BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 31 No. 2, 2024: 339 - 348
DOI: 10.11598/btb.2024.31.2.2207

Research Article

SURVEILLANCE OF β-LACTAMASE GENES AND ANTIMICROBIAL RESISTANCE IN *Salmonella* sp. FROM CHICKEN MEATS TRADED IN WET MARKETS OF METRO MANILA, PHILIPPINES

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ARTICLE HIGLIGHTS

- *bla*$_{CTX-M}$ gene in 24.7% while *bla*$_{TEM}$ gene in 11.2% of *Salmonella* isolates.
- Coexistence of *bla*$_{CTX-M}$ groups II and IV in all *bla*$_{CTX-M}$-positive isolates.
- Dominance of *bla*$_{CTX-M}$ corroborated with phenotypic β-lactam resistances.
- *bla*$_{CTX-M}$-positive isolates had extended-spectrum β-lactamase and multidrug resistance.

Article Information

Received 27 February 2024
Revised 14 July 2024
Accepted 22 July 2024

Reviewers:
Saharuetai Jeamsripong, Ida Bagus Gede Darmayasa

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ABSTRACT

Salmonella sp. is a foodborne pathogenic bacterium causing millions of cases with hundred thousand death incidents. Infection by *Salmonella* can diversely manifest as gastroenteritis, bacteremia, and enteric fever. *Salmonella* can be transmitted through direct consumption of contaminated foods especially animal-based foods, such as chicken meat and its derivatives. Over the years, antimicrobial resistance (AMR) and diverse β-lactamase (*bla*) gene-carrying *Salmonella* strains have been reported. These facts are alarming given that cephalosporins are a major class of β-lactam antibiotics used in clinical settings. Hence, the main objective of this study was to molecularly detect the occurrence of different *bla* genes by Polymerase Chain Reaction (PCR) and profile the phenotypic antimicrobial susceptibility of *Salmonella* collected from various chicken sample types in wet markets of Metro Manila, Philippines. Of the 89 *Salmonella* isolates, *bla*$_{CTX-M}$ had the highest occurrence, detected in 22 isolates (24.7%), while *bla*$_{TEM}$ was detected in 10 isolates (11.2%). Genotypic and phenotypic resistance corroboration was observed in nearly all *bla*$_{CTX-M}$-positive *Salmonella* tested, with all strains showing resistance to ampicillin and nitrofurantoin (100%) and 21 out of 22 (95.5%) exhibiting resistance to both non-extended and extended-spectrum cephalosporins. In addition, *bla*$_{CTX-M}$ groups II and IV genes were co-detected and multidrug resistance (MDR) profiles were also observed in all *bla*$_{CTX-M}$-positive isolates. The high AMR patterns of *Salmonella* isolates suggest potential threats to food safety and public health. Additionally, the corroboration of phenotypic and genotypic resistance and the high occurrence of MDR among *Salmonella* isolates highlight the importance of continued surveillance of AMR genes and regulation of antimicrobial use to combat AMR.

Keywords: antimicrobial resistance, *bla*$_{CTX-M}$ groups, *bla* genes, occurrence, *Salmonella*

INTRODUCTION

Salmonella sp. is a Gram-negative, facultative anaerobic, mesophilic, rod-shaped foodborne bacterium that belongs to the family *Enterobacteriaceae* (Ethelberg *et al.* 2014). Given that poultry is a significant reservoir for *Salmonella*, the risk of transmission increases through improper handling, trade, or slaughter of raw chicken (Eng *et al.* 2015). Infection can occur through the fecal-oral route, involving the ingestion of contaminated food, especially animal-based foods, such as chicken meat, or through water containing *Salmonella* (Eng *et al.* 2015). Due to their easy transmission from raw/improperly cooked meat, the high consumption of chicken in the Philippines can pose a risk for *Salmonella* infection. In the Philippines, the average annual poultry consumption per capita in the period of 2009-2018 was 13.4 kg (DA-BAI 2022).

