Colonies of red to violet were re-incubated at 37°C for three (3) hours for confirmatory test. The pink zones present in every red to violet colonies were counted as Staphylococcus aureus (Murray-Brown Laboratories Inc., 2019).

Estimation was done since the colonies were too many. The counting was done with only one (1) representative square and multiplied by 20. Accordingly, it is recommended to determine
the average count in two (2) or more unaffected squares of the 3M Petrifilm Aerobic Count Plate and multiple the average counts by 20 for an estimated count (3M Science, 2015).

Table 1 shows the summary of the research procedures and the materials used.

Table 1. Summary of the research procedures and the materials used for *Salmonella* sp. and *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Research Procedure</th>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>Sample Preparation</td>
<td>-25 grams of fresh “miki” noodles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3M Salmonella Enrichment Supplement</td>
</tr>
<tr>
<td></td>
<td>Enrichment of Samples</td>
<td>-3M Salmonella Enrichment Base</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Sample bag</td>
</tr>
<tr>
<td></td>
<td>Hydration of Plates</td>
<td>-2.0ml sterile diluent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Spreader</td>
</tr>
<tr>
<td></td>
<td>Inoculation and Incubation</td>
<td>-10μL loop</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Incubator with 42ºC</td>
</tr>
<tr>
<td></td>
<td>Biochemical Confirmation</td>
<td>-Confirmation Disk</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Sample Preparation</td>
<td>-25 grams of fresh “miki” noodles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-225ml of Buffered Phosphate Dilution Water</td>
</tr>
<tr>
<td></td>
<td>Inoculation</td>
<td>-Petrifilm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Pipette</td>
</tr>
<tr>
<td></td>
<td>Incubation and Interpretation</td>
<td>-Incubator at 37ºC/Water bath at 37ºC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-disk for confirmation</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

For the detection of *Salmonella* sp., the red to dark-red colony color with yellow zone found on the Petrifilm after the incubation for 24 hours at 42ºC indicated a presumptive positive result (Fig. 2 left) while the right picture indicated a negative result which did not show green colony and without associated gas bubbles after confirmatory test for *Salmonella* sp.
Figure 2. Presumptive positive result (left) and negative result (right) of confirmatory test seen after the incubation of Salmonella Petrifilms.

These presumptive positive results underwent confirmatory test to which confirmation disk was inserted inside the Petrifilm (see methods for the biochemical confirmation procedure). Table 2 shows the summary of results before and after the Salmonella sp. confirmatory test.

Table 2. Summary of results for Salmonella sp.

<table>
<thead>
<tr>
<th>Month</th>
<th>Colony Color</th>
<th>Colony Metabolism</th>
<th>Presumptive test (+,-)</th>
<th>Confirmatory test (+,-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>AM</td>
<td>Red</td>
<td>Yellow zone</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red</td>
<td>Gas Bubbles</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>Red</td>
<td>Gas Bubbles</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
<tr>
<td>August</td>
<td>AM</td>
<td>Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
<tr>
<td>September</td>
<td>AM</td>
<td>Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>Red</td>
<td>Yellow Zone</td>
<td>+</td>
</tr>
</tbody>
</table>

As seen in Table 2, miki samples collected from the three (3) different stalls of the Zamboanga Old Public Market all showed positive results for presumptive test for Salmonella sp. but were negative for confirmatory test; therefore, colony counting was eliminated. However, if the results gave a green colony associated with bubbles after confirmatory test, it would be an indication that the miki samples were contaminated with Salmonella sp.

For the detection of Staphylococcus aureus, the red to violet colony seen on the Petrifilm after 24 hours of incubation at 37ºC indicated a presumptive positive result for Staphylococcus aureus which were pink zones (Fig. 3 left). Confirmatory test (Fig. 3 right) also showed positive result for S. aureus.

Figure 3. Presumptive positive (left) and confirmatory result (right) for S. aureus.

Presumptive positive results underwent the confirmatory test where confirmation disks were inserted into the Petrifilm and incubated for three (3) hours. It appeared red to violet colony
with pink zones. Hence, colony counting needed to be done. Figure 4 shows the average of the estimated colony counts of *Staphylococcus aureus* among the three (3) stalls during morning and afternoon collection.

**Figure 4.** Average estimated colony counts for *Staphylococcus aureus* among the three (3) stalls during morning and afternoon collection.

The three (3) stalls for both morning and afternoon samples were all contaminated by *S. aureus* but higher in the afternoon. Afternoon samples from stall 3 had the greatest number of *S. aureus* colonies compared to the others. Based on the researchers’ observations, afternoon samples from stall 3 did not use any tools for safe food handling. *S. aureus* is used as an indicator of food contamination and its probable sources of contamination include improper handling, distribution, and the period of when the samples were taken.

