Antibiotic Resistant Bacteria in Raw Chicken Meat Sold in a Public Market in Quezon City, Philippines

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RESEARCH ARTICLE

Abstract

Background: The existence of antibiotic resistant strains of bacteria in both the hospital and community settings is a threat that can plague humanity. There are now increasing evidences that even livestocks for human consumption harbor antibiotic resistant bacteria. To date, there is a limited information on the presence of antibiotic resistant bacteria isolated from raw food, particularly from chicken, for human consumption in the Philippines.
Objective: This study aimed to determine the presence of antibiotic resistant bacteria from raw chicken meat sold in a public market in Quezon City, Philippines.
Methodology: Twenty-five raw chicken meats (leg part) were purchased from randomly selected stalls in a public market. Selective and differential media were used to isolate *Escherichia. coli, Salmonella spp.*, and *Staphylococcus aureus* from the purchased poultry meat. The isolated bacteria were subjected to several morphological and biochemical tests, to confirm their identities. Twenty-five colonies from each of the three isolated genera were selected to be used in the antibiotic susceptibility screening using the Kirby-Bauer disk diffusion method.
Results: This study confirms the presence of antibiotic resistant strains of *Escherichia. coli, Salmonella spp.,* and *Staphylococcus aureus* from raw chicken legs bought in a public market. In the disc diffusion method, 100% of the isolated *E. coli* were resistant to amoxicillin, ampicillin, penicillin, clindamycin; 96% were resistant to erythromycin, 92% to clarithromycin, and 20% to tetracycline. For the isolated *Salmonella spp.*, 100% were also resistant to amoxicillin, ampicillin, penicillin, clindamycin; 96% were resistant to clarithromycin, and 16% were resistant to ciprofloxacin. Of the 25 isolates of *S. aureus*, 100% were also resistant to amoxicillin, ampicillin, penicillin, tetracycline; 20% were resistant to clarithromycin and chloramphenicol; 16% were resistant to clindamycin and norfloxacin; and 12% were resistant to erythromycin.
Conclusion: The presence of antibiotic resistant *E. coli, Salmonella* spp., and *S. aureus* in animal food sold at a public market in Quezon City to not just one antibiotic but more, may pose a serious threat to human health. The livestock industries should also look into the use of antibiotics for nontherapeutic purposes, since these animals can also lead to the emergence of antibiotioc resistant bacteria that can be transferred to humans when they are consumed as food or by direct contact with the farm animals. Therefore, vigilant monitoring and stricter policies and regulations must be implemented on the use and marketing of antibiotics in food animals to ensure food safety in the Philippines.

Keywords: *antiobiotic resistance, foodborne pathogens, Escherichia coli, Salmonella spp., Staphylococcus aureus*

Introduction

Bacterial contamination in raw meat destined for human consumption has been reported to be appearing worldwide and is responsible for illnesses and food-borne outbreaks [1]. Even if the well-established pathogens are being controlled, there are, however, the emergence of pathogens due to ecological and technological changes that connect the emerging potential pathogen to the food chain [2]. It is well-documented that contamination of meat for human consumption with antibiotic resistant bacterial pathogens constitutes a major public health issue, since it is responsible for gastrointestinal illnesses in man that cannot be cured with the existing medications [1,3,4,5]. This growing problem of resistance to well-known and trusted antimicrobial is widely recognized as one of the greatest challenges that physicians face in the management of adult and pediatric infections [6], and most especially in third world countries [7]. The use of antimicrobials in poultry raising has been implicated as one of the causes of increasing the prevalence of antimicrobial resistance in foodborne pathogens coming from livestock industries [8,9].

In the Philippines, these chicken meats are commonly sold in public markets that are just usually placed on tables for display and kept at temperature of the surrounding environment. The contamination of these meats can actually occur at any of several stages of processing, for example, raising in the farm, transporting to the market, handling of the meat, and the surfaces where they are placed for display. These points of chicken meat processing to be sold in an open market are most of the time unhygienic and can possibly contaminate the meats, aside from the fact that these meats are presumed to come from livestocks treated with antibiotics when raised in the farm.