Although *Salmonella* consists of only two species, *enterica* and *bongori*, with *S. enterica* containing six subspecies, most infections are caused by *S. enterica* subsp. *enterica* (Desai *et al.* 2013). Infections can manifest as enteric fever, gastroenteritis, bacteremia, or a chronic carrier state depending on the *Salmonella* serovars. Enteric fever is caused by *S.* Typhi or *S.* Paratyphi A, B, and C which are typhoidal *Salmonella*. All other *Salmonella* strains are designated as nontyphoidal *Salmonella* (NTS), which causes mild infections, including gastroenteritis. Although typhoidal *Salmonella* is more likely to follow a human-to-human transmission route, NTS transmission is more likely associated with animal reservoirs, such as contaminated chicken meat. The most common serovars responsible for NTS are *S.* Enteritidis, *S.* Typhimurium, and *S.* Newport. The symptoms of enteric fever include headache, abdominal pain, diarrhea/constipation, fever, rose spots, and in severe cases, bloody diarrhea. Conversely, the symptoms of gastroenteritis are usually self-limiting and include headache, abdominal cramps, vomiting, non-bloody diarrhea, nausea, and muscle ache (Eng *et al.* 2015).

NTS and typhoidal *Salmonella* may eventually lead to bacteremia and a chronic carrier state. Bacteremia occurs when *Salmonella* penetrates the intestinal barrier and invades the bloodstream, while a chronic carrier state is characterized by fecal shedding of *Salmonella* more than one year after the acute stage of *Salmonella* infection (Eng *et al.* 2015). In 2017, there were approximately 14.3 million cases of typhoidal *Salmonella* and approximately 95.6 million cases of NTS globally (Stanaway *et al.* 2019). Approximately 136,000 died from typhoidal *Salmonella* in the same year, and 128,000 died from NTS (Stanaway *et al.* 2019). In the Philippines, Santos *et al.* (2020) found *S. enterica* in meat sold at wet markets in Metro Manila, highlighting the need to investigate the presence of *bla* genes among *S. enterica* isolates.

Due to numerous *Salmonella*-related deaths annually, antibiotic treatment becomes important for treatments against invasive diseases. Extended-spectrum cephalosporins (ESC) are some of the antibiotics used to treat *Salmonella* infections (Calayag *et al.* 2021). The treatments for NTS also include ciprofloxacin and ceftriaxone, and for severe complications, may include cefixime and cefotaxime (Gut *et al.* 2018). Cephalosporins rely on cell wall synthesis interference through the inhibition of transpeptidases, which causes bacterial cell lysis and death (Cantón 2007). However, antimicrobial resistance (AMR) threatens the efficacy of these treatments. Some *Salmonella* strains have acquired resistance to these antibiotics because of their ability to produce β-lactamase enzymes that hydrolyze class A β-lactam antibiotics, including cephalosporins (Cantón *et al.* 2012).

β-lactamase enzymes, such as temoneira (TEM), cefotaximase (CTX-M), and sulfhydryl variable (SHV) are encoded by bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} genes, respectively, and are among the most common ESBL genes (Ejaz *et al.* 2021). Additionally, $bla_{CTX\text{-}M}$ genes are highly diverse and prevalent among *Enterobacteriaceae*, due to transmissions, mutations, and recombinations. They include numerous groups that confer resistance to different generations of cephalosporins, primarily against cefotaxime and ceftriaxone, with some enhanced variants capable of acting even against ceftazidime (Rossolini *et al.* 2008). Some extended-spectrum β-lactamases (ESBLs) also have the capability to hydrolyze broad-spectrum third- and fourth-generation cephalosporins (Cantón 2007), posing a significant threat in clinical treatment settings. Determining the occurrence of these genes and phenotypic resistance of *S. enterica* is important for understanding its transmission and dynamics. Hence, this study determined the occurrence of bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV}, in *S. enterica* isolated from raw chicken from wet markets in Metro Manila, Philippines. Moreover, this study is among the first in the Philippines to detect the simultaneous occurrence of $bla_{CTX\text{-}M}$ gene groups and determined the phenotypic antimicrobial susceptibility profile of *S. enterica* possessing those gene groups.

