Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Raw Chicken Meat in a Selected Wet Market in Manila City, Philippines

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ABSTRACT

Background and Objective: Antimicrobial resistance (AMR) is a leading global public health concern as it resulted in more difficult-to-treat infections and fatalities. In the Philippines, drug-resistant *E. coli*, including multidrug-resistant (MDR), extended-spectrum beta-lactamase (ESBL)-producing, carbapenemase-producing carbapenem-resistant (CP-CR) *E. coli*, have been isolated from common food animals, increasing the risk of cross-contamination between humans, animals, and the environment. However, there is a lack of data on the distribution of *E. coli* in chicken meat in public wet markets. This study aims to describe the AMR profile of *E. coli* in raw chicken meat from retail stalls in a selected wet market in Manila City.

Methods: This quantitative descriptive study characterized the AMR profile of *E. coli* isolated from 25 raw chicken meat samples from a wet market in Manila City. Antimicrobial susceptibility was determined through disk diffusion method against 23 antimicrobial agents in 16 antimicrobial classes. MDR *E. coli* were identified based on the resistance patterns. ESBL- and carbapenemase-producing capacities of the bacteria were tested through double disk synergy test and modified carbapenem inactivation method, respectively.

Results: Twenty-four out of 25 (96%) chicken samples contained *E. coli* isolates. Of these, 23 (96%) were classified as MDR. High resistance rates were observed against ampicillin (92%), tetracycline (88%), trimethoprim-sulfamethoxazole (83%), chloramphenicol (79%), ampicillin-sulbactam (75%), amoxicillin-clavulanic acid (67%), fosfomycin (67%), and streptomycin (54%). The majority of the *E. coli* isolates were still susceptible to a wide range of selected antimicrobial agents, including carbapenems (100%), ceftriaxone (100%), cefepime (100%), cefuroxime (96%), cefotaxime (96%), ceftazidime (96%), piperacillin-tazobactam (96%), aztreonam (96%), cefoxitin (92%), and nitrofurantoin (83%), among others. Meanwhile, none of the 24 isolated *E. coli* samples were classified as ESBL- and CP-CR *E. coli*.

Conclusion: Among the 25 chicken samples, 24 *E. coli* colonies were isolated that exhibited 0% to 92% resistance rates against selected antimicrobial agents. Most isolates were classified as MDR, but none were considered ESBL- and CP-CR *E. coli*. This study suggests that chickens in wet markets can potentially serve as reservoir hosts for drug-resistance genes, which could transfer to other bacteria and contaminate humans, animals, and the environment within the food production and supply chain. These findings emphasize the need for AMR surveillance and strategies to combat AMR in the Philippines through the One Health approach.

Keywords: drug resistance, multi-drug resistance, ESBL, carbapenemase, Escherichia coli

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INTRODUCTION

Antimicrobial resistance (AMR) is described as the phenomenon in which medicines are ineffective in inhibiting the growth and development of organisms due to specific modifications in the organisms' characteristics over time.[1] The proliferation of drug-resistant organisms has resulted in more difficult-to-treat infections and greater risks of severe hospital cases, disease transmission, and fatalities. The emergence and

widespread distribution of these organisms urged the World Health Organization (WHO) to include AMR in the list of the leading global public health concerns affecting mankind.

AMR can be attributed to various mechanisms; one of which is through drug inactivation.[2] This mode of resistance is exemplified in extended-spectrum beta-lactamase (ESBL)-producing and carbapenemase-producing bacteria. ESBL-positive strains can produce enzymes capable of hydrolyzing beta-lactam-containing antibiotics such as penicillins and cephalosporins.[3] However, one notable limitation of ESBL-producing strains is that they are unable to metabolize cephamycins and carbapenems, which are then used to treat ESBL-related infections.[4] Meanwhile, the evolution of microorganisms has resulted in the emergence of carbapenemase-producing strains which manufacture carbapenemase enzymes that are able to inactivate carbapenems.[5] Studies have also observed that ESBL-producing and carbapenemase-producing carbapenem-resistant (CP-CR) organisms can exhibit horizontal gene transfer of ESBL and carbapenemase enzymes to other bacteria through plasmid-mediated mechanisms.[6,7]

Consequently, an increasing trend in the prevalence of multidrug-resistant (MDR), ESBL-producing, and carbapenem-resistant *Escherichia coli* isolated from both humans and animals has been reported across multiple countries.[3,8-10] The WHO has already classified carbapenem-resistant and ESBL-positive species of Enterobacteriaceae as Priority 1 or critical strains that are in need of urgent development of new and effective antibiotics.[11] Additionally, the Centers for Disease Control and Prevention (CDC) classified carbapenem-resistant and ESBL-producing Enterobacteriaceae under the categories "Urgent Threats" and "Serious Threats", respectively, to human health.[12]

Drug-resistant *E. coli* strains can infect animals, thereby increasing the risk of human infections through direct animal-human exposure or through the food chain and food supply.[7,9] Notably, these microorganisms are recognized as one of the primary pathogens associated with outbreaks of foodborne diseases worldwide that contribute to the significant rise in morbidity and mortality rates globally.[1,3,7,9]

In the Philippines, multiple reports have documented the widespread distribution of AMR *E. coli* in various settings. MDR *E. coli* have been detected in samples taken from slaughterhouses, livestock farms (e.g., poultry, buffalo, and swine farms), and raw chicken meat and animal-derived food products from open markets and supermarkets across the country.[13,14] ESBL genes were also detected in selected small-scale urban agricultural farms. *E. coli* isolates collected from irrigation water, vegetable, and soil samples in Metro Manila, specifically in Quezon City, Marikina City, and Pasig City, were found to be positive for ESBL production.[15] ESBL-producing *E. coli* (ESBL-EC) have also been identified in poultry broiler farms across four provinces in the central region of Luzon.[16] In addition, a small percentage of the *E. coli* isolates from animal-related food products and swab-collected specimens from poultry farms, buffalo farms, swine farms, abattoirs, open markets, and supermarkets in the Philippines were observed to be resistant to carbapenems.[14] Carbapenem-resistant carbapenemase-producing *E. coli* (CP-CREC) have been detected as well in fish meat from Metro Manila wet markets.[17] The presence of carbapenem resistance genes was also identified from poultry broiler farm isolates in Central Luzon.[16] Despite these findings, there has been an observed lack of data on the distribution of drug-resistant bacteria in meat products in the Philippines. There are currently little to no available data on the contamination of MDR *E. coli*, ESBL-EC, and CP-CREC, specifically, from chicken meat in public markets in the country. As a result, limited knowledge is known regarding the impact of MDR *E. coli*, ESBL-EC, and CP-CREC in the transmission of drug-resistant pathogenic bacteria from chicken meat products in wet markets.

Considering the detection of these drug-resistant bacteria in livestock production and supply, there is an increased threat of transmission in public markets where bacteria can easily be spread among different food products and individuals. Hence, constant surveillance of ESBL-EC and carbapenem-resistant *E. coli* is critical in indicating the status of AMR evolution in the country.

This study, therefore, aimed to describe the AMR profile of *E. coli* in raw chicken meat from retail stalls in a selected wet market in Manila City. Specifically, this research aimed to characterize the susceptibility of isolated *E. coli* against selected antimicrobial agents and describe the antimicrobial resistance patterns (i.e., MDR, ESBL-producing, carbapenemase-producing) of isolated *E. coli* in raw chicken meat through absolute counts and proportions.

The detection and characterization of the AMR profile of *E. coli* in raw chicken meat in a selected wet market in Manila City could provide baseline information, which could later be utilized for the surveillance of AMR in the Philippines.