Foodborne outbreaks are commonly caused by *Escherichiacoli (E.coli), Salmonella spp*., and *Staphylococcus aureus (S. aureus)* [1,2,10,11]. Based on the report of the World Health Organization (WHO), diarrheal diseases are responsible for more than half of the global burden of foodborne diseases, causing 550 million people to fall ill and 230,000 deaths every year. Children are at particular risk of foodborne diarrheal diseases, with 220 million falling ill and 96,000 dying every year. Diarrhea is often caused by eating raw or undercooked meat, eggs, fresh produce, and dairy products contaminated by norovirus, *Campylobacter*, non-typhoidal *Salmonella* and pathogenic *E. coli.* [12]. In industrialized nations, *Salmonella* serotypes rank as the highest frequency of occurrence in foodborne diseases. It can be acquired from the ingestion of contaminated poultry products, such as eggs or meat, that also plays a role in the transmission and spread of the organism from animals to humans [13]. *S. aureus* is known to cause a number of pathological conditions in humans and animals that includes mild skin infections, bacteremia, systemic diseases, osteomyelitis, and to the more complicated toxic shock syndrome and staphylococcal food poisoning [14]. It can cause an invasive infection and considered to be one of the medically important human pathogens [15,16]. The numerous reports on hospital-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and community-associated MRSA predominantly affect humans, and in recent years, MRSA has been identified as an emerging pathogen in livestock and companion animals, as well as some other farm animals, as cited by Basanini *et al*. [17].

Antibiotics have greatly enhanced the human life expectancy, reduced mortality, improved the quality of life, and almost won the war against many infectious diseases. There are a lot of common antibiotics used widely to treat various diseases caused by these pathogenic bacteria, such as amino-glycosides, macrolides, chloramphenicol, tetracyclines, and fluoroquinolones [18]. However, the indiscriminate and/or inappropriate use of antibiotics and extensive use of these agents as growth enhancers in animal feed have induced the emergence of antibiotic resistant bacteria [19]. *Escherichia coli* has become resistant to amoxicillin, oxytetracycline, streptomycin, sulphamethoxazole, trimethoprim, and ciprofloxacin [20]. On the other hand, *Salmonella spp.* has developed resistance to streptomycin, sulfonamides, tetracycline, ampicillin, and chloramphenicol [21]. Lastly, *S. aureus* strains are said to have become difficult to attack due to emerging resistance to methicillin [22]. Reports of methicillin-resistant *S. aureus* (MRSA) isolated from farms, the livestocks, and their meats sold in the markets have been increasing in numbers [4,11,23,24,25]. Antibiotics are usually added to animal feed or drinking water of cattle, hogs, poultry, and other food-producing animals to help them gain weight faster or use less of food for them to gain weight, and in so doing, contribute to the development of antimicrobial resistance [26]. Through the mutation or acquisition of resistance genes, the treatment of infections is increasingly compromised by the ability of bacteria to develop resistance to antibiotics [27].

The ocurrence of antibiotic resistant strains from raw chicken meat sold in an open market has not been reported here in the Philippines. This study, therefore, was an initial investigation on the presence of antibiotic resistant strains of three common foodborne pathogens (*E. coli*, *Salmonella spp.*, and *S. aureus*) from chicken meat (leg part) sold in one of the big public markets in Quezon City. Consequently, the results of this study can become the baseline data for further studies on providing more information about the occurrence of antibiotic resistant *E. coli, Salmonella spp*., and *S. aureus* in meats sold at the public market. The results of this study can also promote awareness not just to vendors and buyers on the importance of sanitation in areas where food is sold and bought, but also to clinicians who can properly manage cases of infections caused by antibiotic resistant foodborne pathogens. The possibility also of livestock farms as sources of antibiotic resistant bacteria cannot be discounted, because these farms use antibiotics not just for treatment of their diseased animals but also to enhance the growth of their livestocks [28].

Methodology

All of the procedures done in this study were in aseptic conditions and in triplicates.

