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Research Article

Detection and Antimicrobial Susceptibility of *Escherichia coli* in Retail Chicken Breast Meat in General Santos City, Philippines

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Abstract | Antimicrobial resistance (AMR) is a critical concern under the One Health framework. An alarming phenomenon, which is the global spread of AMR among Enterobacteriaceae in poultry. This preliminary, descriptive study investigated the presence of *Escherichia coli* (*E. coli*) in raw chicken breast (n=9) sold in different public markets in General Santos City and assessed the isolates' antimicrobial resistance profile. Samples were purchased on "lean," "peak," and randomly selected market days. The isolates' antimicrobial resistance was tested using the Kirby-Bauer disk diffusion assay. All five isolates exhibited similar and differing resistance profiles against amoxicillin (AMX) , doxycycline (DOX), and norfloxacin (NOR). *E. coli* M_3D_1 is resistant to AMX, *E. coli* M_2D_1 is resistant to AMX and NOR, both *E. coli* M_2D_3 and M_1D_2 are resistant to AMX and DOX, while *E. coli* M_3D_3 exhibited a multidrug-resistant (MDR) phenotype, showing resistance to all tested antibiotics (AMX, DOX, and NOR). These findings underscore the need for large-scale surveillance to determine the prevalence and risk factors for antimicrobial-resistant *E. coli* in General Santos City.

Keywords | Chicken breast, Multidrug resistance, Amoxicillin, Doxycycline, Norfloxacin

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INTRODUCTION

In the Philippines, chicken is a primary source of animal protein due to its affordability, low fat content, and minimal religious or cultural restrictions. The shift in consumer preference from pork to chicken has been further driven by supply shortages and rising pork prices associated with recurrent African Swine Fever outbreaks (Kaye, 2019). As a result, the poultry industry has become one of the country's fastest-growing livestock sectors (Espino and Bellotindos, 2020).

Antibiotics are extensively used in poultry production for disease prevention and treatment. However, their inappropriate and excessive use has driven the emergence and spread of antimicrobial resistance (AMR), representing a major global public health concern within the One Health framework (Abreu *et al*., 2023; Pineda-Cortel *et al*., 2024). In 2019, antimicrobial-resistant bacterial infections were associated with an estimated 4.95 million deaths worldwide, of which approximately 1.27 million were directly attributable to resistant pathogens (Tang *et al*., 2023). In this context, there is a possibility that resistant bacteria in retail poultry could indirectly contribute to the problem.

Escherichia coli (*E. coli*) is a commensal bacterium commonly found in the intestinal tract of chickens (Koutsianos *et al*., 2021). During slaughtering and processing, intestinal contents may contaminate carcasses, potentially transferring *E. coli* to raw poultry meat. Consumption of improperly handled or undercooked poultry products contaminated with *E. coli* can therefore transmit these bacteria to humans (Wardhana *et al*., 2021). Pathogenic strains of *E. coli* are associated with a range of illnesses, including hemorrhagic enteritis, dysentery, meningitis, pneumonia, and urinary tract infections (Astuti *et al*., 2021). *E. coli* is also a significant cause of foodborne infections and outbreaks. Among its pathogenic variants, enterohemorrhagic *E. coli* (EHEC) has emerged as a major public health threat, accounting for an estimated 9,000 illnesses and 70 deaths annually in the United States alone (Amalia *et al*., 2020).

Despite the public health implications, data on AMR in *E. coli* from raw chicken meat sold in public markets in General Santos City remain scarce. Thus, this preliminary and descriptive study aimed to isolate and identify *E. coli* from raw chicken breast samples obtained from selected public markets in the city and to determine the antimicrobial resistance profiles of the isolates using the disk diffusion method. Specifically, the study evaluated resistance against amoxicillin, doxycycline, and norfloxacin by measuring zones of inhibition and characterizing resistance patterns among the isolates.

