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Profiling Antibiotic Resistant Bacteria and Antibiotic Residues in Raw Chicken Products Sold around Kenyatta University, Kenya

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Abstract

Antibiotic resistant bacteria and antibiotic residues are a serious safety problem for animal food products. Poultry products have been long recognized as a reservoir for antibiotic resistant commensals and pathogens. Antibiotic residues ingested via food animal products expose gut micro-flora to low concentrations of antibiotics, which promote antibiotic resistance. However, there is limited knowledge regarding the potential of chicken products to act as a transmission corridor for the spread of the antibiotic resistant bacteria and antibiotic residues. The present study aimed at profiling antibiotic resistant bacteria and antibiotic residues in raw chicken products sold around Kenyatta University, Kenya. A total of 32 meat and egg samples were randomly collected from two study sites; KM and KU. Antibiotic residues in the study samples were detected using two microbiological techniques with *Bacillus subtilis* , *Staphylococcus aureus, Escherichia coli* , and *Salmonella typhi* as test organisms. Selective and differential media were used to isolate *Escherichia coli* , *Salmonella* and *Shigella* from the samples. Antibiotic susceptibility testing of these isolates against commonly used antibiotics was done using Kirby-Bauer antibiotic discs diffusion method. Of the total samples tested, 87.50% meat and 100% egg samples showed presence of antibiotic residues. The mean colony forming units (CFUs) of meat samples from KM (190.25×10^2 CFU) was higher than that of KU (104.96×10^2 CFU). Eggs from KM showed contamination (158.88×10^2 CFU) than those sampled (108.29×10^2 CFU) within the university outlets. *Escherichia coli* , *Salmonella* and *Shigella*, were resistant to ampicillin. *Escherichia coli* showed intermediate resistance to tetracycline while *Escherichia coli* and *Shigella* showed intermediate resistance to amikacin. This study reveals the presence of antibiotic residues and antibiotic resistant bacteria in chicken meat and eggs sold in the study area. Knowledge generated from this study is helps to develops effective strategies to control antibiotic resistance.

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antibiotics; antibiotic resistance; antibiotic residues

Introduction

The burden of antibiotic resistance (ABR) has a negative impact on the health and socio-economic status of the people (Ferri *et al*., 2017). The emergency and spread of ABR is

associated with the selective pressure exerted by antibiotic use in the community, farm agriculture, veterinary health, aquaculture, hospitals, and the environment. The World Health Organization has warned that inappropriate use of antibiotics in agriculture and food systems may lead to increased food insecurity and food safety hazard (WHO, 2017). The most substantial use of antibiotics worldwide is in the production of animals where they are used for therapeutics, prophylaxis or growth promoters in animal feed (Silbergeld *et al*., 2008; Cháfer-Pericás *et al*., 2010; Abdullahi *et al*., 2015). The practices risk exposure of human consumers to ABR food-borne pathogens and commensals via animal food products and the environment. Already, about 95% of the world food-borne infections are associated with ingestion of contaminated animal food products, poultry products and seafood products (Akbar and Anal, 2011; Jans *et al*. 2018; Sugrue *et al*., 2019).

Furthermore, incorrect application of antibiotics has led to noticeable deposits of xenobiotics in meat, milk, eggs, cheese and other livestock products. Chronic exposure of antibiotics and their derivatives to commensal microorganisms has triggered the development of resistant strains of bacteria because of bioaccumulation of antibiotic residues in animal tissues (Pavlov *et al*., 2008). Although humans are not the target organism for these drugs, they consume copious amounts of them as residues in food, which potentially destabilizes and eliminate normal intestinal microflora. Other possible pathological effects of these xenobiotics include allergies, carcinogenicity, mutagenicity, teratogenicity, nephropathy, immunological disorders, hepatoxicity, and reproductive disorders (Panigrahi *et al*., 2017).

Commensal bacteria like *Escherichia coli* and *Salmonella* found in livestock are frequently present in fresh meat products and they may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans. In other cases, the intestinal microflora adapts to these antibiotics and their metabolites by developing resistance and consequently transfer antimicrobial resistance genes to clinically relevant pathogens (Manyi-Loh *et al*., 2018). The pathogens can be transferred from animals to humans, indirectly through food or directly during handling and processing, thereby posing a threat to public health (Nyamboya *et al*., 2013). Mathur and Singh (2005) noted that the food chain is a major route of antibiotic resistance transmission between animals and human populations. Subsequently, commensal bacteria are suspected of serving as resistance reservoirs as they can transfer resistance genes to pathogenic bacteria.

