Bacterial contamination of *Salmonella* spp. and *Escherichia coli* (Migula, 1895) in fresh chicken meat and chicken-based street food sold in the City of Mati

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ABSTRACT

The increasing popularity of chicken-based street food among low-to-middle-income consumers in the City of Mati, Davao Oriental, Philippines, raises serious health concerns due to the potential risk of foodborne illnesses. Despite rising poultry consumption, microbial assessment of street-vended chicken products is not routinely conducted, creating a significant knowledge gap in local food safety monitoring. This study aimed to determine the presence of *Salmonella* spp. in fresh chicken meat and isaw and *Escherichia coli* (Migula, 1895) in fried chicken, "isaw," and "kwek-kwek" sold by randomly selected street vendors. Samples were collected using standard aseptic techniques. Salmonella Shigella agar and Eosin methylene blue (EMB) agar were used to identify the target bacteria. The most probable number (MPN) method, based on Department of Agriculture-National Meat Inspection Service (DA-NMIS) Circular No. 9-2008-5, was used to detect the presence of *E. coli* in fresh chicken meat. Total plate count (TPC) was used to detect the presence of *Salmonella* spp. in fresh chicken meat and isaw and to detect *E. coli* in fried chicken, "isaw," and "kwek-kwek," following standards set by DA-NMIS and the Department of Health-Food and Drugs Administration (DOH-FDA) Circular No. 2022-12-2. The results showed *Salmonella* spp. in all fresh chicken and "isaw" samples, exceeding the DA-NMIS absence requirement in 25 g. *Escherichia coli* in fresh chicken meat was within the 500 MPN g^{-1} limit. However, TPC values in fried chicken, "isaw," and "kwek-kwek" exceeded the 100 CFU g^{-1} DOH-FDA limit. These findings revealed significant bacterial contamination in fresh chicken meat and popular chicken-based street foods, underscoring the urgent need for stronger implementation of regulation, regular microbial monitoring, and food safety education to support local public health efforts and guide future policy enhancement.

Keywords: bacterial load, food hygiene, food safety, health risk, microbial limit, street food

INTRODUCTION

Poultry meat is an important industry that flourished out of the necessity for affordable and low-fat protein sources. Currently, Philippine poultry production has increased and currently dominates the food industry, which reflects the growing demand for poultry products (DA-BAR 2022). Poultry meat is also a popular main ingredient for several street foods, such as barbecue, "isaw," (an offal-based food product made from processed and fried poultry intestines) and fried chicken, while chicken eggs are the base material

of the popular street food, "kwek-kwek" (deeped fried hardboiled egg coated with flour). These street foods are popular with low-to-middle-income consumers and students because of their cheap and ready-to-eat nature. However, bacterial contamination has been well established in these kinds of foods, thereby raising public health concerns (Rouger et al. 2017). For example, *Escherichia coli* (Migula, 1895) contamination was documented in "kwek-kwek" sold by vendors along the University of Eastern Philippines (Dagalea et al. 2021), while *E. coli* and *Salmonella* (Lignières, 1900) were detected in Laguna street-vended grilled and fried meat (Manguiat and Fang 2013).

Bacterial contamination in food is a public health threat because it can cause illness and death. The World Health Organization (2015) estimates 600 million foodborne disease cases, with 420,000 deaths a year resulting from unsafe food globally. The 2005-2018 consolidated data in the Philippines documented 209 foodborne disease outbreaks, and meat-based dishes account for 14.35% of these outbreaks (Azanza et al. 2019). Among the foodborne pathogens are coliform bacteria, which have notorious variants that can lead to health complications in humans. One such variant is the Shiga toxin-producing (STEC) strain, which produces toxins that can damage the intestinal lining, causing cramps and bloody diarrhea, and may progress into a life-threatening kidney problem called Haemolytic uraemic syndrome (HUS) (Costa 2013; Ishi and Sadowsky 2008; WHO 2018). *Salmonella* is also a top foodborne bacteria causing diarrheal outbreaks. The WHO reported that 17,000 people in the Philippines were recorded to have acute bloody diarrhea in 2018 (WHO 2018).