MATERIALS AND METHODS

Research Design

The study utilized a quantitative descriptive cross-sectional research design to characterize the AMR profile and antimicrobial resistance patterns of *E. coli* isolated from raw chicken meat from a selected wet market in Manila City.

Study Area

The raw chicken meat samples were bought from retail stalls in a selected wet market located in Manila City. The selection of the study site was primarily due to the population density of Manila City as it was known as the most densely populated city among the Highly Urbanized Cities (HUC) in the National Capital Region (NCR) with a population density of 73,920 persons per square kilometer in 2020.[18] The feasibility and logistics concerns of the researchers were also considered in choosing the study site. Due to ethical

considerations, the exact location of the wet market was not disclosed.

Study Population

The study population consisted of retail stalls located within the selected wet market that sold raw chicken meat. There was a one-to-one correspondence between the stall and raw chicken meat as only one chicken sample was obtained from each selected retail stall.

Inclusion Criteria

Retail stalls that sold raw chicken leg meat within the selected wet market in Manila City were included in the study. The stalls were located along the boundary streets or within the halls of the wet market. These stalls also sold chicken legs that were already cut from the chicken body prior to buying.

Exclusion Criteria

Hung chicken legs or those already placed in plastic containers were not selected for this research. This exclusion criterion controlled and minimized the effects of environmental exposure as a confounding variable to the detection of AMR *E. coli*.

Sampling Design

This study employed a probability sampling design in selected retail stalls selling chicken leg meat products. Specifically, the study site was divided into three areas to avoid duplication of selected stalls, and simple random sampling was performed in each location per collection batch to select chicken retail stalls. For chicken meat collection, convenience sampling was conducted wherein one raw chicken leg sample was collected from each retail stall. Then, specimen sampling was employed from each chicken leg sample for homogenization and bacteria isolation.

Sample Size

Considering the financial resources, 25 stalls were included in this study. In each stall, one raw chicken meat was obtained; therefore, a total of 25 raw chicken samples were tested.

Preparation, Collection, and Assay Procedures

The processes in describing the AMR profile of *E. coli* from chicken samples were (1) preparation of media, (2) sample collection, (3) homogenization, (4) isolation of *E. coli*, (5) identification of *E. coli*, (6) antimicrobial susceptibility testing, (7) ESBL production screening and confirmation, (8) carbapenemase production screening and confirmation, and (9) data analysis.

Preparation of Media

The media used in the study were MAC agar, EMB agar, Brain Heart Infusion (BHI) Broth, MHA, Triple Sugar Iron (TSI) slant, and nutrient agar slant.

These media were prepared under the guidance of personnel from the Department of Medical Microbiology (DMM) at the College of Public Health University of the Philippines Manila. Expiration dates and the quality of the materials were thoroughly ensured prior to creation. The procedure for the procurement of the media was strictly aligned with the standard instructions, and every step was constantly monitored by the personnel.

Biosafety measures were followed throughout the duration of the process to avoid contamination and to assure the excellent quality of the media. In cases of defective media, these were not utilized and were properly disposed of.

Quality control organisms were used in ensuring the accuracy of the media for supporting or inhibiting the growth of bacteria. Expected results were based on the growth and inhibition properties in line with proper morphological characteristics, and production of biochemical reactions. All media used *E. coli* ATCC 25922 for positive control.

Sample Collection

Raw chicken leg meat, specifically the drumstick part, was collected from each of the 25 stalls in a selected wet market in Manila City. Chicken legs were selected as the study sample due to the high isolation rates of drug-resistant *E. coli* in whole legs and drumstick portions of the chicken.[13,19] The stall owners were not informed regarding the purpose of buying the chicken samples to eliminate the effects of demand bias by providing chicken samples that are less likely to have been exposed to conditions associated with AMR. The collection of chicken specimens began in September 2022, with three batches bought within the span of 2-3 weeks at around 7:00 to 8:00 in the morning. Five samples were collected in the first batch, and 10 samples in each succeeding batch. After buying the raw chicken meat, the sample was placed in separate sterile plastic bags and stored in an ice-filled cooler. The specimens were transported within five hours to the DMM Laboratory at the College of Public Health University of the Philippines Manila.

Homogenization

Each meat sample was homogenized prior to isolation and identification of *E. coli*. About 25 grams of chicken meat were cut into very small pieces using aseptic techniques. These were placed inside a second sterile bag containing 225 mL of peptone water and vigorously shaken before being mixed using a homogenizer for one minute. About 5 mL of each homogenized sample was transferred to a sterile bottle containing 45 mL of peptone water and mixed gently to create a mixture of the processed chicken meat sample. The resulting solution was then incubated at 37°C for 18 to 24 hours.[14,19,20]

Isolation and Identification of E. coli

After incubation of the processed chicken meat sample, two sets of triplicates of 10-μL loopful of the sample were

simultaneously prepared. One set was streaked and subcultured onto MAC agar and the other set on EMB agar. The plates were incubated at 37°C for 18 to 24 hours. The colonies with pink coloration and metallic green sheen on MAC and EMB agar, respectively, were considered presumptive *E. coli* isolates and were subjected to phenotypic identity confirmation.[21] Identification of *E. coli* from each set-up was conducted using morphological and biochemical techniques, specifically, gram staining, IMViC, oxidase, and triple sugar iron (TSI) tests. Gram-negative rods bacteria with a negative oxidase test; a positive, positive, negative, negative (++--) IMViC test; and a yellow slant yellow butt (A/A) which indicates glucose and lactose fermentation along with positive gas production and negative hydrogen sulfide production for the TSI test were considered positive of *E. coli*.[21-23] All tests were performed using a positive control of *E. coli* ATCC 25922.

Isolates that had inconsistent results in the morphological and biochemical tests underwent identification VITEK® MS in the Microbiology section of the Department of Laboratories, Philippine General Hospital (PGH), University of the Philippines Manila. This automated method uses Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology in the determination of microorganisms.

Only one confirmed *E. coli* colony from the triplicate set-ups was randomly selected to proceed to the characterization of the AMR profile. These colonies were maintained on nutrient agar for storage at 4°C.[24]

Antimicrobial Susceptibility Test

Selected *E. coli* isolates underwent antimicrobial susceptibility testing according to the CLSI's Kirby–Bauer disk diffusion susceptibility test protocol.[25] The antimicrobial susceptibility test consists of multiple steps, presented in order starting from inoculum preparation, inoculation to MHA, the addition of antimicrobial disks, incubation, measurement of inhibition zones, and interpretation of inhibition zones according to the CLSI standard protocol.

Stored *E. coli* from the nutrient agar were cultured in BHI broth and incubated overnight prior to inoculum preparation. To prepare the inoculum standard, isolated colonies of *E. coli* from the BHI broth were picked up by a sterile inoculating loop and placed into a test tube with 5 mL of sterile saline solution. The tube was then vortexed to mix the solution. Depending on the turbidity of the solution, the inoculum density was adjusted to the 0.5 McFarland standard. In cases of a light suspension, organisms were added into the tube, while in a heavy suspension, it was diluted with sterile saline solution to achieve the standard equivalent. The prepared 0.5 McFarland standard was used to finally confirm the resulting suspension. Within 15 minutes of preparation, the confirmed inoculum was inoculated onto the MHA.