Poultry farming is one of the most important small-scale agricultural businesses in sub-Sahara Africa. The population in this region depends partially on poultry farming for home consumption and monitory value from the sale of poultry and poultry products. In Kenya, small scale poultry farming is practiced in most cities with a per capita consumption of poultry meat and eggs at 1.1 Kg and 37.5 Kg per annum respectively (McCarron *et al*., 2015). Nonetheless, poultry production in Kenya is still constrained by ABR. Antibiotics are used indiscriminately on poultry treatment and in their feed, raising a lot of concern over their effects on the quality of poultry products. While antibiotic use in food animals may represent a risk to human health, the degree and relative efforts to combat this have not offered optimum solution (Darwish *et al*., 2013). Accurate information on the flow of ABR strains in food products is still lacks in Kenya. There is a need to carry out investigation whose results can help understand the local ABR and antibiotic residues in poultry food products to ensure food safety and food security.

Materials and Methods

Study area

Sample collection was carried out at Kenyatta University and adjacent Kiwanja market in Nairobi, Kenya (1°10'59.0"S; 36°55'34.0"E) (Geographic positioning system (GPS, eTrex, USA). These sites constitute the leading vendors of chicken meat and poultry products to the University community of more than 70,000 people. The chicken used for meat and other poultry products in the study area are outsourced from small scale farmers and other national suppliers across the country.

Sample collection

Overall, 32 samples of which 16 raw chicken meat and 16 eggs were collected from randomly selected outlets in KM open market and KU. The samples were collected in sterile zip-lock bags for two weeks. The samples were labelled and immediately placed in a cool box and transported to Kenyatta University Microbiology Research Laboratory for analysis. All the samples collected were within the required date for consumption.

Detection of antibiotic residues in chicken meat

First, 10 g of each meat sample was soaked in 100 ml ethyl acetate and crushed using a pestle in a sterile mortar. The solvent was then centrifuged at 6000 revolutions per minute (rpm) for 10 minutes. The supernatant was then transferred into a fresh sterile bijou bottle and sterile filter paper discs placed inside. The set up was left to stand until the solvent completely evaporated. These paper discs were then placed on spread plates of *Salmonella typhi*, *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* and incubated for 18 hours at 37°C (Kehinde *et al*., 2012). Paper discs dipped in ethyl acetate and subsequently evaporated were used as a negative control. In the second method, 1 mg pieces of fresh meat samples were cut and placed directly on spread plates containing the test organisms and incubated at 37°C overnight. Inhibition zones were observed and measured in millimeters (Myllyniemi *et al*., 2001).

Detection of antibiotic residues in chicken eggs

Presence of antibiotic residues on eggs was done using two methods. First, pieces of the eggshells were placed on spread plates containing the test organisms and incubated at 37°C for 24 hours. In the second method, wells were punched into spread plates of Muller Hinton agar with the test organism. A 0.1ml of egg albumen from each egg sample was placed in the wells. The plates were then incubated at 37°C for 24 hours. Zones of inhibition around the agar wells on each plate were observed and the absence or presence of antimicrobial residues recorded (Idowu *et al*., 2010).

Bacterial counts in raw chicken meat and eggs

One gram of the meat sample was aseptically homogenized in 99 ml of 0.85% saline; this effected a 10-2 dilution which was further serial diluted to effect a 103 diluent. From the homogenate, 0.1 ml was spread plated on nutrient agar plates in triplicate and incubated at 37°C for 24 hours. The colony forming units (CFUs) were then counted from each plate using a colony counter (Akusu and Wemedo, 2016). For microbial contamination of eggs, each was placed in a separate sterile plastic bag. A 100 ml sterile isotonic saline was added and washed thoroughly. A 0.1 ml of the washing was then spread plated on nutrient agar plates and incubated at 37°C for 24 hours. Viable cell counts were determined by

counting the CFU. The washing was also used for microbiological isolation of other contaminating organisms (Englmaierová *et al*., 2014).