MATERIALS AND METHODS

Meat sample collection

Raw chicken breast meat was collected from vendors in different public markets in General Santos City. Public markets included in this study were selected based on the following criteria: (1) the market has high foot traffic with a high number of customers; (2) the market is in proximity and accessible to at least three highly populated barangays; and (3) the market has at least five (5) raw chicken meat vendors. Details of the vendors and the public markets were not disclosed to protect vendor privacy. This limits the traceability of results and potential follow-up by regulatory agencies

Samples were collected on three separate days. The first was a "lean" day, defined as a day with a relatively lower number of customers as compared to other days of the week; another sampling day represented a "peak" day which is defined as the busiest day of the week due to having the most number of customers as compared to other days of the week; and another sampling day was chosen randomly, excluding the other identified sampling days. Sampling across "lean" and "peak" days was conducted to increase temporal coverage and not intended to assess differences in storage time or market-day effects.

After selecting the marketplace and day of the week based on the parameters of this study, raw chicken breast samples were randomly collected from vendors. Five vendors per marketplace were randomly selected by lot. One chicken breast was purchased from each vendor and pooled into a single sterile polyethylene bag. This method was used to maximize vendor coverage within each market. The study was limited only to nine samples in total.

All samples were coded by market (M_1, M_2, M_3) and collection day (D_1, D_2, D_3). The market and day codes were merged to create an isolate code, such as M_1D_1, M_2D_2, M_3D_3. Samples were stored in a freezer prior to transport to PhilExport Quality Control Laboratory, Fish Port Complex, General Santos City, where the samples were analyzed for the presence of *E. coli*. The isolation, purification, and identification followed the methods of the US Food and Drug Administration Bacteriological Analytical Manual (US FDA BAM) (Feng *et al*., 2020). Upon receipt, all *E. coli* isolates from the PhilExport Quality Control Laboratory were streaked onto Eosin Methylene Blue Agar (EMBA), and microscopy was performed to further confirm the identification of *E. coli*.

Antibiotic susceptibility test (Kirby-Bauer disk diffusion assay)

All *E. coli* isolates obtained from the samples were tested for antibiotic susceptibility. Three antibiotics representing

different antimicrobial classes, β-lactams, tetracyclines, and fluoroquinolones were used in this study. The antibiotics were selected based on: (1) their frequency of use in the Philippine poultry industry (Barroga *et al.*, 2020); (2) their availability as listed in the Philippine Veterinary Drug Directory (2024); and (3) the availability of corresponding resistance breakpoints in the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). The CLSI M07 guidelines (CLSI, 2024) were used as the reference for selecting the antibiotics and their corresponding disk concentrations. The antibiotics tested were amoxicillin (10 µg), doxycycline (30 µg), and norfloxacin (10 µg).

All *E. coli* isolates were streaked onto sterile nutrient agar (NA) plates and incubated for 18–24 hours at 37 °C (Liaqat *et al.*, 2022). After incubation, morphologically similar colonies were selected and transferred into sterile tubes containing 10 mL of normal saline solution (NSS) (Wiegand *et al.*, 2008). The turbidity of the suspension was adjusted to match a 0.5 McFarland standard by visual comparison using a Wickerham card. Bacterial colonies were added as necessary to adjust the turbidity to match the standard (Hudzicki, 2009; Wiegand *et al.*, 2008).

Subsequently, a sterile cotton swab was immersed in the adjusted suspension and used to streak the entire surface of a Mueller–Hinton Agar (MHA) plate. Streaking was performed three times, rotating the plate approximately 60° between streaks to ensure uniform bacterial distribution. The plates were then left to dry at room temperature for 5 minutes (Hudzicki, 2009). The antibiotic discs were aseptically placed equidistantly on the inoculated MHA plates in accordance with CLSI M07 standards (CLSI, 2024). Three antibiotic discs and one blank disc, which served as a negative control, were placed on each plate. The plates were then inverted and incubated at 37 °C for 24 hours. Three replicates per antibiotic were prepared for each of three independent trials. Following incubation, antimicrobial activity was assessed by measuring the zones of inhibition (ZOI) surrounding each disc. The diameters of the inhibition zones were measured, recorded, and analyzed.