Table 1. Antimicrobial Disks Used Against the Isolated *E. coli*

Antimicrobial Classes	Antimicrobial Agents	Disk Content
Aminoglycosides	Streptomycin (STR)	10 µg
Antipseudomonal Penicillin with beta-lactamase inhibitors	Piperacillin-Tazobactam (TZP)	100/10 µg
Carbapenems	Ertapenem (ETP)	10 µg
	Imipenem (IPM)	10 µg
	Meropenem (MEM)	10 µg
Non-extended spectrum cephalosporins	Cefazolin (CFZ)	30 µg
	Cefuroxime (CXM)	30 µg
Extended-spectrum cephalosporins	Cefotaxime (CTX)	30 µg
	Ceftriaxone (CRO)	30 µg
	Ceftazidime (CAZ)	30 µg
	Cefepime (FEP)	30 µg
Cephamycins	Cefoxitin (FOX)	30 µg
Fluoroquinolones	Ciprofloxacin (CIP)	5 µg
Quinolone	Nalidixic acid (NAL)	30 µg
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (SXT)	1.25/23.75 µg
Monobactams	Aztreonam (ATM)	30 µg
Penicillins	Ampicillin (AMP)	10 µg
Penicillins with beta-lactamase inhibitors	Amoxicillin-clavulanic acid (AMC)	20/10 µg
	Ampicillin-sulbactam (SAM)	10/10 µg
Phenicols	Chloramphenicol (CHL)	30 µg
Phosphonic acids	Fosfomycin (FOF)	200 µg
Tetracyclines	Tetracycline (TET)	30 µg
Nitrofurantoin	Nitrofurantoin (NIT)	300 µg

A sterile cotton swab was dipped in the tube containing the inoculum and rotated on the test tube side above the fluid level to remove excess fluid. The bacteria on the cotton swab were inoculated onto the MHA by streaking the swab three times in a back-and-forth motion across the entire surface while rotating the plate 60 degrees between each pass. Afterwards, the rim of the MHA plate was also swabbed. The plate was left for three to five minutes with the lid slightly ajar to allow the surface of the agar plate to dry prior to adding the antimicrobial disks.

The antimicrobial susceptibility was determined using the disk diffusion assay with 23 antimicrobial agents belonging to 16 antimicrobial classes as summarized in Table 1.

The antimicrobial-impregnated disks were pressed gently onto the surface of the MHA using sterile forceps. Each plate contained five antimicrobial disks placed at a minimum distance of 24 mm apart. All antimicrobial disks were tested against a control organism of *E. coli* ATCC 25922.

Within 15 minutes of adding the antimicrobial disks, the plates were stacked upside down, with no more than five plates on top of each other, and kept at 37°C for 18 to 24 hours.

After incubation, ZOI was measured to the nearest millimeter using a ruler or caliper. All measurements were made by observing the backs of the dishes with the naked eye. The diameter of the ZOI from edge to edge across the disk's center was measured using reflected light while holding the plate a few inches above a non-reflective background. Whenever there is an overlapping ZOI, the radius was used to measure the ZOI of a particular antimicrobial agent.

The CLSI guidelines for Enterobacteriaceae were used to interpret the ZOIs of the tested antimicrobials into three categories: susceptible, intermediate, and resistant.

ESBL-EC Detection

E. coli isolates tested for ESBL production were initially screened through the use of antimicrobial susceptibility testing to cefotaxime, ceftriaxone, ceftazidime, and aztreonam. Results from previous AST of antibiotic disks were used. Isolates with ZOI of ≤27 mm for cefotaxime, ≤25 mm for ceftriaxone, ≤22 mm for ceftazidime, or ≤27 mm for aztreonam were further subjected to DDST. Prior to the confirmatory DDST, sample strains of *E. coli* from the nutrient agar were used to create a new set of inoculum. Similar protocols were applied from the previous AST inoculum preparation and inoculation to MHA for the preparation of media with the lawned organisms.

The disks used for the MHA were amoxicillin-clavulanic acid (20 μg/10 μg) placed at the center of the MHA plate, three disks of third-generation cephalosporins [i.e., cefotaxime (30 μg), ceftriaxone (30 μg), and ceftazidime (30 μg)], and one-fourth generation cephalosporin [i.e., cefepime (50 μg)] placed with equal spaces of 15 mm and 20 mm from the center disk, respectively.[26] After adding the disks, the MHA plate was inverted and incubated for 18-24 hours at around 35°C ± 2°C. Positive results were indicated by distortions or increased extensions in the intersection of ZOI between the amoxicillin-clavulanic acid and cephalosporins.[27] Positive and negative quality control organisms were used to check the validity of the results.

CP-CREC Detection

Isolates that underwent confirmatory tests for carbapenemase production were initially screened through the AST. All intermediate and resistant colonies to at least one carbapenem—imipenem (i.e., ZOI of ≤ 22 mm), meropenem (i.e., ZOI of ≤ 22 mm), or ertapenem (i.e., ZOI of ≤ 21 mm)—were subjected to confirmatory testing of carbapenemase production using mCIM.[28]

Stored *E. coli* were cultured in BHI broth. One microliter loopful of each isolate from the BHI broth was aseptically transferred into a test tube with 2 mL TSB. The tube was vortexed for 10-15 seconds. With sterile forceps, a 10-μg meropenem disk was totally immersed into each test tube. All set-ups were incubated for 4 hours ± 15 minutes at around 35°C ± 2°C. Prior to the end of the incubation period, a 0.5 McFarland suspension of *E. coli* ATCC 25922 was inoculated onto an MHA dish within 15 minutes. Upon completion of the incubation, the meropenem disk was removed from the tube with the use of a 10-μL loop—the disk was pulled out through surface tension by positioning the flat part of the inoculating loop against the flat portion of the antimicrobial disk and then dragging and pressing the loop on the tube edge to remove excess fluid from the disk. Ensuring that the plates were air-dried for around 3-10 minutes, the meropenem disk was positioned on the MHA plate lawned with *E. coli* ATCC 25922. The agar was inverted and incubated for 18-24 hours at around 35°C ± 2°C. After incubation, ZOI was measured and interpreted using the CLSI standards.[28] ZOI of 6-15 mm or the growth of pinpoint colonies within the disk diameter of 16-18 mm was indicative of carbapenemase-producing *E. coli*. Carbapenemase-negative colonies were characterized by a clear ZOI of at least 19 mm. Meanwhile, indeterminate organisms for carbapenemase production were those with ZOIs of 16-18 mm or with growth of pinpoint colonies within a ZOI of at least 19 mm.[28] Positive and negative quality control organisms for mCIM were used to check for the validity of the results.

Data Processing and Analysis

E. coli isolates were labeled using specific codes to represent the stall source (letter codes) and bacteria number (number codes).

Descriptive statistical analysis was implemented to characterize the susceptibility of *E. coli* from raw chicken meat against selected antimicrobial agents and to describe their AMR patterns. Specifically, absolute counts and proportions were calculated, tabulated, and presented as shown in the appendices. A susceptible result suggested that the antimicrobial agent is effective against *E. coli*. An

intermediate result implied that its effects on *E. coli* have an uncertain therapeutic impact, thereby requiring an increased dose. A resistant result suggested that the effect of the antimicrobial agent was not reliable to treat *E. coli*. Breakpoint values for the susceptibility of *E. coli* to each antimicrobial agent were detailed in Appendix Table 1. Meanwhile, resistance patterns refer to AMR classification (i.e., MDR or nMDR), and ESBL-producing and carbapenemase-producing characteristics of the isolates.

Ethical Considerations

The protocol of this research study requested and was approved for exemption from an ethical review of the Research Ethics Board (REB). Nonetheless, the framework of this study was performed in compliance with the ethical guidelines stipulated in the National Ethical Guidelines for Health and Health-Related Research 2017.