Given the risks of ingesting bacteria-contaminated food, the public must be informed and assured of the safety of the food they buy and consume. Republic Act 10611 (Food Safety Act of 2013) prescribes a mechanism to ensure this. An essential step in establishing this state is through microbiological assays and monitoring procedures of food items, such as the most probable number (MPN) test for detecting *E. coli* and the total plate count (TPC) method for assessing *Salmonella* spp. contamination. To date, local health authorities reported that their monitoring has not reached a rigorous level (B.H. Catbagan, personal communication, 04 May 2022). These bacteriological studies on chicken meat and selected street foods were done to assist the local health authorities and the local government in setting and implementing programs and actions. The study examined the presence of bacterial contamination, particularly *E. coli* and *Salmonella*, in fresh chicken meat and selected chicken-based street food sold in the City of Mati, Davao Oriental, Philippines.

METHODS

Sampling Areas and Sample Collection Sites

The locale of the study was the community of the City of Mati., Davao Oriental, Philippines. Samples of fresh chicken meat were bought from Madang Public Market (Site 1). Samples of chicken-based street food were bought from Burgos Street along Baywalk Park (Site 2) and from the stalls and eateries near the main campus of Davao Oriental State University (DOrSU) (Site 3) (Figure 1). A total of 45 pieces of samples were collected from these sites per sampling period (Table 1). These areas were selected due to their popularity among low-to-middle consumers and students, attributed to their accessibility and the ready-to-eat nature of the food offerings. Baywalk Park, situated in the center of the city, is a common gathering place for people to socialize and relax. Students frequently visit the eateries near DOrSU because of their proximity to the campus and the affordability of the food. Studies have shown that taste, convenience, and price significantly influence street food consumption among students and low-middle-middle income groups (Chang et al. 2020; Beniwal and Mogra 2023; Tacardon et al. 2023).

Sample Data-gathering procedure

Table 1 presents the replication of meat samples used for the assay in the laboratory. Sample collection strictly observed the aseptic process. Each bought/collected fresh/cooked sample from each stall packed in labeled sterile plastic zip-lock bags, sealed, and placed in an ice box. These were immediately transported to the laboratory for microbiological analysis. The number of stalls and replications varied due to the sampling limits specified in the Food and Drugs Administration Circular No. 2022-12-2 (DOH-FDA 2022) and Department of Agriculture-National Meat Inspection Service Circular No. 9-2008-5 (DA-NMIS 2008), which require five sample units. Additionally, the number of stalls included in the study was influenced by the limited availability of open stalls at the time of sampling.

Laboratory Analysis of Samples

Measure of presence. The laboratory analyses of the samples began within 60 min of collection. The MPN method was used to analyze the occurrence of *E. coli* in the fresh chicken (drumsticks) meat. The MPN method is a statistical, multistep assay consisting of presumptive, confirmed, and completed phases (Capuccino and Sherman 2013).

In the presumptive test, 20 g of meat from each drumstick were homogenized using a sterile mechanical blender. The homogenized sample was then diluted to 180 mL of sterile water and thoroughly mixed by manually shaking the Erlenmeyer flask. A total of 200 mL mixture was transferred to the test

