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Antimicrobial Resistance of *Escherichia coli* in Urban Gardens and Wet Markets in Two Selected Cities in Metro Manila, Philippines

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Abstract

Urban gardening defined as growing and harvesting in an urban environment has become apparent in times of the COVID-19 pandemic. However, this unique ecosystem promotes proliferation of antibiotic resistant bacteria (ARB). Thus, this study aims to check the impact of antibiotic resistance due to anthropogenic activities in agricultural setting within Metro Manila, Philippines. Using molecular analysis, results showed presence of *Escherichia coli* in 32 out of 117 isolates from urban gardens and 41 of 348 isolates from wet markets. Confirmed *E. coli* isolates were tested against antibiotics within the fluoroquinolone, aminoglycoside, penicillin, tetracycline, quinolone, and sulfonamide groups. It was found that urban garden isolates had high percentage of resistance to ampicillin (46.9%) and tetracycline (68.8%), while wet market isolates had 56.1% ampicillin and 31.7% tetracycline resistance. Multidrug resistance (MDR) was detected in 13 out of 32 urban gardens isolates (41%) and 8 out of 41 (20%) wet markets isolates. Except for tetracycline, Mann-Whitney U and Spearman tests revealed shared antibiotic resistance patterns among isolates, an indication that urbanization and its anthropogenic activities may indirectly increase antibiotic resistance.

Keywords: Antibiotics; Antimicrobial resistance; *Escherichia coli;* Urban gardens; Wet markets

1. Introduction

Antibiotics have been used to either kill or halt bacterial infections in many different fields, including public health, industry, and agriculture. They have been a vital part of our lives since their discovery; however, with the rapid emergence of antimicrobial resistant bacteria (ARB), and with the influence of natural (Darwinian) selection in the evolution of resistance (Baquero *et al.,* 2009), antibiotics are becoming less effective against bacteria. Another contributing factor to this emerging problem is the overuse and misuse of these medications. Several measures, such as rational use of antibiotics need to be implemented to mitigate resistant bacteria (Mutagonda *et al.,* 2022). Likewise, the lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Gould *et al.,* 2013) also contributes to the emergence of resistant strain bacteria.

With the increase of ARB, sources of these bacteria are commonly found in the environment, and because antibiotics are not completely absorbed, they settle in excrement and are used as fertilizer in the soil. This in turn exposes other bacteria in the environment to the absorbed antibiotic and promotes mutation against it. As a result, faecal contamination in the environment is being extensively researched and a common indicator is *Escherichia coli*. *E. coli* is a rod-shaped, Gram-negative bacterium, belonging to the family Enterobactericeae,

with primary habitat in the lower intestine of warm-blooded animals, including humans (Savageau *et al.,* 1983). This makes the bacterium an indicator of faecal contamination in the environment as it has become "naturalized" members of soil communities (Ishii *et al.,* 2008).

As microorganisms naturally produce and secrete antibiotic compounds (Geetanjali *et al.,* 2016), it only takes a single bacterial cell with a genetic or mutational change that confers resistance to cause subsequent proliferation of ARB (Pruden *et al.,* 2006). While the environment serves as both the source (Forsberg *et al.,* 2012; Berendonk *et al.,* 2015; Hu *et al.,* 2018) and potential dissemination route of antimicrobial resistance genes (ARG) (Martinez, 2009; Bengtsson-Palme *et al.,* 2018), contact with human pathogens increases the rate of mutation of these environmental bacteria. One significant example is antibiotic residues as well as ARB and ARG found in hospital effluents which make their way to wastewater treatment plants (Harris *et al.,* 2012) and may contribute to increased ARB in the environment through time. In relation to this, anthropogenic activities such as urban gardening promotes this kind of typical environment. Defined as growing and harvesting produce in an urban environment, this environment is a unique ecosystem with particular biological, chemical, and physical properties different from any natural environment and is characterized by its close relationships with public health and daily life (Su *et al.,* 2017; Li *et al.,* 2018). Part of this activity is irrigation with reclaimed water in urban environment, (Li *et al.,* 2018) and with antibiotics existing in reclaimed water or sewage sludge, this could increase antibiotic resistance in the environment (Pei *et al.,* 2006; Pruden *et al.,* 2013; Su *et al.,* 2014; Xie *et al.,* 2018). Furthermore, relatively small concentration of heavy metals might be sufficient to induce antibiotic resistance through co-selection (Mulder *et al.,* 2011), which urban soil is very rich of.