RESULTS

Presence of AMR *E. coli* in Raw Chicken Meat

All 25 raw chicken meat samples were processed and subcultured on MAC and EMB agar plates. Samples that yielded green metallic sheen colonies on the EMB agar plates were subjected to morphological and biochemical testing. Among the 25 samples, 17 (68%) were confirmed to be presumptive *E. coli* isolates. The remaining 8 (32%) isolates with inconsistent biochemical tests underwent microbial identification through VITEK® MS, which uses MALDI-TOF technology. This automated process was able to provide data regarding the identification and antimicrobial susceptibility profile of bacteria and fungi. Among the 8 isolates, 7 were confirmed to be *E. coli*, while one was identified as *Citrobacter freundii*. In total, 24 of the 25 (96%) chicken samples contained *E. coli* isolates.

Antimicrobial Susceptibility Profile of *E. coli* Isolates

The study characterized the susceptibility of 24 *E. coli* isolates against 23 selected antimicrobial agents as shown in Figure 1 (see Appendix Table 2 for detailed proportions). Notably, high resistance rates were observed against ampicillin (92%, 22/24), tetracycline (88%, 21/24), trimethoprim-sulfamethoxazole (83%, 20/24), chloramphenicol (79%, 19/24), ampicillin-sulbactam (75%, 18/24), amoxicillin-clavulanic acid (67%, 16/24), fosfomycin (67%, 16/24), and streptomycin (54%, 13/24). It was also observed that 79% (19/24) of the *E. coli* isolates had intermediate sensitivity to cefazolin. Despite these findings, the majority of the *E. coli* isolates were still susceptible to a wide range of selected antimicrobial agents, including all kinds of carbapenems (100%, 24/24), ceftriaxone (100%, 24/24), cefepime (100%,

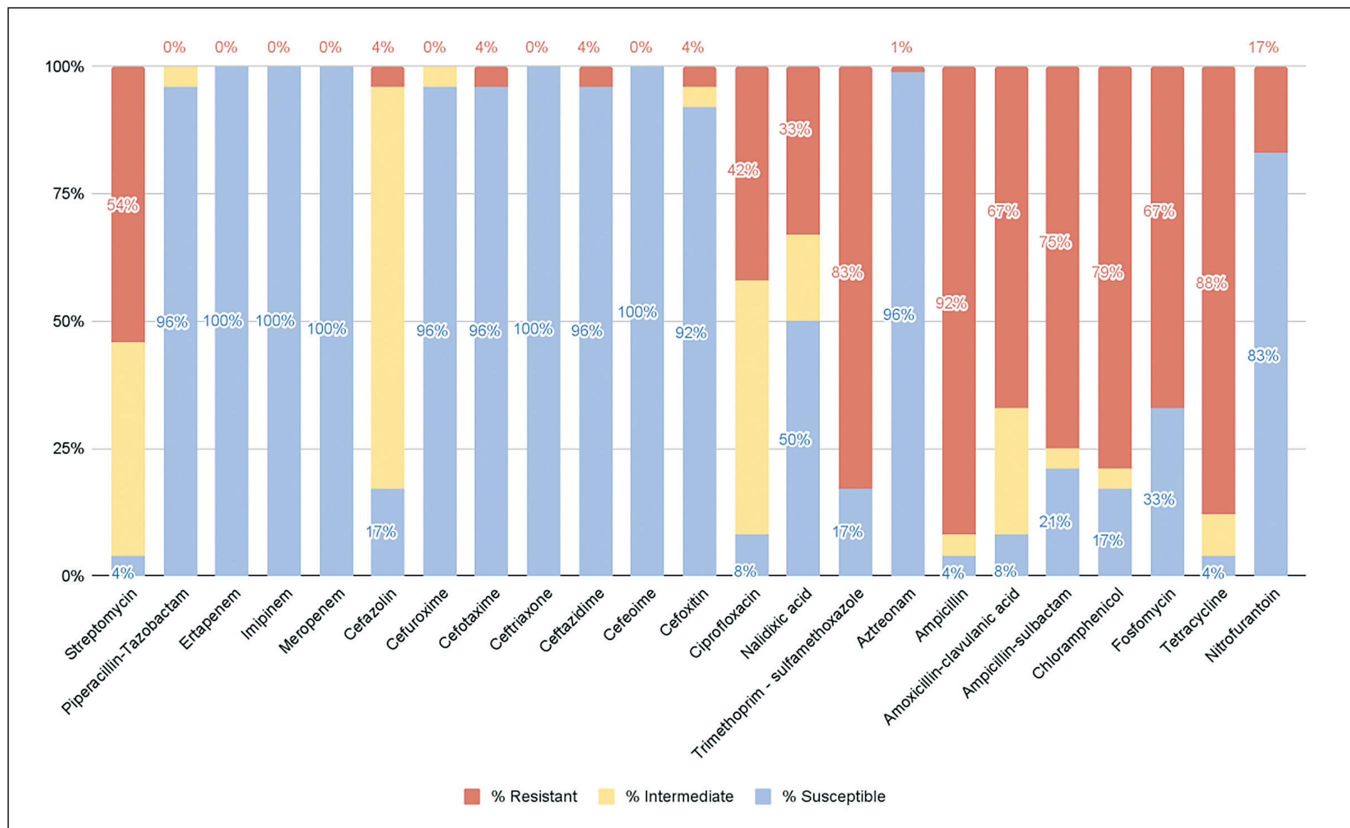

Figure 1. Proportion of Susceptible, Intermediate, and Resistant *E. coli* isolated from raw chicken meat samples (n = 24).

Figure 2. Cumulative relative frequency of AMR *E. coli* based on number of antimicrobial agents (n = 24).

24/24), piperacillin-tazobactam (96%, 23/24), cefuroxime (96%, 23/24), cefotaxime (96%, 23/24), ceftazidime (96%, 23/24), aztreonam (96%, 23/24), cefoxitin (92%, 22/24), and nitrofurantoin (83%, 20/24), among others.

Patterns of Antimicrobial Resistance of *E. coli* Isolates

Out of the 24 *E. coli* isolates, 23 isolates (96%) were resistant to at least one antimicrobial agent (Figure 2), while the remaining one was found to be susceptible to all antimicrobial agents except for its intermediate resistance against tetracycline. Additionally, there was one isolate (4%) that was resistant to at least 14 antimicrobial agents.

Furthermore, isolates that exhibited either resistant or intermediate sensitivity to at least one antimicrobial agent in at least three antimicrobial classes were classified as MDR.[29] This study was able to detect 23 (96%) MDR *E. coli* isolates among the chicken samples.

Among the different resistant patterns identified in the study (Appendix Table 3), one of the notable patterns was the resistance of 13 isolates to seven antimicrobial agents, namely trimethoprim-sulfamethoxazole, ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, chloramphenicol, fosfomycin, and tetracycline. Fifteen isolates were also reported to be co-resistant to ampicillin, amoxicillin-clavulanic acid, and chloramphenicol. Furthermore, co-resistance was also observed among 17 isolates against trimethoprim-sulfamethoxazole, ampicillin, and tetracycline.

For the detection of ESBL-producing *E. coli*, five out of the 24 isolates exhibited ZOIs that passed the initial screening using the antimicrobial susceptibility test. Upon confirmation through DDST, it was found that none of the five isolates exhibited observable zone extensions, thus none of the *E. coli* isolates in the study were considered ESBL enzyme producers.

Similarly, no CP-CREC was detected in the samples as none of the *E. coli* isolates passed the initial screening for carbapenemase production.