MATERIALS AND METHODS

Salmonella Isolates

Following standard procedures under ISO 6579-1:2017 (ISO 2017) and Santos *et al.* (2020), 25 g of raw chicken meat samples from leg, thigh, and breast parts were aseptically minced and placed in sterile Whirl-Pak® bags. Then, 225 mL of buffered peptone water (BPW) (BD Diagnostics System, NJ, USA) was added, followed by homogenization for 30 seconds and incubation at 37 °C for 18-24 hours. Subsequently, 100 μL of the BPW culture was transferred to 9 mL of Rappaport Vassiliadis (RV) (BD Diagnostics System, NJ, USA) and incubated at 42 °C for 18-24 hours. The resulting RV cultures were then streaked on xylose lysine deoxycholate

(XLD) agar (BD Diagnostics System, NJ, USA) and incubated at 37 °C for 18-24 hours. Black colonies on red XLD agar were then subcultured on nutrient agar (NA) (BD Diagnostics System, NJ, USA) plates and incubated at 37 °C for 18-24 hours. The resulting colonies were then subjected to DNA extraction, *Salmonella* confirmation, and *bla* gene detection. A total of 89 *S. enterica* isolates from five cities in Metro Manila, namely, Quezon, Manila, Pasay, Malabon, and Valenzuela, were randomly selected.

DNA Extraction

Three to four colonies from NA were transferred into 100 μL of 1× TE buffer (10 mM Tris, 1 mM EDTA at pH 8.0) for DNA extraction through boil lysis. The suspension was boiled at 100 °C for 10 minutes on a heat block to lyse bacterial cells. After boil lysis, suspensions were subjected to centrifugation at 6,000 rpm for 5 minutes (Calayag *et al.* 2017). The supernatant containing DNA extract was transferred/decanted into a sterile microcentrifuge tube and stored at −20 °C prior to use in further experiments.

PCR Confirmation of *S. enterica*

Each PCR reaction consisted of 1 μL of previously extracted DNA as the template, 6.25 μL of GoTaq® G2 Green Master Mix (Promega, WI, USA), 0.5 μL each of 10 μM forward and reverse primers for *invA* gene, and 4.25 μL of nuclease-free water. DNA extracted from *S. enterica* subsp. *enterica* American Type Culture Collection (ATCC) 14028 served as the positive control, and *Escherichia coli* ATCC 25922 served as the negative control (Ng & Rivera 2015). A no template control (NTC) was used to check for PCR master mix contamination with nuclease-free water to substitute the DNA template. The details of the primer sequence, amplicon size, PCR protocols, and references for the *invA* gene for *S. enterica* detection are shown in Table 1.

Multiplex PCR of *bla* Genes

invA-positive (confirmed *Salmonella*) DNA samples were subjected to PCR-based bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} detection. The volumes and concentrations of MyTaq™ HS Red Mix (Bioline, London, UK), forward and reverse primers, and nuclease-free water were the same as those for the *invA* gene. The details of the primer sequence, amplicon size, PCR protocols, and references for the *bla* genes are also shown in Table 1. For bla_{TEM} and $bla_{CTX\text{-}M}$, the positive controls were *S. enterica* isolates from the Pathogen-Host-Environment Interactions Research Laboratory (PHEIRL) that tested positive for these genes (Calayag *et al.* 2021) and the bla_{SHV}-positive control was *Klebsiella pneumoniae* ATCC 700603. The negative control for all *bla* genes was *E. coli* ATCC 25922 (Pitout *et al.* 2004). An NTC was also used to check for PCR master mix contamination. PCR products were then subjected to agarose gel electrophoresis (AGE).