Urban gardening has become popular especially during COVID-19 pandemic. In the Philippines, there has been a proliferation of home gardens during the pandemic, making some observers believe that this trend could continue after the pandemic and enhance food security to the community (Montefrio *et al.,* 2020), as food insecurity arose due to income disruption, prompting the demand for food aid from the government.

In addition to this, open air markets remain a possible source of ARB in the country. According to Vital *et al* (2017), a greater percentage of antibiotic resistance was found among open air markets than supermarkets. Factors that can contribute to this are poor sanitation practices involved in packaging, washing, cleaning, and other post-harvest practices prior to selling (Abakpa *et al.,* 2015).

For this study, various samples (fresh produce, feces, water, and soil) were collected from urban gardens and wet markets within Metro Manila, Philippines (particularly in Quezon City and Manila City). Samples were processed to detect the presence of *Escherichia coli* using culture and molecular techniques. Antibiotic resistance was tested through disk diffusion assay using six antibiotics. Statistical analyses were performed to check for relationships among antibiotic groups and if anthropogenic activities between the sampling groups have an impact to antibiotic resistance.

2. Materials and methods

2.1 Sampling sites and sample processing

The sampling areas are shown in Figure 1. Various urban garden samples were collected in different locations in Quezon City, Metro Manila. These sites were selected and contacted through social media or by recommendations; those who replied to the inquiries were the ones chosen as sampling sites: these include the Krunali urban garden, Botocan urban garden, and New Greenlands urban garden (Figure 2). Random species of vegetables were collected depending on the availability in the sampling sites. For each vegetable species, three samples were obtained from one plot. 71 fresh produce, 11 faecal,

17 water, and 18 soil samples were collected (Table 1). For wet markets, samples were collected from large wet markets within Quezon City and Manila City. These were the sites where agricultural products from provinces and urban gardens are dropped off to be sold. These included the Nepa Q market, Farmer's market, Balintawak market in Quezon City, and Blumentritt market in Manila City. Various sets of fresh produce (Table 2) were collected from all wet markets; the quantity of fresh produce per wet market was based on the availability of the samples within the location, with a maximum of 12 per sample type.

All samples were collected using sterile containers, placed in an ice box, and processed within 3 hours after collection. Each fresh produce was cut or shredded by approximately 25 grams and 30 mL of 0.1% buffered peptone water (BD, Germany) was added, followed by vigorous shaking for at least 30 seconds. The solution was then subjected to 10-fold serial dilutions; with the last 2 dilutions filtered separately using a 0.45 µm membrane filter and plated on modified mTEC agar (Millipore, USA). The plates were incubated at 37 °C for 2 hours and then at 44.5 °C for 22 hours. After incubation, deep violet colonies were considered presumptive *E. coli*. Presumptive *E. coli* colonies were also plated on Eosin Methylene Blue (EMB) (BD, Germany) plates and incubated at 37 °C for 24 hours; colonies with a green metallic sheen and black colonies were further considered presumptive of *E. coli.* Colonies were stored in Tryptic Soy Broth (TSB) with 50% glycerol in a -20 °C freezer until further molecular processing.

2.2 Revival and culture of previously isolated samples

Previously isolated samples were stored in -20 °C freezer were thawed and inoculated onto TSB and incubated for 24 hours at 37 °C. Following incubation, samples were inoculated on Eosin Methylene Blue Agar (EMB) (BD, Germany) and incubated for another 24 hours. Green metallic sheen or black colonies on EMB presumably indicate *E. coli* colonies.