Isolation and identification of *Salmonella*, *Shigella* and *Escherichia coli*

The fresh meat and egg washing from samples were each pre-enriched in buffered peptone water at 370C for 16 hours. The pre-enriched samples were sub-cultured and incubated for 24 hours at 37°C in Selenite F broth for selective enrichment of *Salmonella* and *Shigella*. This was then followed by plate streaking on DCA and *Salmonella* agar (Oxoid, Basingstokes, UK) and incubation was done at 37°C for 24 hours. To isolate *Escherichia coli*, the samples were enriched in lactose broth and after incubation sub-cultured on EMB agar (Oxoid, Basingstokes, UK) at 37°C for 24 hours. Typical characteristic colonies of *Escherichia coli*, with a green metallic sheen on EMB agar (Oxoid, Basingstokes, UK) and those of *Salmonella* and *Shigella* on DCA were purified by sub-culturing on nutrient agar (HiMedia, Mumbai India). The identity of the isolates was further confirmed by subjecting them to biochemical tests; Urease test, Triple Sugar Iron and IMViC (Indole, Methyl red, Voges-Proskauer and Citrate). All the media were inoculated with suspected isolates and incubated at 37°C for 18-48 hours, and the result interpreted according to the manufacturer's instructions (Kyung-Min *et al*., 2015).

Antibiotic sensitivity testing

Disc diffusion technique was used to determine the susceptibility of isolates to commonly used antibiotics in animal and human medicine as recommended by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015). The antibiotic-impregnated discs used include tetracycline (TE 30), chloramphenicol (C 50), ciprofloxacin (CIP 5) ampicillin (AX 10), Amikacin (AK 30) and gentamycin (GEN 10). Representatives of the isolates were spread plated on Muller Hinton agar and antibiotic discs placed on the agar and incubated at 370C for 24 hours. After incubation period, the diameter of the zones of inhibition was measured to the nearest millimeter using a digital calliper (0-150 mm).The isolates were classified as resistant, susceptible or intermediate.

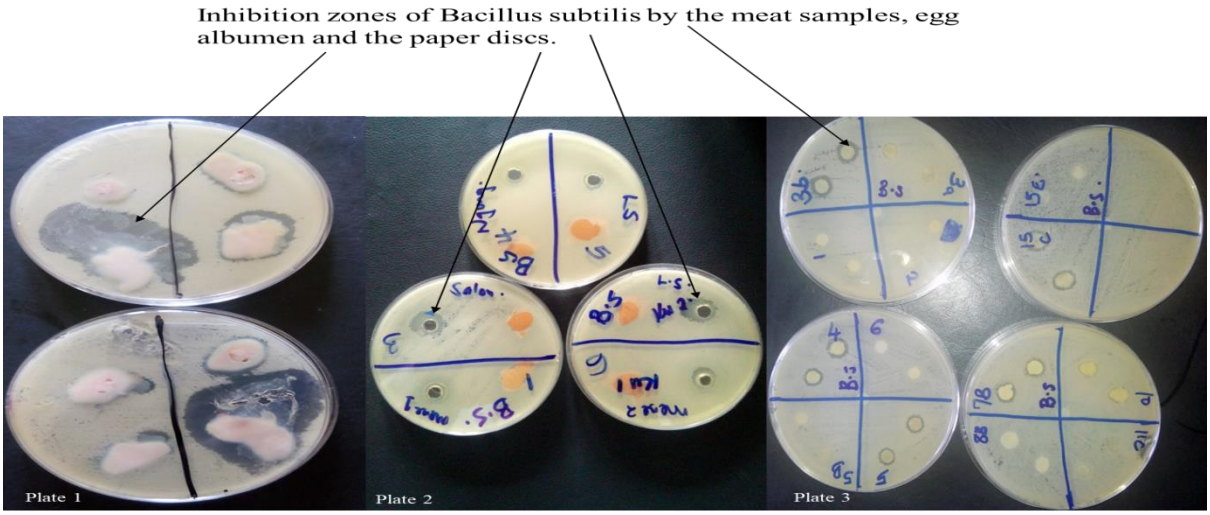
Data analyses

Antibiotic susceptibility means of inhibition zones, values for the abundance of the isolated pathogen and bacterial load values were subjected to ANOVA with significant difference determination among means using Tukey's Honesty Significant Difference test at P≤ 0.05. The analysis was done using Statistical Analysis System version 9.1 (SAS Institute, Cary, NC).