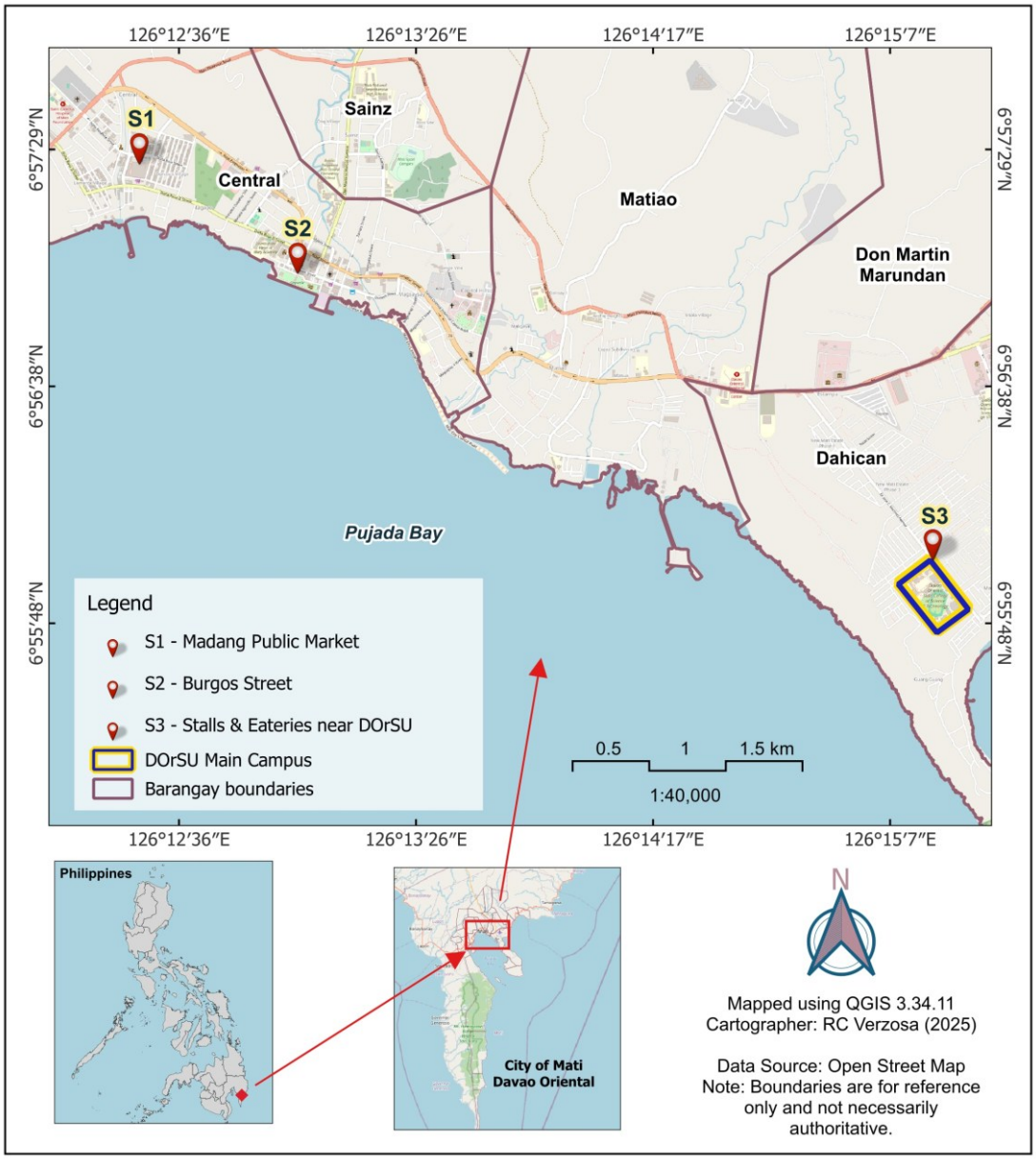

Figure 1. The research area showing the three sampling sites within the City of Mati, Davao Oriental, Philippines. **S1**-Madang Public Market (enclosed public marketplace) located in the central area of the city; **S2**-Burgos Street (open-air vending along Baywalk Park); and **S3**- includes stalls and eateries near Davao Oriental State University (DOrSU) Main Campus in Barangay Dahican. Red location pins indicate sites, while DOrSU is outlined in yellow. Barangay boundaries are shown in purple, and Pujada Bay is depicted in blue. Insets show the location of the study area within Mindanao and the Philippines.

Table 1. Sample sources (Madang Public Market and DOrSU) and replication.