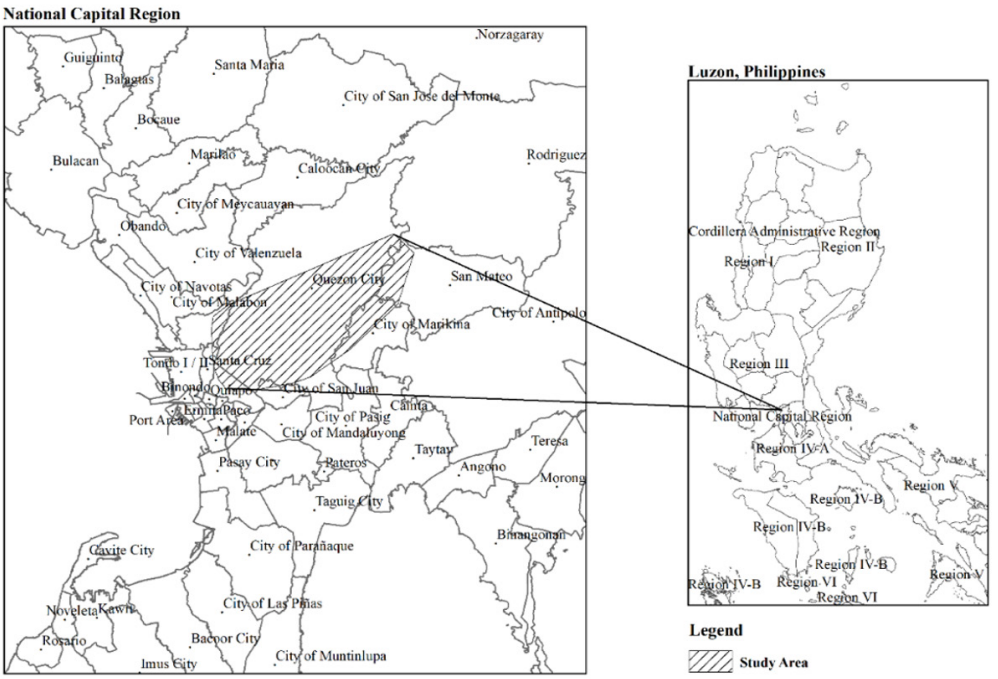

Figure 1. Map showing the study area where fresh produce, fecal, water, and soil samples were collected in urban gardens located in Quezon City and wet markets located in Quezon City and Manila City, National Capital Region, Philippines

Figure 2. Representative sampling locations for the collection of different samples to detect antibiotic resistant *Escherichia coli* in urban farms in Metro Manila, Philippines. (A) New Greenlands Urban Garden, Quezon City; (B) Botocan Urban Garden, Quezon City; (C) Krunali Urban Garden, Quezon City

Table 1. Samples collected from three urban gardens in Metro Manila, Philippines

Sampling sites	Sample types				
	Fresh Produce	Feces	Water	Soil	Total
Botocan	21	5	6	6	38
Krunali	20	6	5	6	37
New Greenland	30	0	6	6	42
Total	71	11	17	18	117

Table 2. Various fresh produce collected from the three large wet markets within Metro Manila, Philippines

Sampling sites	Vegetables	No. of samples collected
Nepa Q Market	Lettuce	9
	Cucumber	10
	Snow Cabbage	9
	Tomato	11
	Carrot	10
	Cabbage	9
	Spring Onion	10
	Water Spinach	10
	Sweet Potato Leaves	10
Blumentritt Market	Lettuce	8
	Cucumber	12
	Snow Cabbage	9
	Tomato	11
	Carrot	11
	Cabbage	10
	Spring Onion	11
	Water Spinach	10
	Sweet Potato Leaves	6
Balintawak Market	Lettuce	9
	Cucumber	10
	Snow Cabbage	9
	Tomato	8
	Carrot	11
	Cabbage	10
	Spring Onion	10
	Water Spinach	9
	Sweet Potato Leaves	8
Farmers Market	Lettuce	11
	Cucumber	10
	Snow Cabbage	10
	Tomato	10
	Carrot	10
	Cabbage	9
	Spring Onion	10
	Water Spinach	10
	Sweet Potato Leaves	8
Total		348

2.3 DNA Extraction and Escherichia coli confirmation

Molecular confirmation of *Escherichia coli* isolates was done by amplifying and detecting the 75-bp *uidA* gene that encodes for β-D-glucuronidase, an enzyme produced by 94 – 96% of *E. coli* strains (Perin *et al.,* 2010). Bacterial colonies were prepared for DNA extraction using a commercially available DNA extraction kit (G-Spin Genomic DNA extraction kit, iNtRON Bio, South Korea), following the manufacturers' instructions and guidelines. The resulting DNA extracts were then subjected to polymerase chain reaction using the MiniAmp Plus (ThermoFisher, USA) thermocycler. Primers that were used were adapted from Takahashi *et al.* (2009), with forward primer ECN 1254F (GCAAGGTGCACGGGAATATT), and ECN 1328R (CAGGTGATCGGACGCGT). PCR mix contain 6.25 µL of GoTaq Green Mastermix (Promega, USA), 0.5 µL of each 1 mM

of forward and reverse primers (Oligo, Macrogen, South Korea), 0.75 µL of DNA template, and 4.25 µL of nuclease free water, with a total of 12.25 µl reaction mix. The reaction mix was amplified with PCR condition of initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 15 seconds, annealing at 55 °C for 1 min, extension at 74 °C for 1 min, and with a final extension of 74 °C for 5 mins. The resulting amplicons were visualized using 3% agarose gel, set at 100v for 45 mins with 50 bp DNA ladder as reference.