PCR detection of $bla_{CTX\text{-}M}$ gene groups

Singleplex PCR was performed for $bla_{CTX\text{-}M}$ groups I, II, and IV because of the similarity in their molecular weight. Each PCR reaction consisted of 1 μL of previously extracted DNA as the template, 6.25 μL of GoTaq® G2 Green Master Mix, 0.5 μL each of 10 μM forward and reverse primers, and 4.25 μL of nuclease-free water. The details of the primer sequence, amplicon size, PCR protocols, and references for $bla_{CTX\text{-}M}$ groups are shown in Table 1. Positive controls were isolates that show positive result in PCR amplification and sequenced for confirmation, while *E. coli* ATCC 25922 served as the negative control. An NTC was also used to check for PCR master mix contamination. PCR products underwent AGE to visualize results. To confirm PCR results, representative amplicons from each $bla_{CTX\text{-}M}$ group underwent Sanger sequencing (Macrogen, Inc., South Korea). Sequence trimming and alignment were performed using BioEdit v 7.2.5 and MEGA v 11.0.13, respectively. The identities of the nucleotide sequences were confirmed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST).

Agarose Gel Electrophoresis

PCR products were visualized through AGE in 2% (w/v) agarose (Vivantis, Malaysia) stained with GelRed® Nucleic Acid Gel Stain (Biotium, CA, USA). Amplicons were separated under 280 V for 30-40 minutes. PCR product molecular weight was estimated using HyperLadder™ 100bp (Bioline, Meridian Bioscience, London, UK).

Table 1 Primers, product size, PCR conditions, and references of *invA* and *bla* genes

Gene	Primers (5′–3′)	Product size (bp)	PCR condition	Reference
bla$_{CTX-M}$	F: ATG TGC AGY ACC AGT AAR GTK ATG GC R: TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	Initial denaturation: 95 °C (3 minutes) 30 cycles denaturation: 95 °C (30 seconds) Annealing: 55 °C (30 seconds) Extension: 72 °C (1 minute) Final extension: 72 °C (10 minutes)	Monstein *et al.* (2007)
bla$_{TEM}$	F: TCG CCG CAT ACA CTA TTC TCA GAA TGA R: ACG CTC ACC GGC TCC AGA TTT AT	445		
bla$_{SHV}$	F: ATG CGT TAT ATT CGC CTG TG R: TGC TTT GTT ATT CGG GCC AA	747		
bla$_{CTX-M}$ group I	F: GAC GAT GTC ACT GGC TGA GC R: AGC CGC CGA CGC TAA TAC A	499	Initial denaturation: 96 °C (3 minutes) 30 cycles denaturation: 96 °C (30 seconds) Annealing: 56 °C (30 seconds) Extension: 72 °C (1 minute) Final extension: 72 °C (10 minutes)	*Pitout et al.* (2004)
bla$_{CTX-M}$ group II	F: GCG ACC TGG TTA ACT ACA ATC C R: CGG TAG TAT TGC CCT TAA GCC	351		
bla$_{CTX-M}$ group IV	F: GCT GGA GAA AAG CAG CGG AG R: GTA AGC TGA CGC AAC GTC TG	474	Initial denaturation: 96 °C (3 minutes) 30 cycles denaturation: 96 °C (30 seconds) Annealing: 60 °C (30 seconds) Extension: 72 °C (1 minute) Final extension: 72 °C (10 minutes)	
invA	F: ACA GTG CTC GTT TAC GAC CTG AAT R: AGA CGA CTG GTA CTG ATC GAT AAT	244	Initial denaturation: 95 °C (2 minutes) 30 cycles denaturation: 95 °C (30 seconds) Annealing: 60 °C (30 seconds) Extension: 72 °C (30 seconds) Final extension: 72 °C (5 minutes)	Chiu & Ou (1996)

Antimicrobial Susceptibility Tests

The Vitek® 2 Compact 60 ID/AST System (AST-GN70 card panel, bioMérieux, Marcy-l'Étoile, France) was used to generate antimicrobial susceptibility profiles of 22 isolates to 16 antimicrobial agents including ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, tigecycline, nitrofurantoin, and trimethoprim/sulfamethoxazole and an ESBL test. The ESBL test included the following antimicrobials alone and in combination with clavulanic acid: cefepime, ceftriaxone, and ceftazidime. Preparation of isolates for Vitek® 2 followed standard procedures (Calayag *et al.* 2021). An isolate was considered multidrug-resistant (MDR) when it displayed nonsusceptibility to at least one antimicrobial agent in three or more antimicrobial categories (Magiorakos *et al.* 2012). Interpretive criteria and breakpoints were based on the Clinical and Laboratory Standards Institute (CLSI) (2022) 32nd Edition. The negative control used was *S. enterica* ATCC 25241.