Results

Antibiotic residues in chicken meat

Bacillus subtilis and *Staphyloccocus aureus* were inhibited (Plate 1). Of the total meat samples, 14 (87.50%) showed inhibition zones on *Bacillus subtilis* , 7 from each sampling site. This was observed on both meat pieces tested and the paper discs, although the paper discs showed more distinct and clear inhibition zones. Out of the total meat samples, 6 from KU and 5 from KM showed inhibition against *Staphyloccocus aureus* (Figure 1.1).



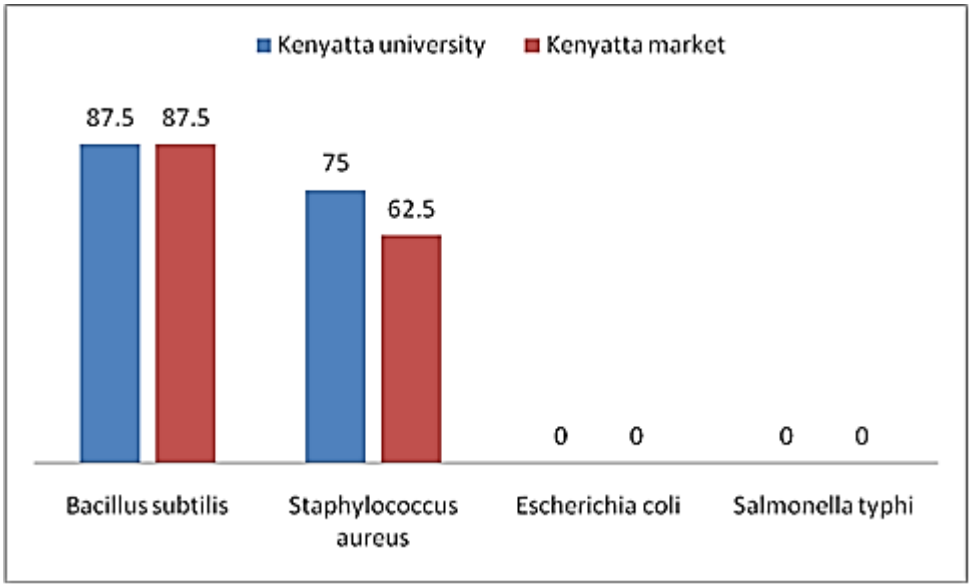


Figure 1.1 Percentage levels of inhibiting samples of antibiotic residues in chicken meat.

Antibiotic residues in chicken egg albumen and shells

Egg albumen of all the sampled eggs showed 100 % inhibition when tested against *Bacillus subtilis* and *Staphyloccocus aureus* (Plate 2). Of the total egg sampled, only two (6.25%), from different outlets, revealed inhibition against *Escherichia coli.* None of the samples inhibited the growth of *Salmonella typhi* (Figure 1.2). The eggshells did not show any inhibition when subjected to the test organisms.

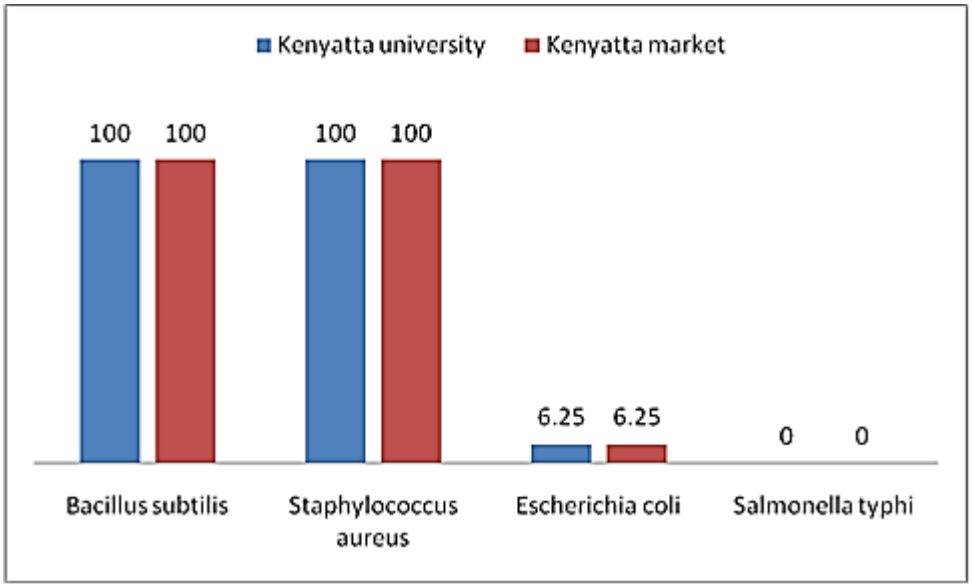


Figure 1.2. Percentage levels of inhibiting samples of antibiotic residues in egg albumen/