2.4 Antibiotic Susceptibility Testing

Isolates that were molecularly confirmed to be *E. coli* were subjected to disk diffusion susceptibility testing. For preparation, the colonies were inoculated into tubes containing 3 mL of 0.85% NaCl, and turbidity was checked using the McFarland 0.5 standard as a reference. The tubes containing the inoculum were then spread evenly using a sterile cotton swab on Mueller-Hinton Agar (MHA) (HiMedia, India) plates; and done in triplicates. After spreading, commercially prepared antibiotic discs (Oxoid™, Bioanalyse™) were placed on the plates; antibiotics used include ampicillin, ciprofloxacin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole; all plates were incubated at 37 °C for 24 hours (Figure 3). A digital caliper was used to measure the zone of inhibition, and the measurement was checked using the CLSI supplement M100 (Wayne, PA: Clinical and Laboratory Standards Institute; 2020) to determine if the values fall under the resistant, intermediate, or susceptible category (Table 3). *E. coli* ATCC 25922 was used as a control strain. A reference range of the quality control of antibiotics can be seen in Table 4.

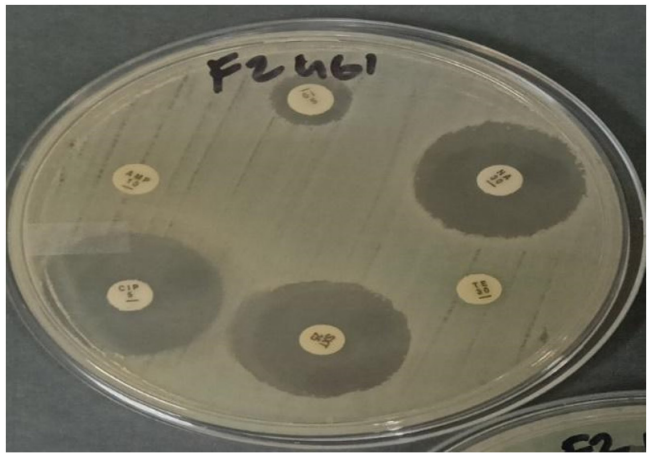

Figure 3. Representative antibiotic susceptibility result of previously isolated *Escherichia coli* colonies. Zone of inhibition indicates effectiveness of the antibiotic. A digital caliper was used to determine the exact measurement of these zones

Table 3. Antibiotic disc interpretative zone sizes in (mm) for Enterobacteriaceae (adapted from CLSI Supplement M100)

Antibiotic	Susceptible	Intermediate	Resistant
Ampicillin	≥ 17	14 - 16	≤ 13
Ciprofloxacin	≥ 26	22 - 25	≤ 21
Nalidixic Acid	≥ 19	14 - 18	≤ 13
Trimethoprim-sulfamethoxazole	≥ 16	11 - 15	≤ 10
Streptomycin	≥ 15	12 - 14	≤ 11
Tetracycline	≥ 15	12 - 14	≤ 11

2.5 Statistical Analysis

To determine significant differences between and among the antibiotic resistant isolates collected from urban gardens and wet markets, Mann-Whitney U and Spearman tests were used. All analyses were performed using Jamovi 2.3.21 software.

3. Results and discussion

3.1 Culture and molecular identification of Escherichia coli

A total of 32 urban garden samples and 41 wet market samples showed black or green metallic sheen on their respective EMB plates (Figure 4). Isolates were confirmed through polymerase chain reaction and gel electrophoresis of the *uidA* gene (Figure 5).

3.2 Prevalence of molecularly confirmed Escherichia coli in urban gardens

117 samples were collected from the three urban gardens for the study, 32 (26%) were molecularly confirmed as *E. coli* (Table 5). Samples from Krunali urban garden had the highest number of *E. coli* positive samples, with 18 out of 37 (49%). This consisted of 7 out of 20 fresh produce (35%), 6 out of 6 fecal samples (100%), 3 out of 5 water samples (60%), and 2 out of 6 soil samples (33%). This was followed by samples from Botocan urban garden with 12 out of 38 samples (32%) being molecularly positive with *E. coli*. Specifically, these were 6 out of 21 fresh produce samples, 4 out of 5 fecal samples (80%), and 2 out of 6 water samples (33%). No positive *E. coli* results was obtained in soil samples collected from Botocan urban garden. Samples from New Greenland urban garden had the lowest prevalence of *E. coli* with only a total of 2 out 42 samples (5%) molecularly confirmed as positive, comprising of two fresh produce samples.