Source of Samples

A total of 25 raw chicken leg samples were purchased from a prominent public market in Quezon City, Philippines. All samples were stored invidually in sterile plastic bags and temporarily stored in a cooler with the temperature maintained at 4ºC. The samples were transported and processed immediately at the Miriam College Science Laboratory, Quezon City.

Isolation and Identification of Bacterial Strains

The isolation procedures of the three bacteria from the chicken leg samples were carried out basically the same for all three except in the selective and differential media used for isolating the specific strains [2]. At first, 20 grams of the chicken meat was aseptically cut and transferred into a sterile homogenizer with 180 mL of sterile peptone broth. After homogenization, a ten-fold serial dilution was made and 0.1 mL from the 10-6 dilution was placed in 10 mL of Nutrient Broth and incubated for 24 hours at 37ºC for cultivation. After 24-48 hours, 1 mL of the culture was transferred and spread plated into MacConkey agar, and pink colonies that grew on it were streaked onto Eosin Methylene Blue agar for the isolation of *E. coli.* In the isolation of presumptive *Salmonella spp.*, 1 mL of the pre-enriched culture was plated onto Salmonella-Shigella Agar (SSA) and incubated at 37ºC for 24 hours. Lastly, for the isolation of *Staphylococcus aureus*, 1 mL from the pre-enriched Nutrient Broth was spread plated onto Mannitol Salt Agar (MSA) and incubated at 37ºC for 24 hours. All the isolated bacterial strains were preliminarily identified by morphological observation using Gram stain based on Bergey's Manual of Systematic Bacteriology [29]. Indole, catalase, methyl red, and nitrate reduction tests were done on green metallic sheen colonies that grew on Eosin Methylene Blue agar to confirm the presence of *E. coli.* The catalase, methyl red, citrate tests were done on black colonies with metallic silver sheen that grew on SSA and was also stab-butt inoculated onto Triple Sugar Iron Agar (TSI) to confirm the presence of *Salmonella spp*. The identity of *S. aureus* was confirmed using the coagulase and catalase tests.

Antibiotic Resistance Screening

The Kirby-Bauer disc diffusion method was done to test the antibiotic susceptibility of the three bacteria isolated from raw chicken meat. There were 10 different antibiotics in discs used in the screening, namely: amoxicillin (30µg), ampicillin (15µg), penicillin (15µg), chloramphenicol (30µg), tetracycline (15µg), clarithromycin (15µg), norfloxacin (30µg), and clindomycin (15µg). After adjusting the concentrations of the bacteria using the 0.5 McFarland standard, 100 µl of each of the bacterial cultures were spread plated on Mueller-Hinton agar (MHA). The antibiotic discs were placed aseptically on the dried surface of MHA and incubated at 37ºC for 24 hours. The zones of inhibition were measured using inhibition zone ruler to determine the susceptibility or resistance of the bacteria to the antibiotics. The obtained measurements were interpreted based on the recommended Zone Diameter Interpretative Standards by the National Committee for Clinical and Laboratory Standard [31].

Results

Isolation and Identification of Bacterial Strains

From the 25 chicken leg samples bought from a public market in Quezon City, a total of 75 colonies were selected from the selective and differential media used in their isolation. By cultural characterization and biochemical tests, 25 colonies with green metallic sheen in Eosin- Methylene Blue agar were *E. coli*, 25 colonies that grew in Salmonella-Shigella agar were *Salmonella spp.*, and 25 colonies that grew in Mannitol Salt agar were *S. aureus* .

Antibiotic Resistance Screening

The results of this study show that antibiotic resistant bacteria were isolated from raw chicken leg samples sold in a public market in Quezon City, Philippines. The sensitivity patterns of the three bacteria isolated from the raw chicken meat is shown in Table 1. The results are quite alarming since all three isolates were resistant to not just one antibiotic but to several antibiotics, a case which we can refer to as multidrug resistance. For *E. coli*, it is resistant to seven out of the 10 antibiotics; *Salmonella spp.* is resistant to five out of the 10 antibiotics; and *S. aureus* is resistant to eight out of the 10 antibiotics used in this study. It is worthy to note however, that there are still some antibiotics that worked well in inhibiting the growth of these three bacteria.