Data analysis

Data analysis was purely descriptive. No statistical analysis was performed due to the exploratory nature of the study and limited sample size. This pilot study was intended to establish feasibility of comprehensive, large-scale surveillance studies. The CLSI M100 guidelines (CLSI, 2023) were used as the reference for assessing the antimicrobial resistance profiles of the isolates. Isolates were classified as susceptible when zone diameters were equal to or greater than the susceptible breakpoint, intermediate when zone diameters fell within the intermediate range, and resistant when zone diameters were equal to or less than the resistant breakpoint. The breakpoint values for the antibiotics evaluated in this study are presented in Table 1.

Table 1: Reference table for the interpretation of zone diameter breakpoints from CLSI M100.

Antibiotic	Zone diameter breakpoint, nearest whole mm		
	Susceptible	Intermediate	Resistant
Amoxicillin, 10 ug	≥17	14-16	≤13
Doxycycline, 30 ug	≥14	11-13	≤10
Norfloxacin, 10 ug	≥17	13-16	≤12

(CLSI, 2023).

RESULTS AND DISCUSSION

Sample collection

A total of nine samples were collected from different markets on different days. Of these, five samples tested positive for the presence of *E. coli*. The positive isolates were M_1D_2, M_2D_1, M_2D_3, M_3D_1, and M_3D_3. Summary is presented in Table 2.

Table 2: Detection of *Escherichia coli (E. coli)* in chicken breast samples.

Market (M)	Day (D)	Sample Code	*E. coli* detected
1	1	M_1D_1	Negative
1	2	M_1D_2	Positive
1	3	M_1D_3	Negative
2	1	M_2D_1	Positive
2	2	M_2D_2	Negative
2	3	M_2D_3	Positive
3	1	M_3D_1	Positive
3	2	M_3D_2	Negative
3	3	M_3D_3	Positive

Isolation and identification of bacteria

All five isolates tested positive for Indole production and Methyl Red tests, and negative for Voges–Proskauer and Citrate utilization tests (Table 3). Figure 1 shows the morphological characteristics of the isolates. When grown on Eosin Methylene Blue Agar (EMBA), all isolates produced green metallic sheen colonies with a black center (Figure 1A), while on Nutrient Agar (NA), all isolates displayed similar morphological characteristics (Figure 1B), except for isolate M_3D_1, which had an undulate margin. Gram staining revealed that all isolates retained a pink coloration, confirming that they are Gram-negative. Under 1000× magnification, all isolates were rod-shaped, with cell lengths varying among isolates (Figure 1C).

Table 3: Biochemical and phenotypic characteristics of *Escherichia coli* isolate.

Isolate	Biochemical properties				Grown in eosin-methylene blue agar				Grown in nutrient agar				Microscopy, 1000x magnification	
	Indole	Methyl Red	Voges-proskauer	Citrate	Color	Margin	Elevation	Texture	Color	Margin	Elevation	Texture	Gram stain	Cell shape
M_1D_2	+	+	-	-	GMS+BC	Entire	Raised	Smooth	Opaque	Entire	Convex	Slimy	Negative	Rods
M_2D_1	+	+	-	-	GMS+BC	Entire	Raised	Smooth	Opaque	Entire	Convex	Slimy	Negative	Short Rods
M_2D_3	+	+	-	-	GMS+BC	Entire	Raised	Smooth	Opaque	Entire	Convex	Slimy	Negative	Rods
M_3D_1	+	+	-	-	GMS+BC	Entire	Raised	Smooth	Opaque	Undulate	Convex	Slimy	Negative	Rods
M_3D_3	+	+	-	-	GMS+BC	Entire	Raised	Smooth	Opaque	Entire	Convex	Slimy	Negative	Short Rods

(+) denotes a positive reaction; (-) denotes a negative reaction; GMS - Green Metallic Sheen; BC - Black Center.