Table 4. Reference range for quality control for antibiotics using ATCC 25922 *E. coli*

Antibiotic	Disk Content	Reference range for ATCC® 25922
Ampicillin	10 µg	15-22 mm
Ciprofloxacin	5 µg	29-38 mm
Nalidixic Acid	30 µg	22-28 mm
Trimethoprim-sulfamethoxazole	1.25 / 23.75 µg	23-29 mm
Streptomycin	10 µg	12-20 mm
Tetracycline	30 µg	18-25 mm

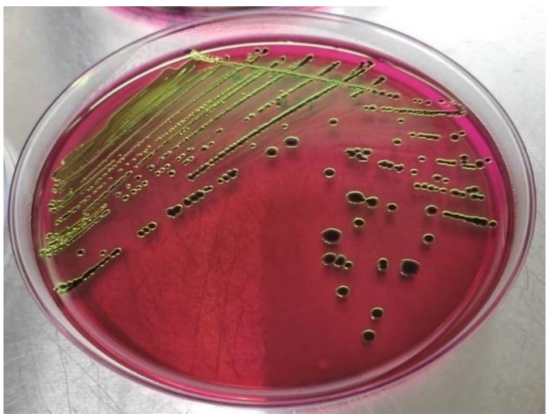

Figure 4. Representative urban garden isolate showing the green metallic sheen on EMB, indicating presumptive *Escherichia coli* colonies

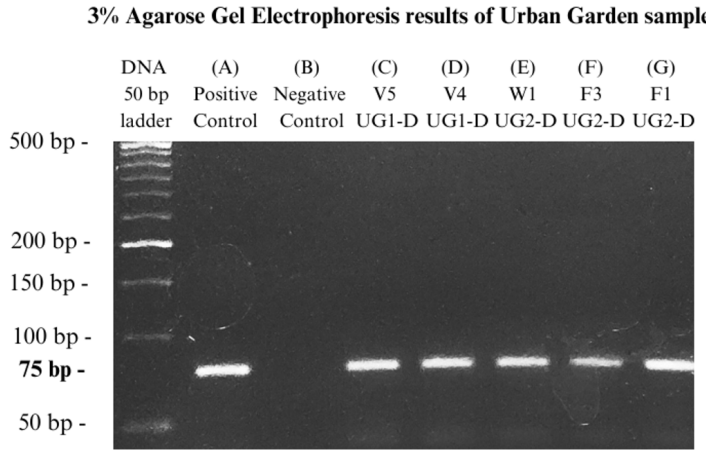

Figure 5. Electrophoresis results of samples collected from urban gardens showing amplification of the 75 - bp *uidA* gene after polymerase chain reaction to confirm *Escherichia coli.* (A) positive control strain of *Escherichia coli*, (B) no template control, (C) water spinach sample and (D) taro sample from Botocan Urban Garden, (E) water pump sample, (F) goat faecal sample and (G) chicken faecal sample from Krunali Urban Garden

Table 5. Number of molecularly positive *Escherichia coli* collected from urban gardens in Metro Manila, Philippines

Sampling sites	Fresh Produces	Feces	Water	Soil	Total
Krunali	7 / 20 (35 %)	6 / 6 (100%)	3 / 5 (60%)	2 / 6 (33%)	18 / 37 (49%)
Botocan	6 / 21 (29%)	4 / 5 (80%)	2 / 6 (33%)	0 / 6 (0%)	12 / 38 (32%)
New Greenland	2 / 30 (7%)	0 / 0 (0%)	0 / 6 (0%)	0 / 6 (0%)	2 / 42 (5%)
Total	15 / 71 (21%)	10 / 11 (91%)	5 / 17 (29%)	2 / 18 (11%)	32 / 117 (27%)

3.3 Prevalence of molecularly confirmed Escherichia coli in four wet markets

348 fresh produce samples collected from four of the wet markets selected, 41 (12%) were positive for *uidA* gene, confirming *E. coli*, as shown in Table 6. Samples from Blumentritt market had the highest prevalence of *E. coli* with 13 out of 88 (15%), followed by Nepa Q market with 11 out of 88 (13%), Balintawak market with 9 out of 84 (11%), and lastly Farmers market with 9 out of 88 (10%).

3.4 Antibiotic Susceptibility Results

Isolates collected from all sample types in both urban gardens and wet markets had high resistance to both ampicillin and tetracycline, followed by streptomycin and trimethoprim-sulfamethoxazole, as shown in Figure 6. There was a difference in resistance to tetracycline and trimethoprim-sulfamethoxazole between the *E. coli* isolates from urban gardens and wet markets, with resistance percentage of 68.8% and 37.5%, respectively. Among wet market isolates, resistance to tetracycline and trimethoprim-sulfamethoxazole were 31.7% and 17.1%, respectively, with a high ampicillin resistance of 56.1% among the wet market isolates. However, urban garden isolates showed intermediate resistance to ciprofloxacin and ampicillin, while resistance to streptomycin was high in both sampling groups as shown in Figure 7.