After 24 hours incubation of the Mueller-Hinton Agar plates, the diameter in the zones of inhibition (mm) were measured. The average of three readings was obtained and the susceptibility or resistance patterns of the three bacteria were interpreted based on the recommended Zone Diameter Interpretative Standards by the National Commitee for Clinical and Laboratory Standard. Representative plates showing the zones of inhibition by the three bacteria isolated from the raw chicken are shown in Figure 1.

Table 1. *Sensitivity patterns of the three bacteria isolated from raw chicken meat sold in a public market against ten antibiotics. These patterns were based on the average zone diameter interpretative standards of the National Committee for Clinical and Laboratory Standard.*

Bacteria	Amox (30 µg)	Ampi (15 µg)	Pen (15 µg)	Chloram (30 µg)	Tetra (15 µg)	Clarith (30 µg)	Eryth (15 µg)	Clinda (15 µg)	Cipro (15 µg)	Norflo (30 µg)
E. coli	R	R	R	S	R	R	R	R	S	S
Salmonella spp.	R	R	R	S	S	R	S	R	S	S
S. aureus	R	R	R	R	R	R	R	R	S	S

R – Resistant **S** – Susceptible
Amox – amoxicillin **Ampi** – ampicillin **Pen** – penicillin **Chloram** – Chloramphenicol **Tetra** – tetracycline
Clarith – clarithromycin **Eryth** – erythromycin **Clinda** – clindamycin **Cipro** – ciprofloxacin **Norflo** - norfloxacin