RESULTS AND DISCUSSION

The occurrence of *bla* genes in *S. enterica* from wet market chicken samples across the five Metro Manila cities is summarized in Table 2. None of those isolates showed positive results for bla_{SHV} gene. The occurrence of bla_{TEM} and $bla_{CTX\text{-}M}$ genes in the tested isolates were 10 (11.24%) and 22 (24.72%), respectively. The highest occurrence for bla_{TEM} was found among the isolates sampled in Valenzuela City, while for $bla_{CTX\text{-}M}$, isolates sampled from Quezon City showed the highest occurrence.

The $bla_{CTX\text{-}M}$ gene showed the highest prevalence among isolates in this study, which contrasted with some studies among *Enterobacteriaceae*. Most studies reported bla_{TEM} as the most predominant *bla* gene over $bla_{CTX\text{-}M}$ and bla_{SHV} among *Salmonella* isolated from raw poultry meat and fecal samples from diarrheic children (Coculescu *et al.* 2015; Wu *et al.* 2015; Ghazaei 2018; Sales *et al.* 2021). In the Philippines, Cruz & Hedreyda (2017) showed the occurrence of bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} in β-lactam-resistant clinical *E. coli* isolates at 56.3%, 18.3%, and 11.3%, respectively. Although the frequency estimates of bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ exist for *E. coli*, the surveillance of the relative frequencies of these genes within the Philippines is limited among *Salmonella* isolates. Calayag *et al.* (2021) estimated the frequency of *bla* genes in *Salmonella* among hog tonsils and jejunum isolates from slaughterhouses in Metro Manila, wherein bla_{TEM} was detected with the highest frequency, followed by $bla_{CTX\text{-}M}$. However, the only detected $bla_{CTX\text{-}M}$ subtypes were $bla_{CTX\text{-}M\text{-}1}$ from group I and $bla_{CTX\text{-}M\text{-}2}$ from group II (Calayag *et al.* 2021). This shows that the occurrence of *bla* genes varies across different geographical areas and sample sources. There are also some studies on other *Enterobacteriaceae* that were consistent with this study. Gundran *et al.* (2019) showed frequencies of 89.9%, 58.0%, and 27.5%, respectively for $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes, among *E. coli* from poultry. Similarly, Li *et al.* (2016) reported *E. coli* isolates from chicken fecal samples and showed that 88.8%, 66.3%, and 3.1% tested positive for $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV}, respectively. These results suggested the high cephalosporin resistance potential of *Salmonella* isolated from retail poultry in Metro Manila.

Upon detection of $bla_{CTX\text{-}M}$ groups I, II, and IV genes, all 22 $bla_{CTX\text{-}M}$-positive isolates were simultaneously positive for $bla_{CTX\text{-}M}$ groups II and IV. Interestingly, none of the tested samples were positive for $bla_{CTX\text{-}M}$ group I. The co-carriage of these $bla_{CTX\text{-}M}$ gene groups had been previously reported and was consistent with findings in other studies (He *et al.* 2013; Li *et al.* 2016; Gundran *et al.* 2019). To confirm the $bla_{CTX\text{-}M}$ group gene identities, PCR products of DNA templates of a $bla_{CTX\text{-}M}$ group I positive control and representative *Salmonella* isolates from $bla_{CTX\text{-}M}$ groups II and IV underwent Sanger sequencing. The BLAST results from the aligned DNA sequences of representative bacterial isolates corresponded to the established sequences of $bla_{CTX\text{-}M}$ groups I, II, and IV, confirming the gene identities. The presence of $bla_{CTX\text{-}M}$ groups II and IV suggested higher resistance potential to cephalosporins which was confirmed by checking the antimicrobial susceptibility of these isolates.