Table 6. Number of fresh produce that were molecularly positive for *Escherichia coli* collected from wet markets in Metro Manila, Philippines

Sampling site	Vegetables	No. of samples collected	No. of Positive Samples (%)
Nepa Q Market	Lettuce	9	1 (11%)
	Cucumber	10	0 (0%)
	Snow Cabbage	9	1 (11%)
	Tomato	11	1 (9%)
	Carrot	10	1 (10%)
	Cabbage	9	1 (11%)
	Spring Onion	10	2 (20%)
	Water Spinach	10	1 (10%)
	Sweet Potato Leaves	10	3 (30%)
Blumentritt Market	Lettuce	8	2 (25%)
	Cucumber	12	2 (17%)
	Snow Cabbage	9	2 (22%)
	Tomato	11	1 (9%)
	Carrot	11	2 (18%)
	Cabbage	10	1 (10%)
	Spring Onion	11	2 (18%)
	Water Spinach	10	1 (10%)
	Sweet Potato Leaves	6	0 (0%)
Balintawak Market	Lettuce	9	1 (11%)
	Cucumber	10	1 (10%)
	Snow Cabbage	9	3 (33%)
	Tomato	8	1 (13%)
	Carrot	11	1 (9%)
	Cabbage	10	0 (0%)
	Spring Onion	10	1 (10%)
	Water Spinach	9	0 (0%)
	Sweet Potato Leaves	8	1 (13%)
Farmers Market	Lettuce	11	2 (18%)
	Cucumber	10	3 (30%)
	Snow Cabbage	10	1 (10%)
	Tomato	10	1 (10%)
	Carrot	10	1 (10%)
	Cabbage	9	0 (0%)
	Spring Onion	10	0 (0%)
	Water Spinach	10	0 (0%)
	Sweet Potato Leaves	8	1 (13%)
Total		348	41 (12%)

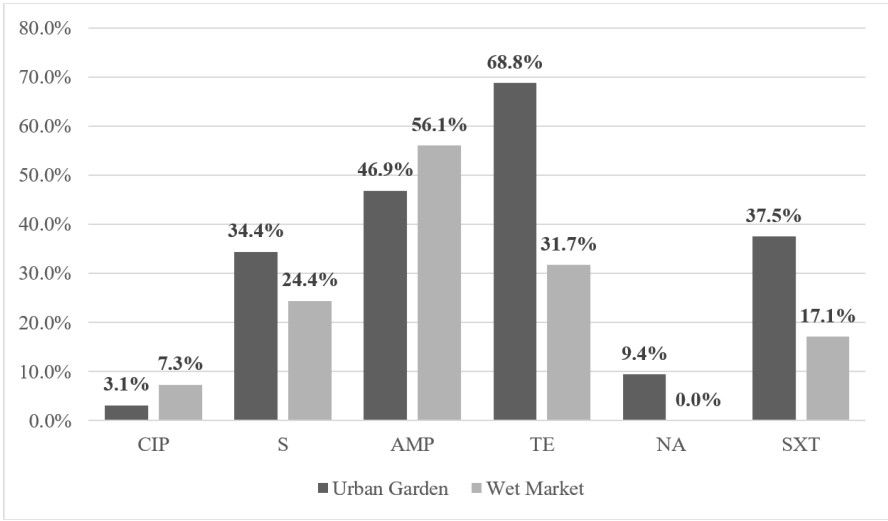

Figure 6. Percentage of antibiotic resistant *E. coli* isolates collected from urban gardens and wet market samples in Metro Manila, Philippines