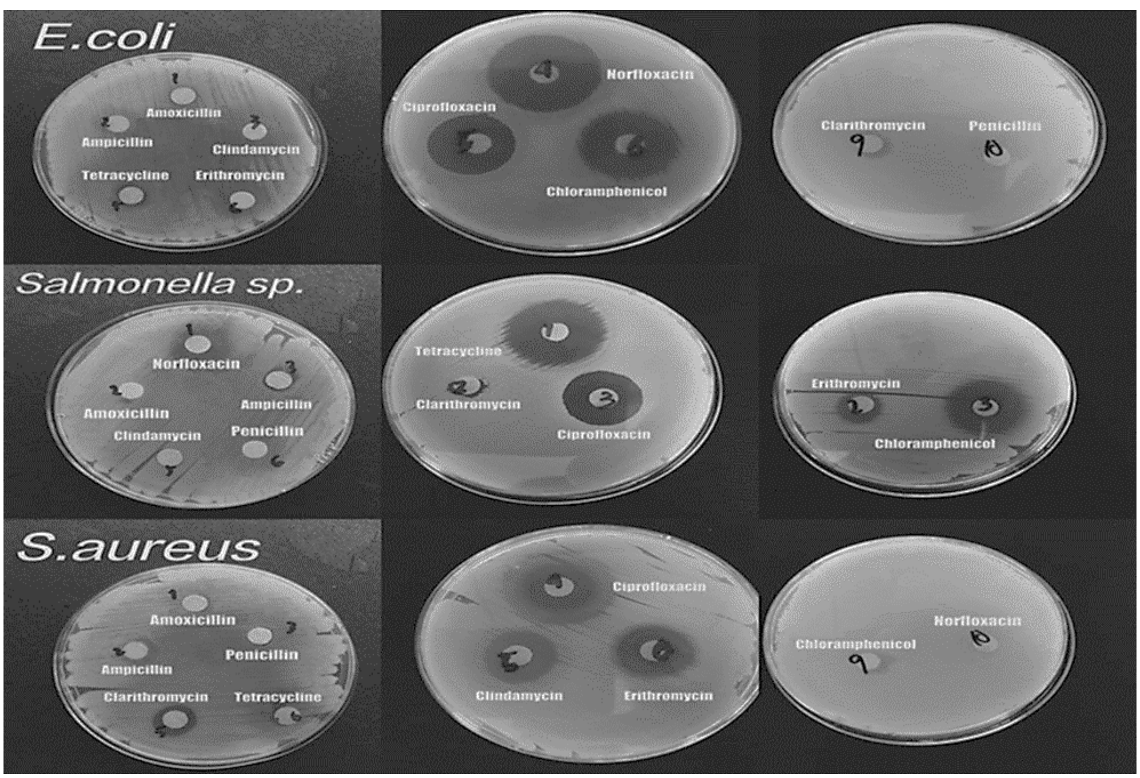

Figure 1. *The zones of inhibition on Mueller-Hinton Agar plates for the antibiotic susceptibility screening to 10 antibiotics by E. coli, Salmonella spp., and S. aureus.*

The antibiotic resistance profile for *E. coli* is shown in Figure 2. It can be seen that 100% of the bacteria were resistant to amoxicillin, ampicillin, clindamycin, erythromycin, and penicillin; 92% of the isolates were resistant to clarithromycin; 88% of the isolates were resistant to tetracycline; 16% of the isolates were resistant to ciprofloxacin; 8% of the isolates were resistant to chloramphenicol; and none of the *E. coli* isolates were resistant to norfloxacin.

Figure 3 shows the antibiotic resistance profile of the isolated *Salmonella spp.* to the 10 antibiotics used in the study. It can be seen that 100% of the *Salmonella* isolates were also resistant to amoxicillin, ampicillin, clindamycin, and penicillin; but only 20% of the *Salmonella* isolates were resistant to erythromycin, unlike for *E. coli* wherein 100% were resistant to erythromycin. Ninety-six percent of the *Salmonella* spp. isolates were resistant to clarithromycin; 16% were resistant to norfloxacin; 12% were resistant to chloramphenicol and ciprofloxacin; and none were resistant to tetracycline.

The antibiotic resistance profile for *S. aureus* is presented in Figure 4. Similarly, 100% of the *S. aureus* isolates were also resistant to amoxicillin, ampicillin, penicillin, and tetracycline; 24% were resistant to clarithromycin, chloramphenicol, and norfloxacin; 16% were resistant to clindamycin; 12% to erythromycin; and none were resistant to ciprofloxacin.