Food Samples	No. of Store/Stall Sources	Replication per Store/Stall (Pieces)
Fresh chicken meat (drumsticks) (MPM)	2	5
Chicken intestines (“isaw”): cooked (MPM)	2	10
Chicken intestines (“isaw”): uncooked (Baywalk)	2	10
Chicken intestines (“isaw”): fried (DOrSU)	3	10
Fried chicken	3	5
“Kwek-kwek”	3	5

tubes containing the double-strength lactose broth (DSLB) and the single-strength lactose broth (SSLB). For each sample, five test tubes for DSLB and 10 test tubes for SSLB were used, amounting to 50 DSLB tubes and 100 SSLB tubes overall. Each culture tube is placed with Durham tubes to trap gas. The tubes with lactose medium were inoculated using a micropipette with 0.1 mL DSLB, 0.01 mL SSLB, and 0.001 mL SSLB aliquots of the homogenized meat sample. Incubation was then carried out at 37°C for 48 h. The presence of gas/bubble formation in Durham tubes indicated a presumptive positive result of coliform bacteria. The number of positive tubes at each dilution allows for the MPN estimation (Blodgett 2020; Capuccino and Sherman 2013).

The confirmed test was conducted to validated the presence of coliform. In this phase, a loopful of inoculum from the positive lactose broth tubes from the presumptive test was streaked into the EMB agar plate (HIMEDIA®). Control plates were also set up to check for cross-contamination. After streaking, the inoculated and the control plates were incubated in an inverted position at 37°C for 24 h. They were then examined for colonies with dark centers and green metallic sheen, which is a positive indication of *E. coli* (Aryal 2019).

The completed test was conducted to examine the coliform colonies in the HIMEDIA® EMB agar plates used in the confirmed test. An isolated colony from the confirmatory test was inoculated into lactose broth tubes and streaked into the nutrient agar slant to perform the gram-staining process. They were then incubated at 37°C for 24 h. The culture tubes that showed acid and gas formation in the lactose broth positively confirmed *E. coli* (Capuccino and Sherman 2013).

The TPC test was used to determine the *Salmonella* population for uncooked and cooked chicken intestines "isaw" and chicken meat to conform to the specified unit in the DA-NMIS guideline (DA-NMIS 2008). It also determined *E. coli* load in other chicken-based street foods. The analysis used 25 g from each sample (chicken meat, uncooked isaw, and chicken-based street food), homogenized, placed in a HIMEDIA® nutrient broth, and incubated for 24 h. This is to recover many bacteria (Elumba et al. 2018). Following incubation, a 10-fold serial dilution was made to decrease a dense cell culture to a more acceptable concentration and facilitate colony counting (Sapkota 2021). In 10-fold serial dilution, the concentration was reduced by a multiple of ten, and it was completed with a ratio of 1:10, of which 1 represents the amount of sample added, and 10 represents the total volume of the final sample. A 1.0 mL aliquot from the stock solution was transferred into a 9.0 mL diluent and mixed by gently shaking the test tubes. The procedure involved five series of 10-fold dilutions to minimize the number of colonies and make them countable (Werner 2023). After serial dilution, 0.1 mL aliquots were plated into dupli-petri HIMEDIA® EMB agar plates and HIMEDIA® Salmonella Shigella (SS) agar plates. The inocula were spread on the surface using a sterile metal spreader. The bacterial cultures were then incubated in a BIOBASE incubator at 37℃ for 24 h and examined for distinctive *Salmonella* and *E. coli* colony features.

The *E. coli* and *Salmonella* colonies were counted per culture plate to determine the degree of contamination. The results are presented as colony-forming units (CFU) per gram of the sample for *E. coli* and per 25 grams for *Salmonella*, following the standards. The number of colonies per plate was calculated based on the dilution factor used to prepare the samples (Capuccino and Sherman 2013). The FDA's Bacteriological Analytical Manual (BAM) (2021) was followed to interpret where, for a plate that surpasses 250 CFU, the counts were recorded as too numerous to count (TNTC) and, if less than 25, the counts were recorded as too few to count (TFTC).