Bacterial counts of meat samples

The mean CFUs for chicken meat from KU and KM were 104.96 x 10^3/gm and 190.250 x10^3/gm, respectively (P≤0.05). Samples obtained from KM were significantly contaminated as compared to those obtained from KU. The highest bacterial load (288.00 x 10^3) was recorded in samples obtained from KM (P < 0.001). The highest bacterial load of the samples obtained from KU was recorded at 157.00 x 10^3 (P < 0.001). The least contaminated sample had a bacterial load of 57 x 10^3gm at P <0.001 and was from KU (Table 1.1).

Table 1.1: Mean values breakdown of CFUs of meat samples for KU and KM

KU		**KM**	
Meat sample	**Mean** ± SE	**Meat sample**	**Mean** ± SE
MU1	111.667 x 10^3±3.28^{cdef}	MM1	127.333 x 10^3±4.67^{cde}
MU2	125.000 x 10^3±12.34^{cde}	MM2	288.000 x 10^3±5.03^{a}
MU3	70.000 x 10^3±7.10^{fg}	MM3	242.00 x 10^3±26.62^{ab}
MU4	100.667 x 10^3±2.19^{edfg}	MM4	286.333 X 10^3±5.93^{a}
MU5	157.000 x 10^3±6.43^{c}	MM5	142.667 x 10^3±3.76^{cd}
MU6	131.667 x 10^3±4.49^{cde}	MM6	210.333 x 10^3±3.53^{b}
MU7	57.000 x 10^3±2.52^{g}	MM7	86.333 x 10^3±7.62^{efg}
MU8	86.667 x 10^3±7.42^{efg}	MM8	138.333 x 10^3±4.67^{cd}
Site mean	104.96 x 10^3±6.77^{b}	Site mean	190.25 x 10^3±15.37^{a}
P-value< 0.001		*P*-value < 0.001	

Key: MU; KU meat, MM; KM meat. Means values followed by the same letter within the columns are not significantly different according to Tukey's Honest Significance Difference (HSD) at 5% level

Bacterial counts on eggs

There was a significant difference the between bacterial population of egg samples obtained from KU and KM at P≤0.05 (Table 1.2). The mean bacterial count from egg washing of samples obtained from KU was 108.292 x 10^3 while that from egg washing of samples

obtained from KM was 158.875 x 10^3 both at P < 0.001. The highest bacterial load (286.667 x 10^3) was recorded at KM while the lowest bacterial load (52.667 x 10^3) was recorded at KU (P < 0.001).

Table 1.2: Mean values ± SE breakdown of CFUs of egg samples for the two study sites

KU		KM	
Egg sample	**Mean ± SE**	**Egg sample**	**Mean ± SE**
EU1	86.667 x 10^3±2.91^{hi}	EM1	286.667 x 10^3±6.36^{a}
EU2	145.333 x 10^3±6.36^{cde}	EM2	156.667 x 10^3±1,76^{cd}
EU3	117.667x 10^3±5.90^{efg}	EM3	93.000 x 10^3±6.56^{gh}
EU4	52.667 x 10^3±1.76^{j}	EM4	247.333 X 10^3±4.33^{b}
EU5	170.333x 10^3±3.84^{cd}	EM5	115.667 x 10^3±5.04^{fg}
EU6	150.667 x 10^3±7.69^{cd}	EM6	136.333 x 10^3±4.49^{def}
EU7	81.000 x 10^3±8.33^{hi}	EM7	156.000 x 10^3±6.43^{cd}
EU8	62.000 x 10^3±3.46^{ij}	EM8	79.333 x 10^3±3.53^{hij}
Site	108.292 x 10^3±8.74^{b}	Site	158.875 x 10^3±14.30^{a}
P-value < 0.001		P-value < 0.001	

Key: EU; KU eggs, EM; KM eggs, 1-8 sampling points. Mean values followed by the same letter within the columns are not significantly different according to Tukey's Honest Significance Difference (HSD) at 5% level.