For phenotypic resistance, the $bla_{CTX\text{-}M}$-positive isolates were subjected to VITEK® 2 automated susceptibility testing. Resistance profiles of *Salmonella* carrying $bla_{CTX\text{-}M}$ to several antimicrobial agents are shown in Table 3. All isolates (100%) displayed resistance to both ampicillin and nitrofurantoin. Meanwhile, 21 isolates (95.5%) were resistant to a non-extended spectrum cephalosporin (NESC) and an ESC. While ESC-resistant isolates expressed nonsusceptibility to a third generation cephalosporin (ceftriaxone), they remained susceptible to a fourth generation

cephalosporin (cefepime). The 21 isolates also displayed resistance to a monobactam and two aminoglycoside class antibiotics. Interestingly, one (4.5%) $bla_{CTX\text{-}M}$-positive isolate did not display resistance to either NESC or ESC antibiotic classes; however, it was resistant to ampicillin with sulbactam, a β-lactamase inhibitor, suggesting other β-lactam resistance mechanisms. Resistance rates of isolates subjected to VITEK® 2 in this study were comparable to the study of Calayag *et al.* (2017) for ampicillin, nitrofurantoin, cefazolin, gentamicin, and tobramycin. In the study of Calayag *et al.* (2017), 70.5% of isolates are nonsusceptible to ampicillin, 93.4% to nitrofurantoin, and 100% to cefazolin, gentamicin, and tobramycin. However, the current study showed higher rates for $bla_{CTX\text{-}M}$ gene with variations in $bla_{CTX\text{-}M}$ gene groups detected.

Corroboration of phenotypic and genotypic resistance was observed in 21 $bla_{CTX\text{-}M}$-positive isolates that displayed resistance to the NESC and ESC antibiotic classes. This was not the case for one isolate that remained susceptible to NESC and ESC. This could suggest that the one isolate susceptible to NESC and ESC is carrying silent copies of $bla_{CTX\text{-}M}$ (Cruz & Hedreyda 2017; Calayag *et al.* 2021). A mutation in the structural gene or regulatory region could prevent the expression of the $bla_{CTX\text{-}M}$, which may lead to the inactivation of phenotypic resistance (Cruz & Hedreyda 2017). Another mechanism for gene silencing is the expression of silencing proteins that interfere with the transcription of the AMR genes. However, gene silencing can be conditional wherein they can be activated depending on the culture media and the presence of antimicrobial selection pressure (Deekshit & Srikumar 2022). In this case, it is possible that the $bla_{CTX\text{-}M}$ genes of one isolate remained silent despite the individual administration of NESC and ESC antimicrobials. Additionally, the ESBL phenotype of this isolate could have been caused by other ESBL genes instead of the detected $bla_{CTX\text{-}M}$ genes.

Shi *et al.* (2021) claimed that $bla_{CTX\text{-}M}$ groups I and IV play roles in ceftriaxone resistance mechanisms. In their study, 64.2% CTX-M-producing isolates that were resistant to ceftriaxone carried $bla_{CTX\text{-}M}$ group I genes, while 35.8% carried $bla_{CTX\text{-}M}$ group IV genes (Shi *et al.* 2021). Although excessive antibiotic use is one of the reasons attributed to the dissemination of $bla_{CTX\text{-}M}$ (Cantón 2007; Cantón *et al.* 2012), it cannot be concluded that excessive use of ceftriaxone was the main reason for the dominance of $bla_{CTX\text{-}M}$ genes in this study. Since cephalosporins are not often used in Philippine poultry farms (Barroga *et al.* 2020), the high occurrence of $bla_{CTX\text{-}M}$ genes warrants further investigation. However, the high resistance rates of the isolates to aminoglycosides and nitrofuran may be associated with excessive antimicrobial usage because these antibiotic classes are among the most utilized antibiotics in Philippine poultry farms alongside fluoroquinolones and tetracyclines (Imperial *et al.* 2022). The predominance of $bla_{CTX\text{-}M}$ may also be due to the mobilization of $bla_{CTX\text{-}M}$ genes and co-selection through resistance to other antibiotics (Cantón 2007; Cantón *et al.* 2012).

