Original Research Article

Occurrence and Antibiotic Sensitivity of *Escherichia coli* and *Salmonella* spp. in Retail Chicken Meat at Selected Markets in Valencia City, Bukidnon, Philippines

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ABSTRACT

Aim: Contaminated retail chicken meat has been implicated with food poisoning caused by *Escherichia coli* and *Salmonella* spp. The present study was aimed to determine the occurrence and antibiotic sensitivity of *E. coli* and *Salmonella* spp. in retail chicken meat at selected markets in Valencia City, Bukidnon, Philippines. **Methods:** A total of 50 retailed chicken meats which comprised of drumsticks (n=25) and wings (n=25) were collected aseptically from three selected markets (2 supermarkets and 1 wet market) in Valencia City, Bukidnon, Philippines and examined for the occurrence of *Escherichia coli* and *Salmonella* spp. Identification of bacterial isolates was through morphological characteristics, Gram-staining, and biochemical tests or growth-dependent identification methods. The antibiotic sensitivity of the pure isolates to amoxicillin (25 µg), ampicillin (10 µg), chloramphenicol (30 µg), and streptomycin (10 µg) was determined through a standard antimicrobial disk diffusion test. **Results:** Results revealed that 68% (17/25) of the drumsticks and 96% (24/25) of the wings samples were contaminated with *E. coli* while 4% (1/25) of the drumsticks and 32% (8/25) of wings samples were contaminated with *Salmonella* spp. In total, 41 pure isolates of *E. coli* and 9 pure isolates of *Salmonella* spp. were obtained. Antimicrobial disk diffusion test revealed that 87.80% (36/41) of *E. coli* isolates and 100% (9/9) Salmonella spp. isolates are resistant to amoxicillin, and 36.58% (15/41) of *E. coli* isolates and 88.88% (8/9) *Salmonella* spp. isolates are resistant to ampicillin. Moreover, 29.27% (12/41) of *E. coli* isolates and 88.88% (8/9) of *Salmonella* spp. isolates are resistant to both amoxicillin and ampicillin. Majority (96%) of isolates are sensitive to streptomycin. **Conclusion:** There was a high occurrence of *E. coli* contamination in retail chicken meat in Valencia City, Bukidnon, Philippines. *E. coli* and *Salmonella* spp. from these retail meat showed high resistance to amoxicillin and ampicillin..

Key words: Antibiotic sensitivity, *Escherichia coli, Salmonella spp*., Chicken meat.

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INTRODUCTION

For decades, microbial food safety particularly on poultry products have drawn concerns from consumers and food safety practitioners worldwide. Rigorous research on this issue has established knowledge that poultry and poultry products are vehicles for food-borne pathogens such as *E. coli* and *Salmonella* spp.[1,2] It is widely accepted that these enteropathogens are zoonotic in origin and acquire their antimicrobial resistance in the food-animal host before onward transmission to humans through the food chain.[3] Meat and eggs were the most implicated vehicles for food-borne disease outbreaks in the United States based from 82 reports during the year 1986-1995 and *Salmonella* spp. and *Escherichia coli* O157:H7 were accounted for 55% agents reportedly isolated.[4] *Salmonella* and *Campylobacter* were also accounted for over 90% of all reported cases of bacteria-related food poisoning worldwide, while *E. coli* O157 emerged as a major food-borne zoonotic pathogen.[2] Recently, the Centers for Disease Control and Prevention[5] reported a multistate outbreak of *Salmonella typhimurium* in the

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US and this outbreak was linked to chicken salad. Food-borne diseases are indeed persistent challenges that we continue to face. In the developing countries, these challenges present even higher health threats to consumers due lenient policies on food safety. Research data indicating trends in food-borne infectious intestinal diseases were said to be limited to industrialised countries.[6] This suggests that research into microbial contamination in food products particularly meat and poultry is scanty in the developing countries.