Total isolated pathogens

Of the total samples analyzed, 14 (43.75%) were positive for *Salmonella*. Eight of these isolates were from chicken meat samples while six isolates were from the surfaces of eggs samples. About 6 (18.75%) of the samples were positive for *Shigella*. Three of the isolates were from chicken meat samples while the other three isolates were from eggs samples. Twenty-one samples (62.63%) were contaminated with *Escherichia coli*. Out of this, 12 were from chicken meat samples, while 9 were from egg samples (Table 1.3).

Table 1.3: Bacterial pathogens isolated from both sites

Site	Sample	*Salmonella sp.*	*Shigella sp.*	*E. coli*
	Meat (n = 8)	5	1	6
KU	Eggs (n = 8)	3	2	4
	Meat (n = 8)	3	2	6
KM	Eggs (n = 8)	3	1	5
Percentage		43.75%	18.75%	62.63%

The abundance of the isolated pathogen

The detection level of the isolated bacteria varied across the sampled outlets within KU at P≤0.05. The abundance of pathogens isolated from KU was significantly different (P= 0.0282). *Escherichia coli* were the most abundant at an average mean of 15.6 followed by *Salmonella* and *Shigella*. The abundance trend of isolates from KM samples was similar to that of KM isolates with E. coli registering the highest abundance (17.19). *Shigella* was the least abundant in KM with a mean of 4.69. Overall, the abundance of the isolates between KU and KM was not significant with both having a mean of 8.203 and 8.984, respectively (Table 1.4).

Table 1.4: Abundance of pathogenic bacteria isolated from the study sites

Isolate	KU	KM
E. coli	15.63±3.13[a]	17.19±1.56[a]
Salmonella sp	12.5±3.13[ab]	9.38±2.56[ab]
Shigellasp	4.69±0.56[ab]	4.69±0.56[ab]
P-value	0.0282	0.0312
SITE	8.203±2.50	8.984±2.16
P-value	0.0729	

Key: Mean values followed by the same letter within a column are not significantly different according to Tukey's Honest Significance Difference (HSD) at 5% level.

Antibiotic sensitivity testing

The results showed that within each isolated bacterial genus, the inhibition was significantly different (P≤0.05) against the tested antibiotics. All the isolates showed significant zones of inhibition against ciprofloxacin, followed by chloramphenicol (Table 1.5). The isolates were 100% susceptible to ciprofloxacin and gentamicin. None of the isolates was susceptible to ampicillin. All *Escherichia coli species* and *Shigella species* were resistant to ampicillin, while 83% *Salmonella* were resistant to ampicillin (Table 1.5)

Table 1.5. Inhibition zones in mm of the isolates to the tested antibiotics

Antibiotic	Test organism		
	Salmonella sp.	***E. coli***	***Shigella sp.***
Amikacin (30)	17.5±0.55[b]	16.833±0.75[b]	17.333±1.53[b]
Ampicillin (30)	7.33±3.27[c]	6.000±0.00[c]	8.67±4.62[c]
Chloramphenicol (50)	28.667±1.12[a]	29.667±1.03[a]	30.667±0.58[a]
Ciprofloxacin (30)	30.667±1.21[a]	29.33±4.23[a]	30.00±4.36[a]
Gentamycin (10)	19.333±1.21[b]	19.5±1.23[b]	19.333±0.58[b]
Tetracycline (50)	19.333±1.21[b]	19.17±4.26[b]	18.333±1.53[b]
P-VALUE	< 0.001	< 0.001	< 0.001

Key: Means followed by the same letter within a column are not significantly different according to Tukey's Honest Significance Difference (HSD) at 5% level.