DISCUSSION

Presence and Prevalence of AMR *E. coli* on Raw Chicken Meat

Antimicrobial resistance in the food animal industry is a serious public health concern considering that the demand for animal protein globally has been continuously increasing.[30] In particular, broiler chickens play an important role in the economy of countries as they are known to contain high-quality protein while having a low-cost price.[30] In order to meet these demands, antimicrobials have been utilized to provide support and maintain the health of food animals, however, this resulted in an increase in AMR bacteria in global food production and supply.[31] Detecting AMR among pathogenic bacteria in these kinds of food animals poses a major public health crisis worldwide as it can limit the treatment and management of life-threatening infections. The presence of AMR bacteria in these settings can also serve as a potential source of drug-resistance genes that can be transmitted to other pathogenic microorganisms through horizontal gene transfer mechanisms.[32]

Commensal bacteria such as *E. coli* are also monitored in poultry in terms of their antimicrobial resistance.[33] The primary habitat of *E. coli* in poultry is located in the lower digestive tract. Some strains of these bacteria normally do not cause harm. However, some strains can either cause intestinal or extraintestinal diseases which depend on the host and site of infection.[34]

In the present study, 24 AMR *E. coli* were isolated among the 25 raw chicken meat samples bought from a selected wet market in Manila City. This relatively high prevalence is consistent with local studies that investigated the presence of AMR *E. coli* in raw chicken meat products sold in public markets. A study in Quezon City was able to detect AMR *E. coli* in all 25 raw chicken meat samples bought from a public market.[13] Another study was able to report similar findings with the detection of AMR *E. coli* from retailed chicken meat bought from selected markets in Valencia City, Bukidnon.[35] MDR *E. coli* was also isolated from chicken carcasses collected from broiler farms in CALABARZON.[36] Moreover, the findings of this study are also comparable to the prevalence of AMR *E. coli* in other countries. Research in Malaysia and Arizona reported AMR *E. coli* from the chicken products bought from retail poultry meat wet markets and grocery stores.[20,37] These data reinforce the need to address the widespread public health issue of AMR, both locally and globally.

Antimicrobial Susceptibility Profile

The present study observed significantly high resistance rates to various antimicrobial agents, including ampicillin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, ampicillin-sulbactam, amoxicillin-clavulanic acid, fosfomycin, streptomycin, ciprofloxacin, and nalidixic acid against the isolated *E. coli* from raw chicken

meat. These findings are aligned with the global findings of *E. coli* resistance rates against tetracycline (60% - 91.8%), trimethoprim-sulfamethoxazole (49.4% - 84%), nalidixic acid (53.4% - 74.1%), ampicillin (34% - 72.9%), and amoxicillin–clavulanic acid (69%) detected from raw chickens in different wet markets.[38-44] Similar results were also reported in previous research conducted in Quezon City, Philippines wherein there was a 100% resistance rate to ampicillin and an 88% resistance rate to tetracycline from raw chicken legs in a public wet market.[13] Additionally, a local study also found resistance rates to tetracycline at 58.33% and trimethoprim-sulfamethoxazole at 41.67% for chicken samples obtained in selected wet markets of Manila.[45] Resistance to tetracycline (66.1%), ampicillin (63.8%), trimethoprim-sulfamethoxazole (46.4%), nalidixic acid (45.1%), streptomycin (41.1%), and chloramphenicol (38.4%) against *E. coli* were also observed from chicken samples bought at abattoirs, supermarkets, open markets, and poultry farms across the Philippines.[14]

One of the factors that might have contributed to the presence of AMR bacteria in chicken meat could be the widespread use of antimicrobials in livestock farms. Evidence suggests that the use and misuse of antimicrobial agents in poultry farms of low- and middle-income countries (LMICs) is rampant for the rapid growth of healthier poultry animals.[46] Among commercial and backyard poultry farms in the four regions of the Philippines (i.e., Central Luzon, South Luzon, Central Visayas, and Western Visayas), 20% of the broiler farms and 42% of layer farms are using three to four antimicrobial agents, while 100% of both broiler and layer farms utilize one to two antimicrobial agents for feed additives as well as for the treatment of respiratory and enteric diseases.[46] Additionally, commercial broiler farms in the Philippines have been using antimicrobials for metaphylaxis, prophylaxis, and growth promotion.[47] Consequently, antimicrobial susceptibility profiles of *E. coli* isolates from different chicken resource samples from poultry farms have shown increased resistance rates against these commonly-used antimicrobial agents. *E. coli* isolates from chickens in broiler farms in CALABARZON have also exhibited high resistance rates to nalidixic acid (97.5%), ampicillin (90%), ciprofloxacin (85%), tetracycline (80%), streptomycin (72.5%), trimethoprim (62.5%), and trimethoprim-sulfamethoxazole (62.5%).[36] These data suggest that AMR *E. coli* from chickens in wet markets might be due to the use of antimicrobials in poultry farms.

In this study, the highest resistance rate was seen in ampicillin (92%). This might be attributed to cross-resistance with antimicrobials in the same class, specifically with its analog, amoxicillin.[48] Amoxicillin is known to be used in broilers in the Philippines for prophylactic and treatment purposes.[36,46] A study also showed that chicks treated with amoxicillin during their growing period resulted in increased resistance rates of *E. coli* isolates to both amoxicillin and ampicillin[49], thus suggesting the possibility of cross-resistance and co-resistance within the two agents.

It was also observed that tetracyclines, fluoroquinolones, quinolones, fosfomycin, and aminoglycosides are commonly used antimicrobials in poultry farms in the Philippines.[45-47] Resistance to these agents, as reported in this study, might be due to their uses as a growth promoter, prophylactic drug, and therapeutic medication for animals.[41,45-47] These findings are concerning considering that fluoroquinolones and quinolones are commonly used as first-line treatment against human foodborne infections caused by *E. coli* and *Salmonella*.[50]

Notably, studies have suggested the possibility of co-resistance to streptomycin and trimethoprim-sulfamethoxazole.[36,51] This co-resistance was identified in *E. coli* from meat products in Norway. It was reported that plasmids commonly harbor both *strA/strB* genes, which encode for streptomycin resistance and *sul2* genes for sulfonamide resistance, implying the presence of co-resistance to these two antimicrobials. The present study also identified 12 isolates that are co-resistant to both streptomycin and trimethoprim-sulfamethoxazole.[51] Another common co-resistance pattern evident in the local setting is the ampicillin-tetracycline-trimethoprim-sulfamethoxazole drug combination.[14,36,45] This study also found 17 isolates with this co-resistance pattern. This might be due to the unregulated use of these antimicrobial agents in Philippine poultry farms.[46]

Meanwhile, this study also exhibited findings inconsistent with existing literature. While this research found a high resistance rate to chloramphenicol at 79%, three local studies have detected much lower rates at 6.67%, 8%, and 38.4%.[13,14,45] The findings of this study are significant considering that chloramphenicol has been banned for usage among food-producing animals in the Philippines since 1990.[52] Potential causes of chloramphenicol resistance might be due to cross-resistance with antimicrobials within the same family or co-resistance with other antimicrobial classes as well as the illegal use of this agent in the agriculture industry.[36]

Aside from chloramphenicol, one study conducted in the Philippines also reported high resistance rates for non-extended- and extended-spectrum cephalosporins[36], which are contrary to the findings of this study. Although there are no reports of cephalosporin use in local poultry farms, it was found that cephalosporins are used in local commercialized swine farms.[46] A probable reason for this unique finding is through cross-contamination with other animals and animal products.

Essentially, these data on AMR *E. coli* in the poultry industry suggest that the food production and supply chain can serve as a reservoir for drug-resistant genes that can be transferred to other animals and humans. In the clinical setting, the 2021 DOH-ARSP reported a comparable antimicrobial susceptibility profile of *E. coli* from clinical samples with the profile of this study.[53] Based on the DOH-ARSP report, percent resistance rates to the following antimicrobials are 78.7% to ampicillin, 54.8% to tetracycline, 54.5% to co-trimoxazole/trimethoprim-sulfamethoxazole, 48.5% to ciprofloxacin, and 45.9% to cefazolin. AMR

bacteria can cross into the human population through direct contact with contaminated live animals and consumption of contaminated food products, or indirectly through the surrounding environment.[54] Considering these findings, strict surveillance and re-aligning of policies on the usage of antimicrobial agents in livestock farms must be prioritized to prevent further worsening of antimicrobial resistance.