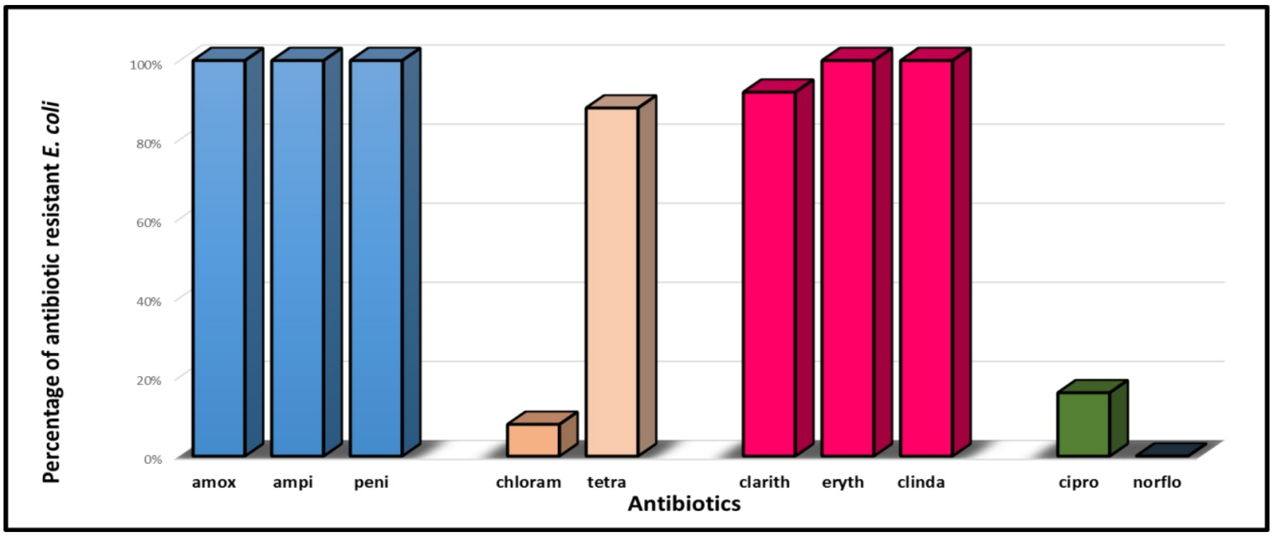

Figure 2. *Antibiotic resistance profile of Escherichia coli (n=25) isolated from raw chicken legs bought in a public market against 10 antibiotics.*

- inhibits cell wall synthesis [37]
- blocks the binding of tRNA to mRNA-ribosome complex [38]
- blocks exit of nascent peptide from the ribosome [39]
- prevents replication of bacterial DNA [37]

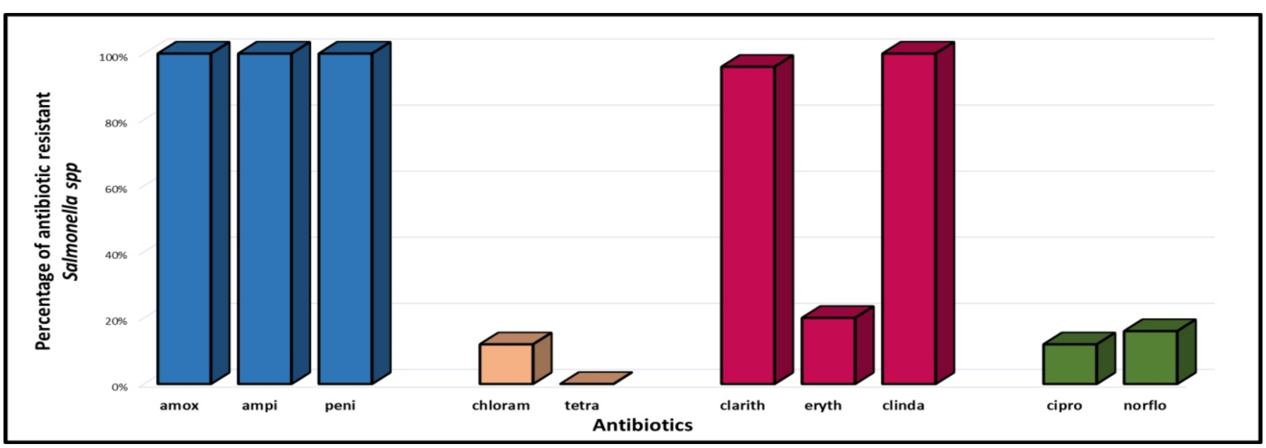

Figure 3. *Antibiotic resistance profile of Salmonella spp isolated from raw chicken legs bought in a public market against 10 antibiotics (n=25).*

- inhibits cell wall synthesis [37]
- blocks the binding of tRNA to mRNA-ribosome complex [38]
- blocks exit of nascent peptide from the ribosome [39]
- prevents replication of bacterial DNA [37]