Data Processing and Analysis

The colony count was compared to the recent Philippine FDA Circular No. 2022-12-2 guidelines. This circular was used as a reference to assess the bacteriological quality of food cooked immediately before sale or consumption. The FDA's acceptable level of chicken-based food for *E. coli* was 20 to 100 CFU g^{-1}, and the DA-NMIS acceptable limit was 100–500 MPN g^{-1}. *Salmonella* must not be detected or absent in chicken meat and in all chicken-based street food. Hence, food cooked immediately before sale must not exceed the microbial limit to avoid potential health hazards.

RESULTS

Evidence of Presence of *E. coli* and *Salmonella* in Samples

Table 2 and Table 3 show the result of the examination of culture plates based on the diagnostic colony features of *E. coli* and *Salmonella*. As shown, it can be seen that all samples examined manifested these diagnostic features, which are a green metallic sheen for *E. coli* colonies and transparent colonies with black centers for *Salmonella* as compared to the bacterial control. *Salmonella* is, thus, detected in fresh chicken meat samples and cooked and uncooked *isaw* sold in one stall along the city's main public market (S1) and in another stall located along the Baywalk area (S2). On the other hand, *E. coli* is detected in all chicken-based street food examined and bought from sellers near the DOrSU main campus (S3).

The fresh chicken meat and "isaw" bought from S1 and in Burgos Street along S2 have

Salmonella spp. exceeding the requirement of absence in the 25 g sample as per DA-NMIS Circular No. 9-2008-5 guidelines (Table 4). Although there were colonies that were TFTC in cooked "isaw" in the first and third sampling period, confirmed presence of *Salmonella* spp. renders the sample non-compliant with DA-NMIS standards, which require complete absence in 25 g samples. In this study, both TNTC and TFTC colony growths were confirmed to contain *Salmonella* spp. and as such all positive samples regardless of bacterial load were classified as exceeding the acceptable limit. The *E. coli* from fresh chicken meat was within the acceptable limit of 500 MPN g^{-1} based on the DA-NMIS set standards. However, the chicken-based street foods, including fried chicken, "isaw", and "kwek-kwek" bought near DOrSU (S3) are generally way above the acceptable limit set by DOH-FDA Circular No. 2022-12-2 (Table 5). The permissible limit for *E. coli* in takeaway food (such as fried chicken, "isaw", and "kwek-kwek"*)* based on DOH-FDA Circular No. 2022-12-2 set standards is 20 CFU g^{-1} and should not exceed 100 CFU g^{-1}.

Table 2. Result of sample examination to detect the presence of *Salmonella* spp.

Sample Source		***Salmonella*** **Diagnostic Colony Features** **(colorless or transparent colonies, usually with black centers in SS Agar plates)**
Fresh Chicken Meat (Madang Public Market)	Stall 1	Positive
	Stall 2	Positive
Uncooked "Isaw"	Stall 1 (Madang Public Market)	Positive
Cooked "Isaw"	Stall 2 (Baywalk Park)	Positive

Table 3. Result of sample examination to detect the presence of *E. coli* in Madang Public Market (MPM) and Davao Oriental State University (DOrSU).

Sample Source		***Escherichia coli*** **Diagnostic Colony Features (Green metallic sheen colonies in EMB Agar plates)**
Fresh Chicken Meat (Madang Public Market)	Stall 1	Positive
	Stall 2	Positive
Fried Chicken meat (DOrSU vicinity)	Eatery 1	Positive
	Eatery 2	Positive
	Eatery 3	Positive
"Isaw" (DOrSU vicinity)	Stall 1	Positive
	Stall 2	Positive
	Stall 3	Positive
"Kwek-kwek" (DOrSU vicinity)	Stall 1	Positive
	Stall 2	Positive
	Stall 3	Positive

Table 4. *Salmonella* spp. mean load in fresh chicken meat and chicken products across three sampling periods (DA-NMIS standard: absent in 25 g sample). Note: Regardless of colony count, the confirmed presence of *Salmonella* spp. in any amount violates DA-NMIS guidelines, which require complete absence in 25 g sample. Thus, even TFTC samples are classified as exceeding the acceptable limit. (TNTC=too numerous to count; TFTC = too few to count).