Multi-drug Resistant E. coli

This study identified 95.83% (23/24) MDR *E. coli* isolates from raw chicken meat samples. This high prevalence coincides with the findings in varying local wet markets ranging from 67.9% - 100% MDR *E. coli*.[13,14] In addition to these, international studies are also aligned with high rates of MDR *E. coli*. Other countries reported the following MDR *E. coli* rates from raw chicken in markets – China with 83.9%, Indonesia with 61.08%, Bangladesh with 76%, Zambia with 55%, and Qatar with 63.4%, among others.[38,41-44] This implies the continuous spread of antimicrobial resistance, specifically MDR *E. coli*, across the globe.

The identified increased MDR *E. coli* rates from chickens in wet markets are also similar to the rates observed in poultry farms in the Philippines. Two studies found prevalence rates of 92.5% and 95.65% of MDR *E. coli* isolated from freshly slaughtered chicken in broiler farms.[16,36] The variety of antimicrobial agents used in different farms across regions creates a wide extent of AMR problems with different scopes as both found locally and internationally.[38,46] Additionally, the demand to produce livestock, specifically chicken, calls for a greater number, larger dosage, frequent, and longer use of antimicrobials, which contributes significantly to MDR development.[38] The high demand for animal products in the Philippines increases the possibility of cross-contamination of MDR bacteria among animals, animal products, and humans.[14]

Environmental factors that affect the spread of MDR *E. coli* in chicken from wet markets include cross-contamination in different stages of production. In market settings, it was observed that the sewage samples from cleaning wholesale chicken meat in markets of Dhaka City in Bangladesh contain MDR *E. coli* with a prevalence of around 80%; higher MDR rates were also recorded in other bacteria.[19] These findings show the possibility of contamination among food products in the market. Therefore, hygiene practices should be given importance from the production site up to the markets.

ESBL-producing E. coli

Out of the 24 *E. coli* isolates examined in this study, none were considered ESBL-producers. This finding is consistent with a local study conducted in Manila City, which reported 0% ESBL-positive *E. coli* among intestinal samples from chickens, pigs, and Nile tilapia sourced from wet markets.[45] This similarity may be attributed to the comparable sampling and environmental conditions considering that both studies utilized meat samples sourced from wet markets in Manila City. It can also be inferred that ESBL genes have not yet been transmitted among *E. coli* in chicken meat obtained in the selected wet market. However, there may also be a possibility of failure in detecting ESBL production as it has been established that phenotypic confirmatory tests are unable to exhibit all kinds of ESBL enzymes as compared with automated and genotypic methods.[55]

However, other studies reported significantly higher ESBL-EC rates. One research identified 52.82% ESBL-EC among various samples (i.e., chicken, pork, and beef) from multiple sources including abattoirs, wet markets, supermarkets, and livestock farms across the Philippines.[56] Additionally, another study detected 60.26% ESBL-positive *E. coli* strains from chickens in farms located in Central Luzon.[16] These significant differences in ESBL-positivity rates from the current study may be due to the greater sample sizes, wider sampling areas, therefore, chickens came from different sources with varying environmental conditions, and the type of samples (i.e., environmental swabs, cloacal swabs, and raw meat samples) used in the two studies.

Multiple studies have also identified possible sources of ESBL-EC in the Philippines. It was observed that surface waters used for irrigation systems in agricultural settings in Metro Manila were contaminated with ESBL-EC.[15] ESBL-positive strains of *E. coli* were also detected among tilapia samples from two wet markets in Manila City.[17] The presence of ESBL isolates in these settings may result in cross-contamination of pathogenic bacteria among animals, the environment, and humans, including the food production and supply chain. Therefore, despite the zero positivity rate observed in this study, continuous monitoring of ESBL-EC among poultry samples in wet markets should still be carried out as ESBL-resistance genes can be transferred from *E. coli* in poultry to humans or vice-versa through conjugative plasmids while harboring other antimicrobial resistance genes.[57]

Moreover, the positivity rates of ESBL internationally and in clinical samples locally are increasing. Although ESBL-positivity rates from chicken cloacal swabs in Indonesian markets remain low at 3.33% (2/60)[58], some countries recorded relatively higher ESBL-positivity rates such as 14.49% in Egypt, 39.2% in Peru, 53% in Cambodia, 53.8% in Malaysia, 76.8% in the Netherlands, and 86% in Bangladesh.[19,20,59-62] Researchers have suggested that travelers, especially those coming from Southeast Asia and India, contribute largely to the spread of ESBL-positive strains to other countries.[63] Hence, the high rates of ESBL-EC in poultry production in other areas can potentially affect the ESBL-EC rates in the Philippines. The DOH-ARSP also published in its 2021 annual report an ESBL-EC-positivity rate of 24.51%, in addition to the 29.53% ESBL-positivity among *K. pneumoniae*, from clinical specimens obtained from sentinel sites spread throughout the Philippines.[53] These further emphasize the need for ESBL surveillance in the country considering that the antimicrobials used on

ESBL-EC are rarely prescribed as first-line treatment against *E. coli* infections.[16]

Carbapenemase-producing Carbapenem-resistant E. coli

In this study, all 24 *E. coli* isolates from chicken samples were susceptible to carbapenems, specifically, imipenem, meropenem, and ertapenem, and were therefore classified as non-CP-CREC. This is despite a local study in the Philippines which found 1.7% (2/117) and 19.2% (43/224) resistance rates against meropenem and imipenem, respectively, among *E. coli* isolates from chickens.[14] An international study also reported resistance rates of 17.8% (5/28) to meropenem and 28.0% (8/28) to imipenem from *E. coli* detected in internal organs of raw chickens in Pakistan.[64]

Nonetheless, the present investigation concurs with earlier research indicating that there are no reports of CP-CREC in poultry meat from wet markets in the Philippines. Similar to Egypt and the United Kingdom, there was also no evidence of CP-CREC isolated from retail chicken.[60,65] However, carbapenemases have been detected from *E. coli* isolated in the internal organs of chickens in Pakistan and in chicken cloacal swabs in Malaysia.[64,66] In addition, researchers in Egypt were also able to detect high rates of carbapenemases among other members of the Enterobacteriaceae family obtained from chicken meat.[60]

In addition, there are a number of potential sources of carbapenemase resistance genes that can transfer to bacteria present in poultry meat in wet markets. One study discovered CP-CREC in fish meat from a wet market in the Philippines.[17] Carbapenem resistance genes were also identified in a poultry broiler farm in Central Luzon.[16] The presence of these genes may be attributed to the origin and manner of transmission of antimicrobial residues, resistance genes, and resistant bacteria, such as those released into rivers.[67] It is possible that these potential sources of carbapenemase enzyme have not yet been transmitted in the samples used in this study due to differences in environmental exposure, sources of poultry meat, and livestock farm production practices.

Moreover, according to the 2021 DOH-ARSP report, there is also a statistically significant change in the annual resistance rate of carbapenems in *E. coli* such as imipenem, meropenem, and ertapenem in clinical isolates collected from sentinel locations in the Philippines.[53] Similarly, *E. coli* with the carbapenemase gene, commonly reported in clinical settings, was found in hospital sewage and river water in the country.[68] Therefore, the existence of carbapenem resistance in the country facilitates the possible spread of these genes between humans, animals, and the environment, as highlighted in the One Health approach. This further necessitates active surveillance among the potential sources of resistance genes to prevent the emergence of carbapenem resistance as multiple studies in a variety of settings have demonstrated the spread of resistant genes and resistant bacteria among the community and the environment.[69-71] Carbapenems are also considered to be the last-resort antimicrobials[72]; hence, the proliferation of resistant microorganisms such as *E. coli* against this class of antimicrobials poses a hazard to public health and safety since it reduces the effectiveness of treating infections.