The use of antibiotics in poultry and other food animals as treatment for diseases and/or as antimicrobial growth promoter (AMGP) has been attributed to the emergence of antibiotic resistant commensal bacteria that may contaminate meat products.[7] This problem may even be worsened because resistance genes of bacteria can be transferred to other bacteria in the poultry setting or in the human gut, creating a huge pool of antibiotic-resistant bacteria. The emergence of these antibiotic-resistant strains are serious human health threats because antimicrobial therapy against these pathogens would most likely to fail and could result to chronic and severe infections. Continuous surveillance of antibiotic resistance and control of antibiotic use in food animals are perceived as important measures to prevent the spread of antibiotic resistant or multiple-drug resistant strains.

The Province of Bukidnon, Philippines is endowed with verdant environment and soothing weather condition that is very advantageous to livestock and poultry industry. In the year 2016, the Province of Bukidnon had produced a total of 53,424 metric tons of chickens and 30,834 metric tons of chicken eggs and the trend of production is increasing per year.[8] Majority of the province' poultry farms (about 78%) is managed by Commercial Poultry Integrators composed of large food companies. This booming poultry industry in the province is advantageous because it provides steady supply of poultry meat and eggs and hence, contributes to food security in the province. However, the occurrence and antibiotic sensitivity profile of bacterial contaminants in retail chicken meat in the province remains understudied. Thus, this study aimed to investigate the occurrence and antibiotic sensitivity of the two most common bacterial contaminants in poultry products, *E. coli* and *Salmonella* spp. in retail chicken meat at selected markets in Valencia City, Bukidnon, Philippines.

MATERIALS AND METHODS

Sampling and collection site

A total of 50 retailed chicken meats which were comprised of 25 drumsticks and 25 wings were collected aseptically from three leading markets (2 supermarkets and 1 wet market) in Valencia City, Bukidnon, Philippines. Collection was done through the use of sterile disposable polyethylene plastic bags which were then sealed and brought to the laboratory for microbiological analyses. Analysis of samples commenced within 60 mins of collection.

Broth enrichment

To recover considerable number of *E. coli* and *Salmonella* spp. isolates, broth enrichment was done following collection of the samples. The surface of each sample was thoroughly swabbed using sterile cotton swab which was then soaked in 20 ml nutrient broth. Additionally, approximately 1 cm^3 of cut meat sample was added to the nutrient broth tube with the swab. The broth tubes were incubated at 35°C for 24 h.

Isolation of *E. coli* and *Salmonella* spp.

To isolate *E. coli* and *Salmonella* spp., 100 µl of previously enriched overnight broth cultures were spread plated in Eosin Methylene Blue (EMB) and *Salmonella*-Shigella (SS) agar plates, respectively and were incubated overnight at 35°C. After incubation, the plates were observed for the presence of green metallic sheen colonies in EMB agar plates and black colonies in SS agar plates which are indicative of *E. coli* and *Salmonella* spp., respectively. These colonies where picked using a sterile wire loop and were streaked plated in another EMB or SS agar plates, incubated overnight at 35°C after which well-isolated colonies were picked and inoculated into EMB or SS agar slants to obtain pure cultures. Finally, pure cultures were maintained in the refrigerator (4°C).

Identification of *E. coli* and *Salmonella* spp.

The presumptive isolates were identified through morphological characteristics, Gram-staining and biochemical tests or growth-dependent identification methods. Morphological characteristics include cell shape and arrangement, while biochemical tests or growth-dependent identification methods include growth in selective-differential culture media (EMB and SS), catalase, oxidase, H_2S and indole production, lactose fermentation, citrate utilisation and methyl red and Voges-Proskaeur reactions. The schematic diagram for the identification for *E. coli* and *Salmonella* spp. is shown in Figure 1.