Sample Source		**1st Sampling**	**2nd Sampling**	**3rd Sampling**	**Standard Limit (Based on DA-NMIS: must be absent in g^{-25})**
Fresh Chicken Meat (Madang Public Market)	Stall 1	TNTC	TNTC	TNTC	Exceeds acceptable limit
	Stall 2	TNTC	TNTC	TNTC	Exceeds acceptable limit
Uncooked "Isaw"	Stall 1 (Madang Public Market)	TNTC	TNTC	TNTC	Exceeds acceptable limit
	Stall 2 (Baywalk Park)	TNTC	TNTC	TNTC	Exceeds acceptable limit
Cooked "Isaw"	Stall 1 (Madang Public Market)	TNTC	TNTC	TFTC	Exceeds acceptable limit
	Stall 2 (Baywalk Park)	TFTC	TNTC	TFTC	Exceeds acceptable limit

Table 5. *E. coli* mean load in the examined fresh chicken meat and chicken-based products across three sampling periods, with reference to Department of Agriculture-National Meat Inspection Service and Food and Drugs Administration microbial limits. (TNTC = too numerous to count; TFTC = too few to count).

Sample Source		Unit	Mean Bacterial Load Per Sampling Period			Standard Limit (Based DA-NMIS: ≤500 MPN g^{-1}) and FDA: ≤100 CFU g^{-1})
			1st Sampling	2nd Sampling	3rd Sampling	
Fresh Chicken Meat (Madang Public Market)	Stall 1	MPN g^{-1}	103.20	105.10	398.00	within acceptable limit
	Stall 2		10.10	86.80	354.00	within acceptable limit
Fried Chicken meat (DOrSU vicinity)	Eatery 1	CFU g^{-25} Sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Eatery 2		TNTC	TNTC	TNTC	Exceeds acceptable limit
	Eatery 3		TNTC	TFTC	TNTC	Exceeds acceptable limit (1st & 3rd); within limit (2nd)
"Isaw" (DOrSU vicinity)	Stall 1	CFU g^{-25} sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 2		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 3		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
"Kwek-kwek" (DOrSU vicinity)	Stall 1	CFU g^{-25} sample	TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 2		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)
	Stall 3		TFTC	TNTC	TNTC	Exceeds acceptable limit (2nd & 3rd); within limit (1st)

DISCUSSION

Presence of *Salmonella* spp. and *E. coli* in Samples

Based on personal communications with students and colleagues, incidents of gastrointestinal discomfort were reported following the consumption of takeaway foods purchased from local food establishments. This study presents preliminary evidence that these takeaway foods are contaminated with *Salmonella* spp. and *E. coli*. However, data gathered did not establish a causal link between the isolated bacteria. Some *E. coli* strains are commensals and natural residents in the gut of warm-blooded animals (WHO 2018). However, some harmful strains cause diarrhea, so its possible link to the reported cases cannot be ruled out. Further studies, such as strain identification and pathogenicity testing, would be necessary to confirm whether the isolated bacteria were responsible for the gastrointestinal symptoms. The presence of *E. coli* and *Salmonella* in the foods examined (Table 2 and Table 3) probably resulted from fecal contamination that could be attributed to poor sanitation and improper food handling (Birgen et al. 2020). Although the takeaway foods are cooked, *E. coli* is generally killed at 70°C (Li and Ganzle 2016; WHO 2018), including *E. coli* O157:H7, the most health-threatening *E. coli* variant to humans (WHO 2018). The presence of *E. coli* and *Salmonella* spp was exceedingly high. Table 4 and Table 5 beyond the permissible limit from set guidelines on these food may be attributed to the ability of these bacteria to withstand various environmental stresses, such as heat and acidity. While *Salmonella* was killed at 74°C (Ansah 2023) or less than this temperature given a more prolonged time exposure, some *E. coli* strains demonstrated heat-resistance such as *E. coli* AW1.7 (Li and Ganzle 2016) or produce toxins that are persistent in food (FSIS-USDA 2021). Moreover, *E. coli* can adapt to heat (Wang et al. 2021) and acidic environments (van Elsas et al. 2011), allowing it to survive under harsh conditions and rapidly reproduce when favorable conditions return. Similarly, enteric *Salmonella* has been found to endure heat treatments and desiccation (Wang et al. 2021), with specific variants exhibiting resistance to heat, acid, and other stresses (Thames and Sukumaran 2020). Given these factors, preparers and vendors must be aware of these survival mechanisms and implement proper food handling, preparation, and storage practices to prevent