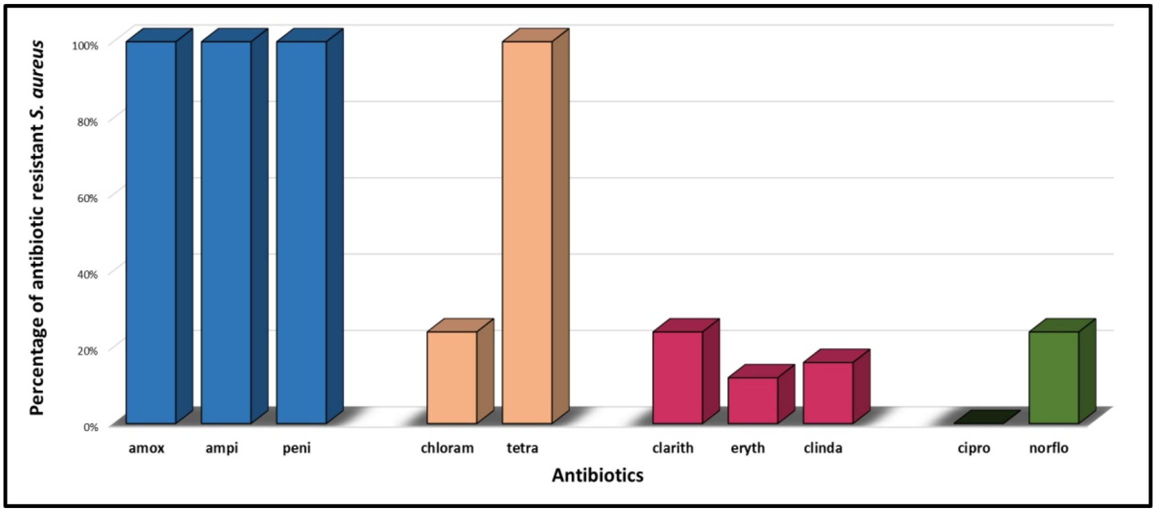

Figure 4. *Antibiotic resistance profile of Staphylococcus aureus isolated from raw chicken legs bought in a public market against 10 antibiotics (n=25).*

inhibits cell wall synthesis [37]
blocks the binding of tRNA to mRNA-ribosome complex [38]
blocks exit of nascent peptide from the ribosome [39]
prevents replication of bacterial DNA [37]

Discussion

This study is a preliminary screening on the presence of three antibiotic resistant bacteria from raw chicken meat in the Philippines, namely*, E.coli, Salamonella spp.,* and *S. aureus.* There are a few studies regarding the presence of antibiotic resistant bacteria isolated from various foods here in the Philippines, for example, street-vended foods [32] and from shrimps in ponds [33]. All three isolates in this study exhibited varying degrees of resistance to the 10 antibiotics used and the results are quite alarming, since the isolates were not just resistant to one antibiotic but to several kinds.

The antibiotics used in this study were based on the common medication prescribed by medical practitioners to treat bacterial infections of *E. coli, Salmonella spp.,* and *S. aureus* [34,35,36]. In general, the actions of the chosen antibiotics work in the following manner: amoxicillin, ampicillin, and penicillin inhibit the synthesis of cell walls [37]; chloramphenicol and tetracycline, on the other hand, are drugs that reversibly bind to the 30S ribosomal subunit of bacteria, blocking the binding of aminoacyl-tRNA to the mRNA-ribosome complex, thus preventing the addition of amino acids to the growing peptide, resulting in inhibition of protein synthesis [38]; clarithromycin, erythromycin, and clindamycin also prevent bacterial protein synthesis by binding to the 50S ribosomal subunit, thus blocking the path by which nascent peptides exit the ribosome [39]. Ciprofloxacin and norfloxacin inhibit the enzyme bacterial DNA gyrase and prevent the replication of bacterial DNA during bacterial growth and reproduction [37]. These antibiotics are broad spectrum that are useful in treating both gram-positive and gram-negative bacterial infections except for the clindamycin, which is a narrow spectrum for gram-negative bacteria only [40].

In the last decades, most bacterial pathogens have exhibited antimicrobial resistance [41]. The indiscriminate use of antibiotics to combat these foodborne pathogens has led to the development of antibiotic resistant bacteria worldwide. Several studies from many countries have reported the presence of antibiotic resistant bacteria from poultry, as well as from other food sources for human consumption, for example, pork, beef, goat meat, and dairy milk [1,2, 4,5,8,9,11,13,18,24,25,35,42]. The countries where these studies have been done were mostly third world countries. The Philippines, also a third world country, is not spared from the presence of antibiotic resistant bacteria.

In this study, the three bacteria were resistant not just to one but to several antibiotics, displaying therefore multidrug resistance (MDR). *S. aureus* was resistant to eight of the 10 antibiotics, *E.coli* to seven antibiotics, and *Salmonella spp*. was resistant to five of the 10 antibiotics.