Antibiotic Sensitivity Test

The antibiotic sensitivities of all isolates were assessed through the standard antimicrobial disk diffusion test protocol by the Clinical and Laboratory Standard Institute.[9] Briefly, the overnight well-isolated colonies of the isolates in nutrient agar plates were inoculated to

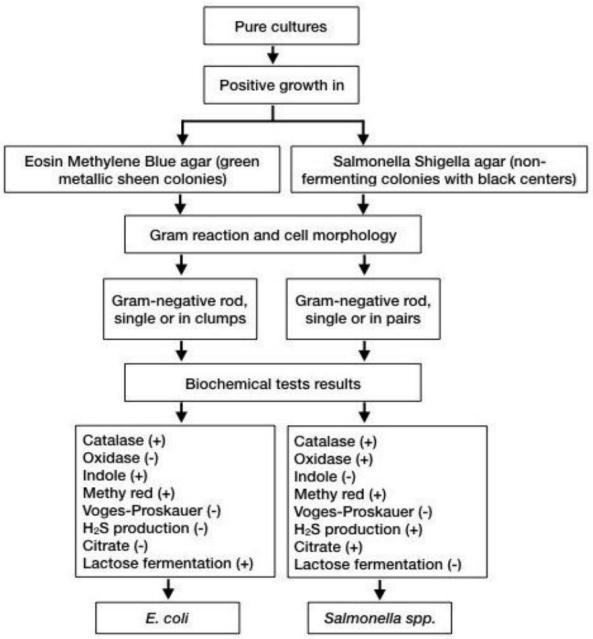

Figure 1: Schematic diagram used for the identification of *E. coli* and *Salmonella* spp.

5 ml sterile saline water until its turbidity is comparable to that of the 0.5 McFarland Turbidity Standards. At this turbidity, the suspension contains approximately 1 to 2 × 10^8 CFU/ml for *E. coli*. Within 15 min after the inoculum suspension has been prepared, a sterile cotton swab was dipped in the adjusted suspension, rotating the swab several times and pressed firmly in the inside wall of the tube. Inoculation was done in the Mueller Hinton agar plates by streaking the swab over the entire agar surface. This inoculation procedure was done 4 times, rotating the plate each time to ensure an even distribution of the inoculum. The inoculated plates were left slightly open for 3 mins to dry-up the agar surface after which the sterile antibiotic disks were dispensed to the plates using sterile forceps. For negative control, a 6 mm ∅ Whatman filter paper No. 2 disk was positioned at the center of each plate and 20 µl of sterile isotonic water was pipetted to each disk. The plates were incubated at 35°C for 18 h and the resulting zones of inhibition (ZOI) were observed. The diameter of the ZOI were measured in millimetre using a vernier caliper and measurements were rounded off to the nearest whole number. The antibiotic sensitivity profiles of the isolates were determined following the zone diameter breakpoints and interpretative categories (susceptible, intermediate or resistant) for Enterobacteriaceae set by the Clinical and Laboratory Standard Institute.[10]

Table 1: Incidence of *E. coli* and *Salmonella* spp. contamination in retailed chicken legs and wings.

Chicken Meat	No. of Samples	No. of samples positive for	
		E. coli	*Salmonella* spp.
Drumsticks	25	17	1
Wings	25	24	8

RESULTS

The occurrence of *E. coli* and *Salmonella* spp. contamination in retailed chicken meat samples is shown in Table 1. Both drumsticks and wings were found contaminated with *E. coli* and *Salmonella* spp. but the occurrence of *E. coli* contamination is high compared to that of *Salmonella* spp. Of the 25 meat samples for both drumsticks and wings, 17 (68%) of the drumsticks and 24 (96%) of the wings were positive for *E. coli* while only 1 (11.11%) of the drumsticks and 8 (88.88%) of the wings were positive for *Salmonella* spp. Antibiotic sensitivity test for all isolates following the protocol of CLSI (2006) revealed that 87.80% (36/41) of *E. coli* isolates and 100% (9/9) of *Salmonella* spp. isolates are resistant to amoxicillin. Further, 36.58% (15/41) of *E. coli* isolates and 88.88% (8/9) *Salmonella* spp. isolates are resistant to ampicillin. Multi-antibiotic resistance were also recorded for 29.27% (12/41) of *E. coli* isolates and 88.88% (8/9) of *Salmonella* spp. isolates as they are resistant to both amoxicillin and ampicillin. However, 97.56% (40/41) of *E. coli* isolates and 44.44%

Table 2: Antibiotic sensitivity profiles of *E. coli* isolates.