E. coli belongs to the Enterobacteriaceae family within the order Enterobacteriales. The cells are straight, cylindrical rods, occurring singly or in pairs, Gram-negative, and aerobic to facultatively anaerobic with a fermentative metabolism. Biochemically, *E. coli* is positive for indole production and negative for citrate utilization as a carbon source (MacWilliams, 2009; Whitman *et al.*, 2012). Assessment of glucose fermentation end products revealed that all isolates tested positive in the Methyl Red test and negative in the Voges–Proskauer test, consistent with the typical metabolic profile of the genus *Escherichia*, which produces lactic and acetic acids as end products (McDevitt, 2009). These biochemical characteristics correspond with the observed morphology and reactions of all isolated *E. coli* strains.

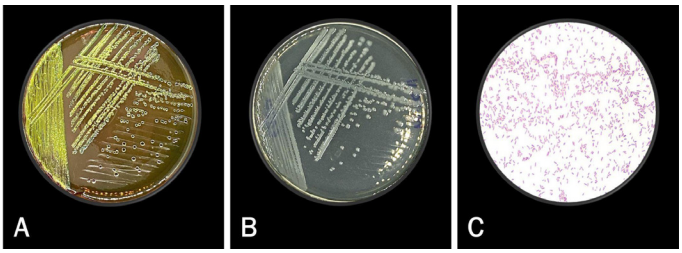

Figure 1: Morphological Characteristics of representative isolates (A) *E. coli* M_3D_3 grown in EMBA showing green metallic sheen with black center; (B) *E. coli* M_2D_3 grown in NA showing similar morphological characteristics; (C) *E. coli* M_1D_2 appearing rod-shaped with pink coloration after Gram staining; viewed in 1000X magnification.

Antibiotic susceptibility test (Kirby-Bauer disk diffusion assay)

All five isolates exhibited resistance to the tested antibiotics: amoxicillin (AMX), doxycycline (DOX), and norfloxacin (NOR) (Table 4). *E. coli* M_3D_1 was resistant only to AMX, while *E. coli* M_2D_1 was resistant to AMX and NOR. Both *E. coli* M_2D_3 and *E. coli* M_1D_2 showed resistance to AMX and DOX, whereas *E. coli* M_3D_3 was resistant to all three antibiotics (AMX, DOX, and NOR). Figure 2A shows the plate setup. Figure 2B-C show representative results with zones of inhibition.

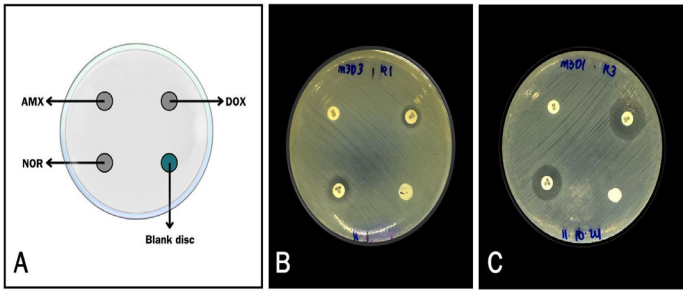

Figure 2: Different zones of inhibition using the kirby-bauer disk diffusion assay. (A) Plate set-up showing the placement of different antibiotics and the blank disc; (B) *E. coli* M_3D_3 resistant to AMX, DOX, & NOR; (C) *E. coli* M_3D_1 resistant to AMX, susceptible to DOX, intermediate to NOR.

Table 4: Antibiotic resistance profile of *Escherichia coli* isolates.