ESC-resistant isolates may acquire AMR through horizontal gene transfer from abiotic surfaces due to the prolonged survival of bacteria harboring AMR genes (Warnes *et al.* 2012; Imperial *et al.* 2022). Warnes *et al.* (2012) showed that cefotaxime-sensitive *E. coli* acquired a $bla_{CTX\text{-}M}$ group I gene via horizontal gene transfer on a stainless-steel surface. Imperial *et al*. (2022) documented the acquisition of a gene that encodes resistance for macrolides, lincosamides, streptogramin B, and oxazolidinones (*ermB*) in bacteria from chicken fecal samples where the chicken host was not subjected to any antibiotic treatments. Hence, the acquisition of the AMR gene via horizontal gene transfer can be attributed to contamination of surfaces during feeding, cage cleaning, and animal handling.

Although $bla_{CTX\text{-}M}$ group I and IV have been considered as the most common groups of $bla_{CTX\text{-}M}$ genes (Cantón *et al.* 2012), this study reported the absence of $bla_{CTX\text{-}M}$ group I, while $bla_{CTX\text{-}M}$ groups II and IV have the highest occurrences. While the absence of $bla_{CTX\text{-}M}$ group I requires further investigation, it is plausible that plasmids and insertion sequences (IS) played a role in the spread and co-existence of $bla_{CTX\text{-}M}$ groups II and IV. Specifically, IS*Ecp1* and IS*903B* are associated with $bla_{CTX\text{-}M}$ group IV, while the IS*CR1* element is associated with $bla_{CTX\text{-}M}$ groups II and IV (Cantón 2007; Cantón *et al.* 2012; Ferreira *et al.* 2014; Nguyen *et al.* 2021; Shi *et al.* 2021). Moreover, genes under these $bla_{CTX\text{-}M}$ groups II and IV might recombine and produce novel β-lactamases as in the case of $bla_{CTX\text{-}M\text{-}123}$, which is a hybrid of group I $bla_{CTX\text{-}M\text{-}15}$ and group IV $bla_{CTX\text{-}M\text{-}14}$ (He *et al.* 2013). This could eventually lead to more β-lactamases that can counter cephalosporins and render antibiotic treatment ineffective.

Additionally, carrying additional AMR genes that encode the resistance to fluoroquinolones and aminoglycosides of bla_{CTX-M}-carrying bacteria favors their survival, which may contribute to the spread of bla_{CTX-M} (Cantón 2007). Co-carriage of bla_{CTX-M}, bla_{TEM}, and a quinolone resistance gene (*qnr*) were observed in *S. enterica* isolates of Calayag *et al.* (2021). Unfortunately, the coexistence of different AMR genes in a bacterial isolate may lead to MDR. Plasmids that harbor ESBL genes may also carry genes encoding resistance for aminoglycosides, trimethoprim, sulfonamides, tetracycline, and chloramphenicol (Paterson 2000; Shi *et al.* 2021). This is important considering that MDR is observed in all bla_{CTX-M}-positive *S. enterica* isolates where each isolate is resistant to at least six antimicrobial classes. However, this study is limited by not detecting other AMR gene classes. The MDR profile of isolates is shown in Table 4. Among the 22 bla_{CTX-M} positive isolates, 18 (81.8%) were also resistant to six antibiotic classes. Meanwhile, three isolates (13.6%) were resistant to seven antibiotic classes and one (4.5%) was resistant to eight antibiotic classes. Alarmingly, all isolates displayed ESBL phenotypes.

Due to the high occurrences of *bla* genes and MDR among *S. enterica* isolates, it is important to promote national surveillance of AMR and antimicrobial use in the agricultural and veterinary sectors to combat AMR. Although regulations regarding the sale, distribution, and prescription of antibiotics for animal use exist in the Philippines, there is a weak implementation of standards for veterinary medicinal products and enforcement of animal antibiotic use policies (Barroga *et al.* 2020; Imperial *et al.* 2022). As a result, there may be a discrepancy between the declared use of antibiotics in animals and the actual use of antimicrobials in farms (Imperial *et al.* 2022). For this reason, research in phenotypic and genotypic AMR and antimicrobial use in farms is highly critical to assess the current situation of AMR in the Philippines. Doing so will aid in identifying MDR *S. enterica* in poultry and implementing policies that will curb the spread of AMR and MDR. Continuing surveillance of AMR genes can aid in policymaking that will counter the spread of AMR and, hopefully, prevent cases of invasive *Salmonella* infections that persist despite antimicrobial treatments.