Antibiotic agent	Susceptible		Intermediate		Resistant	
	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates
Amoxicillin (25 µg)	≥ 18	3 (7.32%)	14-17	2 (4.88%)	≤ 13	36 (87.80%)
Ampicillin (10 µg)	≥ 17	14 (34.15%)	14-16	12 (29.27%)	≤ 13	15 (36.58%)
Chloramphenicol (30 µg)	≥ 18	33 (80.49%)	13-17	2 (4.88%)	≤ 12	6 (14.63%)
Streptomycin (10 µg)	≥ 15	40 (97.56%)	12-14	1 (2.44%)	≤ 11	0 (0%)

Table 3: Antibiotic sensitivity profile of *Salmonella* spp. Isolates.

Antibiotic agent	Susceptible		Intermediate		Resistant	
	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates	Breakpoint (Zone diameter in nearest whole mm)	No. (%) of isolates
Amoxicillin (25 µg)	≥ 18	0 (0%)	14-17	0 (0%)	≤ 13	9 (100%)
Ampicillin (10 µg)	≥ 17	0 (0%)	14-16	1 (11.11%)	≤ 13	8 (88.88%)
Chloramphenicol (30 µg)	≥ 18	8 (88.88%)	13-17	0 (0%)	≤ 12	1 (11.11%)
Streptomycin (10 µg)	≥ 15	4 (44.44%)	12-14	3 (33.33%)	≤ 11	2 (22.22%)

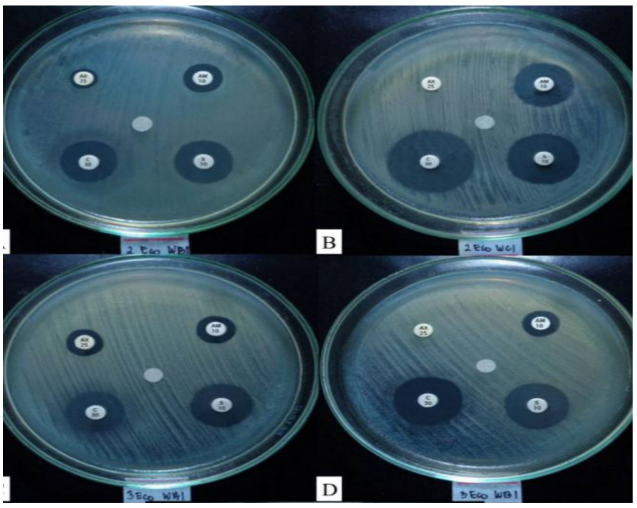

Figure 2: A-D. Representative disk diffusion assay plates of *E. coli* isolates showing the zone of inhibition by different antibiotics. (AM- ampicillin, AX- ammoxicillin, C- chloramphenicol, S- streptomycin)

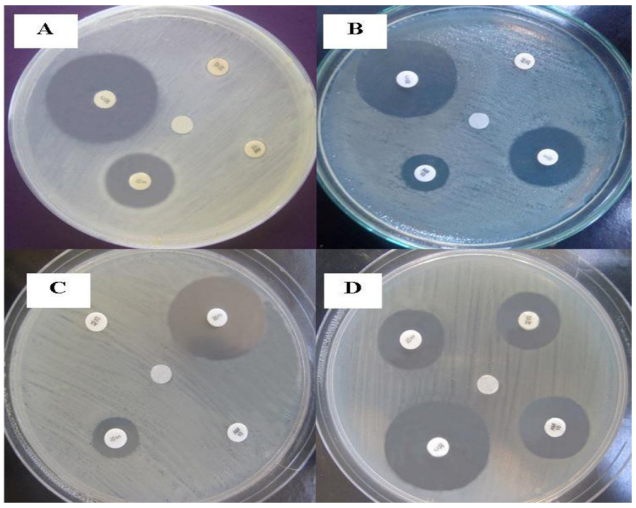

Figure 3: A-D. Representative disk diffusion assay plates of *Salmonella* spp. isolates showing the zone of inhibition by different antibiotics. (AM- ampicillin, AX- ammoxicillin, C- chloramphenicol, S- streptomycin)