According to Corlett (1989), factors noted to be significant on the presence of *S. aureus* could be exposure to contaminants like flies, containers, and other food products that were in contact with the fresh miki, improper handling, distribution and time when the samples were collected. Further, fresh noodles has a high risk for consumer temperature abuse, mishandling and over extending of shelf life. In terms of handling, although stall 2 had the second highest count of colonies, there were times that its employees used materials or tools for safe handling of food while stall 1 consistently used hand protectors and had the least number of colonies.

In terms of period of when the samples were taken, exposures to contaminants for a long period of time may have higher bacterial count. Results showed a higher colony count in the afternoon than in the morning. Accordingly, they have a short shelf life and will deteriorate quickly if not stored under refrigeration (ANZFA, 2001).

According to Kibret & Ahera (2012), a significant source of contamination is the hygiene of an employee. In this study, improper employee hygiene was observed in stalls 2 and 3 which explains its higher colonies than stall 1. *Staphylococcus aureus* is often an inhabitant of the nasal passages, from which it contaminates the hands. From these sources, it can readily enter food. If the microbes are allowed to incubate in the food—a situation called temperature abuse—microbes reproduce and release enterotoxin into the food. This can lead to staphylococcal intoxication. It is stated that the recommended counting limit on a Petrifilm Staph Express Count is 300 *S. aureus* colonies (3M Petrifilm Staph Express Count Plate, 2010). The number of
colonies in the study sample exceeded the recommended counting limit of Petrifilm Staph Express Count Plate which is 150 to 300 S. aureus colonies per 1 mL homogenate. In this regard, this presents high risk (3M Microbiology) for morning trials which is 315.6 colonies and afternoon trials which is >300 colonies.

According to Bennett et al. (2001) and Jensen et al. (2004), the presence of a large number of Staphylococcus aureus organisms in foods may indicate poor handling or sanitation and that one of the primary resources of potentially harmful microorganisms, just like the Staphylococcus aureus, was improper handling and distribution of products that is detrimental to food quality maintenance and safety.

In terms of proper packaging, the fresh miki sold in the three (3) selected stalls of Zamboanga Old Public Market were not packed and were exposed to possible contaminants. Thus, according to Corlett (1989), these fresh noodles have a high risk for mishandling.

*Staphylococcus aureus* is often an inhabitant of the nasal passages, from which it contaminates the hands. From these sources, it can readily enter food. If the microbes are allowed to incubate in the food—a situation called temperature abuse—microbes reproduce and release enterotoxin into the food. This can lead to staphylococcal intoxication. It is stated that the recommended counting limit on a Petrifilm Staph Express Count is 300 S. aureus colonies (3M Petrifilm Staph Express Count Plate, 2010). The number of colonies in the study sample exceeded the recommended counting limit of Petrifilm Staph Express Count Plate which is 150 to 300 S. aureus colonies per 1 mL homogenate. In this regard, this presents high risk (3M Microbiology) for morning trials which is 315.6 colonies and afternoon trials which is >300 colonies.

Regarding *Salmonella* sp. contamination, results revealed that the samples taken from three (3) different stalls at Zamboanga Old Public Market were all safe based on the obtained results of the confirmatory test. According to the Food and Drugs Administration (FDA, 2018), *Salmonella* sp. may be present in foods, but the prevalence and levels are low and is usually less than one percent (<1%).

**CONCLUSION AND RECOMMENDATIONS**

The study showed that the fresh miki noodle samples taken from three (3) different stalls at Zamboanga City Old Public Market were contaminated with *Staphylococcus aureus* but were safe from *Salmonella* sp. The major factors that contributed to this bacterial contamination may be influenced by improper food handling and the period of when the samples were taken. Stall 3 had the highest number of colony counts for S. aureus which may be due to poor sanitation practices of the stall compared to the two (2) other stalls. Higher colony count was also observed in the afternoon than in the morning which verified that the fresh miki was exposed to contaminants for a longer period of time. The city government should impose strict policies on proper food handling and regular monitoring. For the consumers, if food contamination by human handlers cannot be avoided completely, the most recommended reliable method of preventing staphylococcal food poisoning is adequate refrigeration during storage to prevent toxin formation.
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REFERENCES


Food and Drug Administration (FDA, 2018) [Retrieved from http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094147.htm].


3M Science, 2015. 3M™ Petrifilm™ Aerobic Count Plate Liquefiers. [Retrieved from multimedia.3m.com/mws/media/.../3m-petrifilm-aerobic-count-plate-liquefiers.pdf].