Table 2 Occurrence of bla_{TEM}, bla_{CTX-M}, and bla_{SHV} among *S. enterica* isolates obtained from different markets of Metro Manila

City	No. of *S. enterica* isolates	bla_{TEM}	bla_{CTX-M}	bla_{SHV}
Quezon	16	0	7 (43.8%)	0
Manila	16	2 (12.5%)	3 (18.8%)	0
Pasay	18	0	7 (38.9%)	0
Malabon	20	4 (20.0%)	0	0
Valenzuela	19	4 (21.1%)	5 (26.3%)	0
Total	89	10 (11.2%)	22 (24.7%)	0

Table 3 Nonsusceptibility levels of 22 *bla*$_{CTX-M}$-positive *S. enterica* isolates against different antimicrobial agents

Class	Antimicrobial	% Nonsusceptibility
Penicillin	Ampicillin	100
Penicillin/β-lactamase inhibitor	Ampicillin/sulbactam	9.1
Antipseudomonal penicillin/β-lactamase inhibitor	Pipercillin/Tazobactam	0
Non-extended spectrum cephalosporin	Cefazolin	95.5
Extended-spectrum cephalosporin	Ceftriaxone	95.5
	Cefepime	0
Monobactam	Aztreonam	95.5
Carbapenem	Ertapenem	0
	Meropenem	0
Aminoglycoside	Amikacin	0
	Gentamicin	95.5
	Tobramycin	95.5
Fluoroquinolone	Ciprofloxacin	13.6
Glycycline	Tigecycline	4.5
Nitrofuran	Nitrofurantoin	100
Folate pathway inhibitor	Trimethoprim/ Sulfamethoxazole	13.6

Table 4 Multidrug resistance patterns of 22 *bla*$_{CTX-M}$-positive *S. enterica* isolates

Multidrug resistance pattern*	Number of isolates
Pen, Pen/BI, NESC, ESC, Mon, Ami, Flu, Nit	1
Pen, NESC, ESC, Mon, Ami, Flu, Nit	1
Pen, NESC, ESC, Mon, Ami, Nit, FPI	2
Pen, Pen/BI, Flu, Gly, Nit, FPI	1
Pen, NESC, ESC, Mon, Ami, Nit	17

Notes: *Pen = penicillin; Pen/BI = penicillin/β-lactamase inhibitor; NESC = non-extended Spectrum cephalosporin; ESC = extended-spectrum cephalosporin; Mon = monobactam; Ami = aminoglycoside; Flu = fluoroquinolone; Gly = glycoside; Nit = nitrofuran; FPI = folate pathway inhibitor

CONCLUSION

This study reported the predominance of *bla*$_{CTX-M}$ and the coexistence of *bla*$_{CTX-M}$ groups II and IV in all *bla*$_{CTX-M}$ positive *S. enterica* isolates. These suggest high cephalosporin resistance potential. Aside from the corroboration of phenotypic and genotypic resistance among *bla*$_{CTX-M}$-positive isolates, MDR was also observed with ESBL phenotypes detected in all isolates. Given the high occurrence of *bla* genes, the co-existence of two *bla*$_{CTX-M}$ gene groups, and high phenotypic resistance among *bla*$_{CTX-M}$ positive *S. enterica* isolates, the continued surveillance of *bla* and other AMR genes and phenotypic resistances are thus crucial to monitor the extent and dissemination of resistance and combat their emergence and spread through policy recommendations and regulations.

ACKNOWLEDGMENTS

We thank the Department of Agriculture, Biotechnology Program Office, Philippines (Project No. DABIOTECH-R2302) for the financial support. We also acknowledge Vanessa Yvonne B. Meclat for the technical assistance. We also state that Miles G. Madayag and Rance Derrick N. Pavon contributed equally to this work.

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