(4/9) *Salmonella* spp. isolates are sensitive to streptomycin (Table 2 and 3). Representative disk diffusion assay plates are shown in Figure 2 and 3.

DISCUSSION

Microbial food safety is a continuing global concern because food-borne diseases can result to considerable high morbidity and mortality rates. Numerous studies in this field were focused on poultry products and had established knowledge that poultry products are vehicles for food-borne pathogens.[1,2,11-13] *E. coli* and *Salmonella* serovars are the most dominant members of Enterobacteriaceae family causing food-borne infections. *Salmonella* spp. can cause salmonellosis- one of the major food-borne diseases in many countries while *E. coli* can cause a wide variety of illnesses such as diarrhoea, urinary tract infections, respiratory illnesses and pneumonia, among others.[14] The present study revealed the occurrence of *E. coli* and *Salmonella* spp. in retailed chicken meat at Valencia City, Bukidnon, Philippines. This is the first report on the occurrence of bacterial contamination in poultry meat in the area. It is noteworthy to mention that there was a high occurrence of *E. coli* (41 of 50 samples) in meat samples than *Salmonella* spp. (9 of 50 samples). Recently, the high prevalence of *E. coli* in poultry products had been reported from studies across the globe.[11,15-18] For instance, the prevalence of *E. coli* in smallholder chicken flocks in Ontario, Canada was 99% and 47% of the isolates were resistant to one or more of the antimicrobials tested.[15] The high prevalence of *E. coli* in chicken carcasses is considered an indicator of poor meat handling and unsanitary abattoir. Since the intestines and the environment of chicken served as reservoirs for extraintestinal pathogenic *E. coli* (ExPEC) strains,[18] these ExPEC strains are easily contracted to chicken carcasses during butchery and meat handling, especially if sanitary and food quality measures are lenient.

Salmonella spp. is another important food borne pathogen that is also associated with poultry products. The present study reveals the occurrence of *Salmonella* spp. in retailed chicken meat which can be an added information to the prevalence of this bacterium in poultry products. Studies show that the prevalence of *Salmonella* spp. in chicken meat and/or in any poultry products is relatively low compared to that of *E. coli*.[11,13,15] In this study, 18% (9 of 50 samples) prevalence of *Salmonella* spp. in chicken meat was recorded. The study of Lebert *et al*.[15] recorded 0.3% *Salmonella* prevalence at the bird level in smallholder flocks. Moawad *et al*.[11] also reported 8.3% prevalence of *Salmonella* spp. in raw chicken and beef meat, lower than the reported prevalence of *E. coli*. Prevalence of *Salmonella* from swine, chicken and farm workers in farming communities in northern Thailand was 2%-25%, lower compared to that of *E. coli* which was 36.8% to 53%.[19] These relatively high prevalence of *E. coli* than *Salmonella* spp. in poultry products is due to the fact that *E. coli* is a normal flora of chicken intestines and therefore chickens are considered as reservoirs of this bacterium.[18] Recently, however, 100% prevalence of *Salmonella* in chicken meats and in poultry processing environments in wet markets in Penang and Perlis, Malaysia was reported by Nidaullah *et al*.[20] This supports the fact that poultry processing environments can also contribute to the high prevalence of *Salmonella*. Unsanitary environments can be colonised by this enteropathogen and can contaminate chicken carcasses when processed in such environments.