In the study of Greeson *et al*. [1], many of the bacteria isolated from four unprocessed meats purchased from local retail outlets in Riyadh area were resistant to more than one antibiotic. The study by Aly *et al*. [11] also supports the results of our study, which reported MDR strains of *E. coli* isolated from food samples to at least three members of beta-lactam class of antibiotics. Falagas and Karageorgopoulos said that this result is common in gram-negative bacteria either due to the presence of the outer membrane and/or production of beta-lactams [43]. The presence of *E. coli* in the meat samples indicates fecal contamination.

Salmonellosis can result from the ingestion of poultry contaminated with *Salmonella spp.* The isolation of this foodborne pathogen and its screening for antibiotic susceptibility in this study confirm the presence of isolates from raw chicken that were resistant to five of the 10 antibiotics used. Harsha *et al*. mentioned that the administration of antimicrobial agents in chickens allows selection pressure that favors the survival of antibiotic resistant pathogens, and multidrug resistant phenotypes have been increasingly described among Salmonella species worldwide [13].

Staphylococcus aureus has long been recognized as one of the food poisoning bacteria in man worldwide [9]. The study of Yemisi *et al*. has shown that most of the *S. aureus* isolated from raw chicken, pork, beef, and goat meat from a retail store in Osogbo, Nigeria were susceptible to six antibiotics, including erythromycin which was used in this study, but were resistant to amoxicillin and two other antibiotics [4]. This is quite not like the result obtained from this study since the isolated *S. aureus* from the chicken legs were susceptible to only two antibiotics and resistant to eight of the 10 antibiotics used in this study (Table 1). The study of Abdellah *et al.* in 2013, revealed that the *S. aureus* isolated from turkey meat in Morocco was resistant only to two antimicrobial agents out of the eight used in the study [9]. The antibiotic to where our staphylococci isolates were also resistant to was tetracycline (30 µg). The most likely reason why the chicken meat sold in the public market had *S. aureus* contamination may be related to improper personal hygiene of the vendors during handling and processing [9].

Although the pathogenicity of the three isolates were not determined in this study, it can be supposed that some of these isolates may have any of the plasmid genes that encode for proteins that can render them virulent, such as colonization factor antigen (CFA) that confers the ability of bacterial cells to attach to epithelial cells of the small intestine; toxins that can induce extensive secretion of water and salts into the bowel (diarrhea); and outer proteins that allow the bacteria to subvert the host defense functions [44]. Regardless of how these bacteria confer their virulence, what could be a potential threat to the medical world is the increasing reports on the emergence of antibiotic resistant bacteria from animal foods. This increasing number and types of microorganisms resistant to drugs can lead to public health problems, such as morbidity, mortality, and increased cost of treatments [28]. The presence of multidrug resistant bacteria in food animals, such as chicken, may translate into increased resistance in bacteria that can infect humans, which in turn could transfer resistance genes to both commensals and pathogenic bacteria in the human digestive tract, that can be passed along by person to person or contact to fomites [1].

Conclusion and Recommendation

This study on the presence of antibiotic resistant bacteria in raw meat foods, such as chicken, can be considered as a pilot study in the Philippines. The isolation of antibiotic resistant bacteria to not just one antibiotic but to several antibiotics can be used as a baseline data in reporting the presence of multidrug resistant foodborne pathogens in the country. The results of this study can also be used as a warning call not just to consumers, retailers, regulatory agencies, and health care systems but also to farm breeders of livestock, such as poultry. The livestock industries should look into the use of antibiotics for non-therapeutic purposes, since these food animals can also lead to the emergence of antibiotic resistant bacteria that can be transferred to humans when they are consumed as food or by direct contact with the farm animals. Therefore, vigilant monitoring and stricter policies and regulations must be implemented on the use and marketing of antibiotics in food animals to ensure food safety in the Philippines.

Further investigation must be done on the presence of antibiotic resistant bacteria from raw animal foods sold in the market for humans to eat. Molecular methods can also be employed for a rapid identification of isolates and determination of the presence of antibiotic resistant genes in the bacteria isolated from meat food.

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