Isolate Code	Profile	No. of Antibiotics
M_3D_1	AMX	1
M_2D_1	AMX – NOR	2
M_2D_3	AMX – DOX	2
M_1D_2	AMX – DOX	2
M_3D_3	AMX – DOX – NOR	3

AMX, Amoxicillin; DOX, Doxycycline; NOR, Norfloxacin

E. coli M_3D_3 is classified as multidrug resistant (MDR), exhibiting resistance to three different classes of antibiotics: Beta-lactam (AMX), Tetracycline (DOX), and Fluoroquinolone (NOR) (Al-Mustapha *et al.*, 2022). In a larger study by Al-Mustapha *et al.* (2022), 73 out of 181 chicken broiler cloaca samples tested positive for *E. coli*, of which 82% were MDR and 50.6% were extensively drug resistant (XDR), defined as resistance to more than five classes of antibiotics. Similarly, Ranabhat *et al.* (2024) reported *E. coli* in chicken broiler meat samples consisting of a mixture of breast, liver, and thigh tissues. Out of 105 samples, 61 tested positive for *E. coli*, with 29.5% classified as MDR.

Among the antibiotics tested in this study, amoxicillin

(AMX) was the least effective, with all five isolates (5/5) resistant. For doxycycline (DOX), three of the five isolates (3/5) were resistant, and two out of five (2/5) were susceptible. Norfloxacin (NOR) exhibited two out of five (2/5) resistant, two out of five (2/5) intermediate, and one out of five (1/5) susceptible (Table 5). Rafiq *et al.* (2024) reported that *E. coli* isolates from poultry feces were resistant to amoxicillin, with 94.1% classified as multidrug resistant (MDR). Similarly, Gharaibeh *et al.* (2024) found that 360 of 385 broiler chicken cloaca swab samples tested positive for Avian Pathogenic *E. coli* (APEC), with 100% resistant to tetracycline and 99.2% resistant to amoxicillin. Larger studies involving cloacal and internal organ isolates were cited to provide a broader context on the occurrence of AMR in the poultry industry.

Table 5: Antimicrobial susceptibility of *Escherichia coli* isolates.

Antibiotic	Susceptibility classification of *Escherichia coli* isolates based on CLSI M100 guidelines		
	Susceptible, isolate count (n/5)	Intermediate, isolate count (n/5)	Resistant, isolate count (n/5)
Amoxicillin, 10 µg	-	-	5/5
Doxycycline, 30 µg	2/5	-	3/5
Norfloxacin, 10 µg	1/5	2/5	2/5

(CLSI, 2023); A dash (-) indicates that no isolates were classified in that susceptibility category

CONCLUSION AND RECOMMENDATION

The preliminary and descriptive findings of this study indicate the presence of multidrug-resistant *E. coli* in the examined samples of raw chicken breast meat. These findings highlight the urgent need for comprehensive, large-scale surveillance, in collaboration with local regulatory agencies to determine the true prevalence and associated risk factors of antibiotic-resistant *E. coli* within the Philippine poultry production and distribution chain. Future studies should incorporate appropriate statistical reporting. As a general preventative measure, appropriate food safety practices, including thorough washing, proper cooking, and safe handling of poultry products, are recommended to reduce the risk of foodborne illness and cross-contamination

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NOVELTY STATEMENT

This study provides novel insights into the antimicrobial resistance profiles of *E. coli* in raw chicken breast sold in public markets in General Santos City, an area with limited published data on foodborne pathogens. Unlike previous research focusing on broiler cloaca or mixed meat samples, this study specifically targets raw chicken breast intended for human consumption, providing direct relevance to food safety and public health. Furthermore, it characterizes the isolates' resistance against multiple antibiotic classes, identifying the presence of multidrug-resistant strains. The findings not only reveal potential public health risks associated with poultry consumption but also the need for consumer education and regulatory monitoring in the Philippines, thereby contributing to local and global efforts to combat antimicrobial resistance.

AUTHOR'S CONTRIBUTION

ANM and GBF: Contributed to conceptualization, laboratory works, analysis of the data, and manuscript writing. CJGP, HST, MUS, MMS, and GARBC: Assisted in laboratory works. KMBA: Contributed to conceptualization, supervised the study, reviewed protocols, and revised the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that AI-assisted tools (ChatGPT and Grammarly) were used solely to improve English language quality and grammar. All outputs were reviewed by the authors. No AI tools were used for data analysis or interpretation.

Conflict of interest

The authors have declared no conflict of interest.

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