One Health Approach

One Health acknowledges that human health is strongly associated with animal and environmental health.[73] Animals, albeit beneficial to humans in many ways, such as food and livelihood, can transmit diseases caused by AMR bacteria to humans.[74] Therefore, as human, animal, and animal product movement increase, diseases disperse more rapidly around the world.[73] The CDC also emphasizes that geographical boundaries cannot effectively prevent the spread of AMR; this is relevant to note in the context of this study as most of the retailed poultry in Manila City are grown from nearby areas like Central Luzon and CALABARZON.[75]

In animal food products, studies have revealed that the major sources of contamination are the environment, equipment, workers, and contaminated animal. Poor sanitation and hygiene practices favor the cross-contamination of animal food products like chicken meat with microorganisms. For instance, there is a high risk of contamination in retail stalls due to raw chicken drips that spread on other meat and contact surfaces.[20] Additionally, in the context of this research, most of the chicken retail stalls used wooden cutting boards and had tiled or plastic-covered table tops. It was observed that working surfaces have shown to be important in the proliferation of microbial pathogens. Wooden surfaces had the highest risk of ESBL-EC contamination, followed by tiled surfaces and plastic-sheet-covered surfaces, compared to stainless steel surfaces.[20] Also, cutting boards were found to play a role in ESBL-EC occurrence, wherein wooden cutting board was reported to have the highest risk and plastic cutting board was revealed to have twice the risk of contamination compared to stainless steel cutting instruments. Furthermore, the water source for handwashing and utensil cleaning may also be a possible source of contamination. It has been shown there is three times the risk of contamination when only one water container is used for both handwashing and cleaning of utensils during the retail period, as compared to directly using water from a tap source.[20] The sampling site in this study also displayed the raw chicken meat in an open area, which may also contribute to the profile of microorganisms that have been isolated.

Limitations

One methodological limitation in this study was identified to be the usage of conventional methods for identification of isolates (i.e., the use of morphological and biochemical tests). In this study, the phenotypic isolation and identification of *E. coli* from raw chicken meat samples are prone to error because there are organisms that have similar biochemical characteristics to *E. coli*. It was observed that the

characteristic green metallic sheen of *E. coli* on EMB plates may also be exhibited by other organisms, such as *Citrobacter*. Hence, there are potential risks of misidentification of the microorganism using morphological and biochemical parameters.

First, to increase the accuracy of identifying if the isolated organism is *E. coli*, multiple morphological and biochemical tests based on CLSI standards were performed. These biochemical tests include indole, methyl red, Voges Proskauer, and citrate (IMViC); oxidase; and triple sugar iron (TSI) tests. Triplicates and multiple subcultures of isolated samples were also conducted using EMB and MAC agar plates. Vigorous lactose-fermenting gram-negative bacteria create an acidic environment, which produces a green metallic sheen, characteristic of *E. coli*.[76] However, one sample had *E. coli*-like colonies on the EMB plate, which were later confirmed as *Citrobacter*, similar to one study that aimed to isolate *E. coli* from fresh vegetables.[77] Therefore, to confirm the identity of the isolates with green metallic sheen but with biochemical results that are inconsistent with *E. coli*, automated identification through VITEK MS was conducted to verify their identities at the Philippine General Hospital Department of Laboratories. This automated verification procedure decreased the risks of misidentification of the microorganisms.

Another methodological limitation identified was the phenotypic observation of AST and DDST. Both AST and DDST are heavily reliant on subjective interpretation of results, thus affecting the accuracy of the results.[78] To minimize errors, three to four researchers carried out the observation, microbiology experts were consulted to verify the findings, and control organisms were also used to verify DDST results.

Considering that the study only used chicken legs as the source of the specimen, microorganisms present in other parts of the chicken might have been missed out. To minimize this limitation, raw meat pieces were cut from different areas of the raw chicken leg.

CONCLUSION

This study analyzed the AMR profile of *E. coli* isolated from raw chicken meat from retail stalls in a selected wet market in Manila City. Among the 25 chicken samples, 24 *E. coli* colonies were isolated while one was found to be *Citrobacter freundii*. The resistance rates to selected antimicrobial agents range from 0% to 92%. High resistance rates were observed in ampicillin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, ampicillin-sulbactam, amoxicillin-clavulanic acid, fosfomycin, streptomycin, ciprofloxacin, and nalidixic acid, among others. However, there was no evidence of resistance to ceftriaxone, cefepime, or any of the carbapenems, including ertapenem, imipenem, and meropenem. Notably, most isolates (96%) were found to be resistant to at least one antimicrobial agent, with more than half (54%) exhibiting resistance to at least eight antimicrobial agents, and one isolate showing resistance to at least 14 antimicrobial agents.

Of the isolated *E. coli* colonies, 23 (96%) were classified as MDR, and none of the 24 isolated *E. coli* samples were considered ESBL- and CP-CR *E. coli*.

The findings of this study offer preliminary data that can serve as a foundation for a more comprehensive analysis of commercial chicken meat and surveillance of AMR in the country while taking into account the possibility of transmission between humans, animals, and the environment. The reported resistance rates among *E. coli* isolates from chicken meat could be used to support the development of more effective treatment strategies against critical MDR *E. coli* infections.

Recommendations

The researchers recommend the use of automated techniques to improve the identification of *E. coli* as well as the characterization of its AMR profile. These technologies include, but are not limited to, the use of VITEK 2, VITEK MS, and PCR among others. To explore a broader antimicrobial profile, sensitivity against other antimicrobial agents can also be investigated. This can also determine possible extensively drug-resistant and pan drug-resistant microorganisms.

The researchers also suggest performing genotypic methods for future studies regarding ESBL and carbapenemase production. This could be done through PCR identification of the genes bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{OXA}, which are predominant among ESBL-producing *E. coli* in the country.[14,16]

Given the findings of this study, increased collaboration in addressing AMR in food products between various community sectors, including the academic community, government organizations, and food product handlers, can be promoted.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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APPENDICES

Appendix Table 1. Zone Diameter Breakpoints for Enterobacteriaceae (CLSI, 2020)

Test /Report Group	Antimicrobial Agent	Disk Content	Interpretative Categories and Zone Diameter Breakpoints, nearest whole mm			
			S	SDD	I	R
Aminoglycosides						
O	Streptomycin	10 µg	≥15	–	12-14	≤11
Antipseudomonal Penicillin with beta-lactamase inhibitors						
B	Piperacillin-Tazobactam	100/10 µg	≥21	–	18-20	≤17
Carbapenems						
B	Ertapenem	10 µg	≥22	–	19-21	≤18
B	Imipenem	10 µg	≥23	–	20-22	≤19
B	Meropenem	10 µg	≥23	–	20-22	≤19
Non-extended spectrum cephalosporins						
A	Cefazolin	30 µg	≥23	–	20-22	≤19
B	Cefuroxime	30 µg	≥18	–	15-17	≤14
Extended spectrum cephalosporins						
B	Cefotaxime	30 µg	≥26	–	23-25	≤22
B	Ceftriaxone	30 µg	≥23	–	20-22	≤19
C	Ceftazidime	30 µg	≥21	–	18-20	≤17
B	Cefepime	30 µg	≥25	–	19-24	≤18
Cephamycins						
B	Cefoxitin	30 µg	≥18	–	15-17	≤14
Fluoroquinolones						
B	Ciprofloxacin	5 µg	≥26	–	22-25	≤21
Quinolones						
O	Nalidixic acid	30 µg	≥19	–	14-18	≤13
Folate Pathway Inhibitors						
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	–	11-15	≤10
Monobactams						
C	Aztreonam	30 µg	≥21	–	18-20	≤17
Penicillins						
A	Ampicillin	10 µg	≥17	–	14-16	≤13
Penicillins with beta-lactamase inhibitors						
B	Amoxicillin-clavulanate	20/10 µg	≥18	–	14-17	≤13
B	Ampicillin-sulbactam	10/10 µg	≥15	–	12-14	≤11
Phenicols						
C	Chloramphenicol	30 µg	≥18	–	13-17	≤12
Phosphonic Acids						
U	Fosfomycin	200 µg	≥16	–	13-15	≤12
Tetracyclines						
C	Tetracycline	30 µg	≥15	–	12-14	≤11
Nitrofurantoin						
U	Nitrofurantoin	300 µg	≥17	–	15-16	≤14