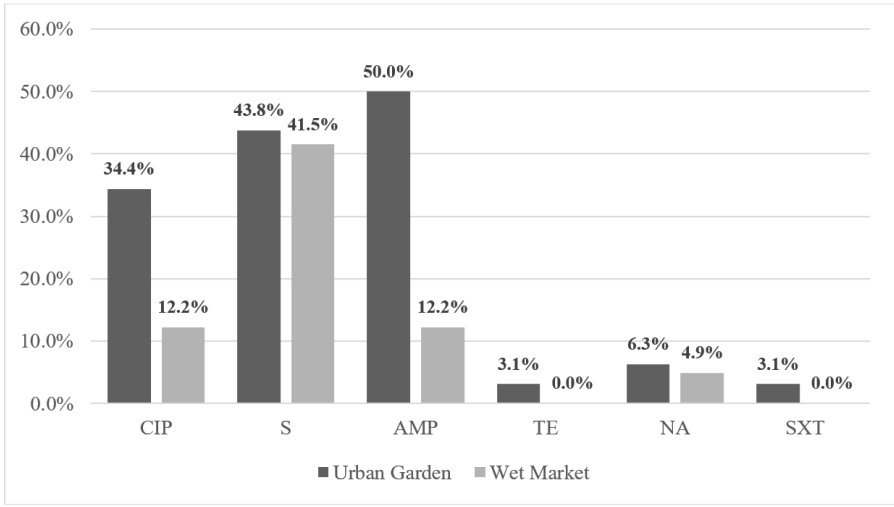

Figure 7. Percentage of intermediate antibiotic resistance of *E. coli* isolates collected from urban gardens and wet market samples in Metro Manila, Philippines

E. coli isolates from both urban garden and wet market samples had the highest susceptibility to nalidixic acid (Figure 8) which was expected as this antibiotic has been rarely used in recent times and may not be introduced to the urban environment. Also, multidrug resistance (MDR), defined as having at least one antibiotic resistance in three or more antibiotic categories (Magiorakos *et al.,* 2012), was apparent in isolates collected in the study as 21 out of the 73 (29%) *E. coli* isolates in both urban gardens and wet markets showed resistance to three antibiotic categories. The most common pattern was resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, while some isolates showed a multidrug resistant pattern in quinolones such as streptomycin and nalidixic acid. Isolates from urban gardens showed the highest percentage of MDR among the two sampling groups, having 13 out of 32 (41%), while only 8 out of 41 wet market isolates (20%).

Antibiotic results of the reference strain also passed the quality control according to the CLSI guidelines for antibiotics against *E. coli* as seen in Table 7. To determine if there is a significant difference between the two groups' antibiotic resistance results, statistical analysis was done.

3.5 Statistical analysis

Mann-Whitney U test was used to see whether the *E. coli* isolates from the two sampling groups came from one population in terms of antibiotic resistance and to determine if there is a significant difference in antibiotic susceptibility between the two groups. The test showed that only tetracycline resistance had statistically significant results ($p < 0.05$), suggesting that *E. coli* samples isolated in urban gardens and wet markets were from different populations (Table 8). On the other hand, while trimethoprim-sulfamethoxazole also showed differences, it was not statistically significant. Spearman correlation test showed that tetracycline and ampicillin have a weak positive correlation ($p < 0.05$).

3.6 Discussion

Escherichia coli isolates collected from both urban gardens and wet markets showed increased resistance to certain antibiotics that are commonly used in agriculture and for human treatment. This study demonstrated increased resistance to tetracycline, a widely used broad-spectrum antibiotic that has many forms being used due to low production costs as even synthetic derivatives like doxycycline can be produced, making this antibiotic class popular. Also, tetracycline antibiotics in animals are not entirely absorbed, and about 30 – 90% of them are excreted as parent compounds (Chee-Sanford *et al.,* 2009) and remain in the environment where they are readily introduced to environmental bacteria, causing mutations. Furthermore,

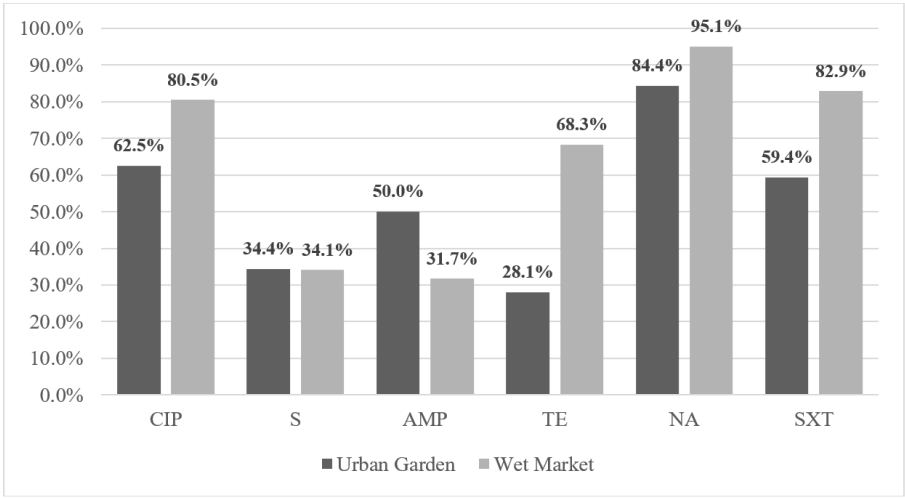

Figure 8. Percentage of *E. coli* isolates that are susceptible to antibiotics collected from urban gardens and wet market samples in Metro Manila, Philippines