contamination and the reintroduction of pathogens during food processing and selling.

Improper food handling increases bacterial contamination anywhere during chicken handling, slaughtering, and meat processing (Bhaisare et al. 2014). Another percentage of bacterial load is added during food preparation and selling (Moloi et al. 2021; Wardhana et al. 2021). The presence and high levels of *E. coli* and *Salmonella* in the samples could be due to unsanitary handling practices, contamination sources, and vendor hygiene conditions. According to WHO (2018), *E. coli* is typically present in the digestive tract of warm-blooded animals; thus, its presence in fresh chicken meat and cooked chicken food indicates fecal contamination. This could result from poor handling practices, such as improper washing of raw chicken, using contaminated water for washing, or cross-contamination from raw to cooked food during preparation. Birgen et al. (2020) established a direct link between high bacterial load and the hygiene conditions of vendors. Factors such as lack of food covers to prevent flies and other vectors, waste accumulation in vending areas, improper hand hygiene, and unclean clothing among food handlers all contributed to bacterial contamination. This supports the idea that *E. coli* and *Salmonella* in the samples may result from inadequate sanitary practices in food establishments in the local community (Marquez and Bureros 2022). Given these findings, food sellers and establishments should be assessed for compliance with health and hygienic standards to prevent foodborne contamination and illnesses.

In early 2022, a policy brief was generated from the findings of these early studies by DOrSU Bachelor of Science Biology students with close supervision of their faculty advisers. The policy brief was presented to the Sangguniang Panlungsod of Mati in March 2022; however, concrete action is yet to be felt by the local authorities. With the findings of additional studies done in 2023 and the current year, as consolidated in this paper, it is hoped that this material provides enough scientific proof that local authorities need to enforce the necessary action to implement sanitary inspections, mandatory food safety training for handlers, strengthening local food safety ordinances, and establishing routine microbial monitoring to protect public health and ensure safer street food practices.

Salmonella is a leading cause of foodborne disease outbreaks in humans. On the other hand, *E. coli*, a natural resident in the digestive tract of humans and warm-blooded animals, indicates fecal contamination in food. Although the study did not identify specific variants of *E. coli* present, the high amounts detected in food samples are still a significant concern. The latter has pathogenic variances that are responsible for a high number of disease outbreaks in humans. Both bacteria are present in fresh chicken meat and chicken-based street food commonly sold in certain stores in Mati to a degree way beyond the acceptable limit set by FDA Circular No. 2022-12-2 and DA-NMIS Circular No. 9-2008-5, which should alert and trigger the local authorities to implement the necessary and immediate action to address the situation. Regular monitoring of food establishments should ensure strict compliance with health standards and sanitary practices must be followed. Further, building the capacity of local/barangay leaders in the assessment of the actions employed by the food establishments as well as people involved in food-based economic activities should be done through information and education. Collaboration with the university and other institutions to assess the strategies implemented by the Local Government Unit is also highly encouraged.

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GENERATIVE AI STATEMENT

The authors affirm that no generative AI was used in the design of the study, data acquisition, analysis, or interpretation of the results. Grammarly profreader was solely used for enhancing the clarity of the manuscript document. All the content, ideas, and conclusions are the work and responsibility of the authors.

ETHICAL CONSIDERATIONS

There were no experimental animals used in this study.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests for any of the authors.

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