Appendix Table 2. Proportion of susceptible, intermediate, and resistant *E. coli* isolates isolated from raw chicken meat samples (n = 24)

	Antimicrobial Agent	*E. coli* isolates tested, n (%)					
		Susceptible		Intermediate		Resistant	
Aminoglycosides	STR	1	4%	10	42%	13	54%
Antipseudomonal Penicillin with beta-lactamase inhibitors	TZP	23	96%	1	4%	0	0%
Carbapenems	ETP	24	100%	0	0%	0	0%
	IPM	24	100%	0	0%	0	0%
	MEM	24	100%	0	0%	0	0%
Non-extended spectrum cephalosporins	CFZ	4	17%	19	79%	1	4%
	CXM	23	96%	1	4%	0	0%
Extended spectrum cephalosporins	CTX	23	96%	0	0%	1	4%
	CRO	24	100%	0	0%	0	0%
	CAZ	23	96%	0	0%	1	4%
	FEP	24	100%	0	0%	0	0%
Cephamycins	FOX	22	92%	1	4%	1	4%
Fluoroquinolones	CIP	2	8%	12	50%	10	42%
Quinolone	NAL	12	50%	4	17%	8	33%
Folate pathway inhibitors	SXT	4	17%	0	0%	20	83%
Monobactams	ATM	23	96%	0	0%	1	4%
Penicillins	AMP	1	4%	1	4%	22	92%
Penicillins with beta-lactamase inhibitors	AMC	2	8%	6	25%	16	67%
	SAM	5	21%	1	4%	18	75%
Phenicols	CHL	4	17%	1	4%	19	79%
Phosphonic acids	FOF	8	33%	0	0%	16	67%
Tetracyclines	TET	1	4%	2	8%	21	88%
Nitrofurantoin	NIT	20	83%	0	0%	4	17%

Appendix Table 3. ZOI per antimicrobial agent of each isolate

Isolate (Stall Code-Isolate No.)	Antimicrobial Class																					
	Aminoglycosides	Antipseudomonal Penicillin with Beta-Lactamase Inhibitors	Carbapenem			Non-extended Spectrum Cephalosporins		Extended Spectrum Cephalosporins				Cephamycin	Fluoroquinolones	Quinolone	Folate Pathway Inhibitors	Monobactam	Penicillin	Penicillins with beta-lactamase inhibitors		Phenicols	Phosphonic Acids	Tetracycline	Nitrofurantoin
	Antimicrobial Agent																						
	STR	TZP	ETP	IPM	MEM	CFZ	CXM	CTX	CRO	CAZ	FEP	FOX	CIP	NAL	SXT	ATM	AMP	AMC	SAM	CHL	FOF	TET	NIT
	Zone of Inhibition (ZOI)																						
Control	19	31	32	31	35	29	29	32	33	29	35	30	28	28	28	35	17	24	21	30	31	31	23
A-3	6	25	30	32	31	23	23	26	29	21	28	20	10	6	6	12	6	17	18	7	18	14	17
B-2	6	21	27	29	29	20	20	28	27	23	26	20	14	6	6	28	6	12	9	8	6	6	14
C-1	13	25	27	29	29	25	24	30	29	25	29	29	23	21	26	32	14	23	23	25	35	11	22
D-3	15	25	27	30	30	24	24	30	30	27	30	22	29	24	26	32	20	22	19	32	33	12	21
E-2	11	26	26	28	29	21	23	27	28	24	28	21	17	6	6	30	6	14	11	8	27	6	20
F-2	6	22	28	28	29	21	23	29	27	25	28	24	23	18	6	31	6	10	8	6	6	99	20
G-2	14	23	28	28	30	20	21	30	29	24	30	28	25	22	6	30	6	15	13	6	6	7	21
H-2	14	23	28	25	28	19	23	30	28	24	29	22	27	20	6	29	6	10	9	8	6	6	19
I-3	14	24	28	27	30	23	21	28	26	23	27	22	9	6	6	28	6	10	9	6	6	6	20
J-1	11	21	27	29	30	22	22	30	29	24	29	22	23	19	6	30	6	11	7	7	6	9	17
K-3	12	20	28	29	31	22	23	29	28	25	27	24	6	6	6	30	6	10	7	6	6	6	17
L-3	11	23	26	28	28	22	23	30	30	24	30	25	22	21	6	30	6	16	16	24	25	25	14
M-1	10	21	30	29	31	21	22	30	26	25	28	22	22	19	6	29	6	12	8	6	6	6	22
N-1	NOT *E. coli* (Bacteria identified: *Citrobacter freundii*)																						
O-3	6	22	28	28	30	21	22	27	28	24	28	24	12	6	6	31	6	12	9	6	6	6	21
P-2	11	23	29	27	29	22	22	29	29	23	29	24	22	19	6	30	6	9	6	6	6	6	17
Q-2	10	24	28	31	31	21	22	29	28	29	29	23	23	21	22	30	6	14	11	6	26	6	18
R-1	6	21	27	28	30	20	19	27	26	22	26	17	18	16	6	29	6	10	9	6	6	6	20
S-2	13	22	29	28	29	21	20	28	27	23	28	25	24	21	6	28	6	13	9	6	6	6	20
T-1	12	23	28	30	30	21	23	31	28	25	31	24	24	23	6	30	6	12	11	15	6	9	19
U-2	9	23	23	23	30	11	16	22	23	15	26	6	21	17	6	25	6	9	10	10	6	6	6
V-3	12	23	28	29	30	21	19	28	28	23	30	20	6	6	6	27	6	12	10	8	6	6	19
W-3	13	21	27	28	29	21	22	30	29	24	29	21	11	6	23	30	6	13	11	10	27	11	14
X-2	12	22	28	30	30	22	22	30	29	29	29	22	22	22	6	31	6	11	10	10	6	6	19
Y-1	9	24	26	28	28	20	21	28	27	23	28	21	22	18	6	29	6	16	15	24	29	6	19

STR – Streptomycin; TZP – Piperacillin-Tazobactam; ETP – Ertapenem; IPM – Imipenem; MEM – Meropenem; CFZ – Cefazolin; CXM – Cefuroxime; CTX – Cefotaxime; CRO – Ceftriaxone; CAZ – Ceftazidime; FEP – Cefepime; FOX – Cefoxitin; CIP – Ciprofloxacin; NAL – Nalidixic acid; SXT – Trimethoprim-sulfamethoxazole; ATM – Aztreonam; AMP – Ampicillin; AMC – Amoxicillin-clavulanate; SAM – Ampicillin-sulbactam; CHL – Chloramphenicol; FOF – Fosfomycin; TET – Tetracycline. Red-colored cells are resistant, blue-colored cells are intermediate, and yellow-colored cells are susceptible.