Table 7. Antibiotic susceptibility results of ATCC 25922 reference control showing a passing value according to CLSI guidelines

Antibiotics	ATCC® 25922
Ampicillin	16.5 mm
Ciprofloxacin	29.3 mm
Nalidixic Acid	24.8 mm
Trimethoprim-sulfamethoxazole	23.6 mm
Streptomycin	13.5 mm
Tetracycline	24.3 mm

Table 8. Mann-Whitney U test using Jamovi 2.3.21 statistical software, to check for statistical differences between the antibiotic susceptibility results of *E. coli* isolates collected from both Urban Gardens and Wet Markets

Antibiotic resistance	p-value
Ciprofloxacin Resistance	0.673
Nalidixic Acid Resistance	0.508
Trimethoprim-Sulfamethoxazole Resistance	0.171
Streptomycin Resistance	0.455
Tetracycline Resistance	**0.006***
Ampicillin Resistance	0.241

waste from cattle or poultry is often used for fertilizer, so residual antibiotics in animal wastes are often transferred into agricultural soils as fertilizer (Wang *et al.,* 2015). This could explain the significant difference in the Mann-Whitney U test where wet market isolates have low resistance to tetracycline because they are no longer in contact with soil, but with anthropogenic activities in wet markets. This could lead to introduction of a new environment for ARB to be introduced into agricultural products, as wet markets are observed to have low-to none hygienic practices and fresh produce is often washed thoroughly only when bought (Lo *et al,* 2019). This promotes bacterial growth that is either already present in the product or will be introduced by the environment; however, this may not always be the case, as another study examined soils from various agricultural systems and concluded that both manure- and nonmanure-amended soils were contaminated with bacteria resistant to tetracycline, streptomycin, and erythromycin (Popowska *et al.,* 2012), an indication that AMR is a natural phenomenon that predates the clinical use of antibiotics, and ARGs occur naturally in soils independently of anthropogenic activities (Perera *et al.,* 2020).

The data also showed low resistance to nalidixic acid, which is promising as nalidixic acid inhibits DNA synthesis to destroy the bacterium, and resistance to this can lead to difficult treatment. Ciprofloxacin and streptomycin showed increased intermediate result of antibiotic susceptibility, an indication of a higher dosage of antibiotic usage needed for efficacy. These may also be associated with higher levels of phenotypic diversity that can have variable levels of antibiotic concentration needed for treatment (Lee *et al.,* 2018).

The weak positive correlation result between tetracycline and ampicillin indicates that the two antibiotic groups share a common resistant pattern, most notably in fresh produce, suggesting that the two antibiotic groups share a link in resistant gene mutations. Several studies support this correlation, as metagenomic data revealed the prevalence and abundance of genes encoding resistance to quinolones, ß-lactams, and tetracycline in soil (Mafiz *et al.,* 2018), and another study recovered *Escherichia coli* from vegetables grown in urban gardens that were ampicillin-resistant (Perera *et al.,* 2020). Genes that interfere with efflux pumps also lead to antibiotic resistance in *Escherichia coli* and can be attributed to having this pattern as well. AcrR is an example of this efflux pump gene that causes antibiotic resistance. It is an autoregulated local repressor of acrAB that binds the acrA promoter region and a mutation of this gene causes inactivation of acrR which causes increased acrAB expression and antibiotic resistance in *Escherichia coli* (Kang *et al.,* 2019).

4. Conclusion

Given the data, it has been found that urban gardens and its anthropogenic activity indirectly skews the antibiotic susceptibility of *E. coli* isolates more towards the resistance category than wet market isolates. This could be due to the excretion of antibiotics used by humans and being aggravated by urban soil contents to promote antibiotic resistance, potentially leading to an increase in ARB in their community. While the degree of

significant activity to increase the ARB in the environment is not readily studied, several studies have shown that usage of animal waste as fertilizer in agricultural soil increases the number of resistant genes and accumulation of ARB horizontal gene transfer to soil bacteria in the environment. In addition, multidrug resistance is more evident in urban garden isolates than in wet markets, implying an instance of ARB proliferation within the community due to horizontal and vertical transfer of resistance genes. Furthermore, understanding the human activities in urban gardening should be studied, as such activities like use of antibiotics in numerous applications in this environment, including for poultry growth, gardening, and human consumption.

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