Along with microbial food safety issues, antimicrobial resistance is also a growing human health concern. Antimicrobial resistant bacteria can cause severe and chronic infections due to failed antimicrobial therapy which may lead to an eventual death. In the European Union, treatment failures by multi-resistant strains were reportedly the responsible for half of the 27,000 annual deaths from infections.[21] There is an increasing evidence that *E. coli* and *Salmonella* spp. from poultry environments and poultry products exhibit antimicrobial resistance due to the incorporation of antimicrobial growth promoter (AMGP) in feeds. The present study revealed high resistance of *E. coli* and *Salmonella* spp. to amoxicillin and ampicillin. It was reported by Diarra *et al*.[22] that multiantibiotic-resistant *E. coli* isolates can be found in broiler chickens regardless of the antimicrobial growth promoters used. This suggests that incorporation of any kind of AMGP in poultry feeds creates selective pressure to bacteria, pushing them to develop resistance. Further, application of antimicrobials in food animals is considered as a major determinant for the presence of antibiotic resistant bacteria in animal reservoir.[23] These antibiotic resistant bacterial strains may spread vertically between generations of flocks and/or may be contracted to humans, creating a huge pool of antibiotic resistant bacterial strains.

The high number of amoxicillin- and ampicillin-resistant *E. coli* and *Salmonella* spp. isolates obtained in this study implies that these antibiotics are commonly applied in poultry. Resistance to both amoxicillin and ampicillin was also recorded for 29.27% (12/41) of *E. coli* isolates and 88.88% (8/9) of *Salmonella* spp. isolates of this present study. Belenguer *et al*.[23] reported that treatment of growing chicks with amoxicillin increased the resistance of *E. coli* not just to amoxicillin but also to other antibiotics such as ampicillin. This may suggest that the use of even a single antibiotic in poultry may lead to the development multiantibiotic-resistant strains. The results of the present study are in agreement with other studies conducted in other countries. For instance, *E. coli* isolates from poultry meat samples collected from Bangkok City and suburb in Thailand were found resistant to ampicillin and tetracycline.[17] High resistance of *E. coli* and *Salmonella* spp. to amoxicillin and ampicillin were also recorded in Ontario, Canada.[15] *Salmonella* spp. isolates from chicken eggs in Dhaka City, Bangladesh also showed the highest percentage of resistance to amoxicillin and ampicillin.[24]

Collectively, the result of this present study adds information on the prevalence of *E. coli* and *Salmonella* spp. in poultry and poultry products. Moreover, the present report on the antibiotic resistant isolates is congruent with the previous reports on the rise of antibiotic-resistant and multiantibiotic-resistant strains from these environments. These should be taken seriously because these antibiotic resistant strains are serious human health threats. The use of antibiotics in poultry as AMGP and/or as treatment for avian diseases must be regulated to control the rise of antibiotic resistant bacterial strains.

CONCLUSION

We reported the occurrence and the antibiotic sensitivity profile of *E. coli* and *Salmonella* spp. isolated from retail chicken meat sold at various markets in Valencia City, Bukidnon, Philippines. Occurrence of *E. coli* in retail chicken meat is higher than that of *Salmonella* spp. and high number of both isolates are resistant to amoxicillin and ampicillin. Multi Antibiotic-resistant *E. coli* and *Salmonella* spp. isolates were also recorded. The results of this study must be taken as baseline information for concerned authorities to come-up with measures to control

the emergence of antibiotic-resistant bacterial strains in poultry farms in the Province of Bukidnon. Food safety measures in terms of poultry product processing from farm to market must also be imposed to eradicate if not lessen the occurrence of contamination along these processing lines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Nil.

SUMMARY

This article reports the high occurrence and antibiotic sensitivity of *E. coli* and *Salmonella* spp. in retail chicken meat at selected markets in Valencia City, Bukidnon, Philippines. *E. coli* and *Salmonella* were isolated from 41 and 9 meat samples, respectively (n = 50). Ninety percent of all isolates (n = 50) are resistant to amoxicillin, 46% are resistant to ampicillin, and 40% are resistant to both amoxicillin and ampicillin. Ninety six percent of the isolates are sensitive to streptomycin.

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