Table 1.6. Antimicrobial susceptibility profiles of the isolates

Antibiotic	Percentage inhibition of all the isolates tested								
	***Salmonella* sp. (n=6)**			***Escherichia coli* (n=6)**			***Shigella* sp. (n=6)**		
	S	I	R	S	I	R	S	I	R
Ciprofloxacin(30)	100	0	0	100	0	0	100	0	0
Tetracycline (30)	100	0	0	84	16	0	100	0	0
Gentamicin (10)	100	0	0	100	0	0	100	0	0
Chloramphenicol (50)	100	0	0	100	0	0	100	0	0
amikacin (30)	100	0	0	67	33	0	67	33	0
Ampicillin (10)	0	17	83	0	0	100	0	0	100

Key: S=sensitive; I=intermediate; R=resistant; n=number of tested isolates

Discussion

The current findings revealed *Bacillus subtilis* as the highly susceptible bacteria to most of the samples of chicken meat and egg albumen. These results correspond with those of a study carried out in Khartoum by Elnasri *et al*. (2014) who reported susceptibility of *Bacillus subtilis* to most of the samples of chicken tissue. These results also suggest that the test organisms were subjected to samples which contained unspecific inhibitory substances (Myllyniemi *et al*., 2001). The potential of chicken meat and egg albumen to inhibit the growth of *Bacillus subtilis*, *Staphyloccocus aureus* and *Escherichia coli* depicts poultry products as have unspecified antibiotic residues and therefore, potential reservoir of antibiotic resistance. Susceptibility of these bacteria to the chicken meat and egg albumen could be linked to the use of antibiotics in poultry production (Mund *et al*., 2017).

Samples obtained from KM were significantly contaminated as compared to those obtained from KU. The samples evaluated in this study came from two different sites whose outlets serve almost the same clients. The difference in the contamination could be attributed to poor handling of poultry food products. According to Jans *et al*. (2018), transmission of bacteria between food animals and humans occurs during the handling of animal food products at production, distribution, and household levels. There is limited sanitary knowledge for most food handlers at KM as opposed to KU food handlers who have some level of knowledge in sanitation as trained caterers. In a related study carried out in Kumasi, Ghana, it was found that street food was more contaminated with bacteria, especially enteric bacteria caused by improper handling of food (Feglo and Sakyi, 2012).

The most common bacterial contaminant in this study was *Escherichia coli*, followed by *Salmonella*, and *Shigella*, which gives evidence of the presence of enteric bacterial contamination in the tested samples within the study areas. Wong *et al*. (2009) documented that *Salmonella* mostly contaminate meat from faecal material during slaughter and processing. The authors added that *Salmonella* sometimes might be present internally in meat tissue of infected animals. The difference in bacterial contamination of samples in the current study can be associated with the preservation methods used. During sampling, most of the meat samples from KM were not refrigerated, while in KU all the samples were kept in a cold room. In a related study, Mensah *et al*. (2002) who investigated the microbial quality of foods sold on streets of Accra, Ghana and factors predisposing to their contamination reported similar findings. Rane, (2011) reported that in developing countries, food sold in open-air markets are mostly contaminated with *Salmonella* sp., *Escherichia coli*, and *Shigella* sp. The researcher associated this with poor handling techniques of food and lack of proper storage facilities.

The results revealed a high percentage of *Salmonella*, *Escherichia coli* and *Shigella* resistance to ampicillin and high susceptibility to ciprofloxacin, gentamicin and chloramphenicol. This result corresponds with those of Nyamboya *et al*. (2013) who reported high resistance against ampicillin and susceptibility to chloramphenicol and gentamicin by bacteria isolates from different abattoirs in Nairobi. Tetracycline and amikacin were moderately resisted by *Shigella* and *Escherichia coli*. The existence of resistant bacteria in the chicken meat and egg albumen samples reflect the potential spread of these bacteria in human consumers as well as the transfer of resistant components among the bacteria in the environment (Singer *et al*., 2016). The incidence of antibiotic resistant bacteria in poultry products as shown in the current study is a threat to public health as the bacteria could disseminate antibiotic resistant genes to other bacteria of human clinical significance (Woolhouse *et al*., 2015). Poultry products could, therefore, act

as conduits for the dissemination of clinically relevant antibiotic resistance to the environment.

Conclusions

There is a high level of antibiotic residues in chicken meat and eggs sold in KU and KM. These residues are in contact with commensals and clinically relevant bacteria on the same chicken products. There is, therefore, a high risk of these microbes adapting to the presence of the antibiotics and thus developing resistance to them. The chicken products in the study area are contaminated with bacteria pathogens; *Escherichia coli*, *Salmonella* and *Shigella*, which pose a health risk to the population. The isolates were resistant to ampicillin and relatively resistant to tetracycline. This study findings form the basis upon which intervention tools for monitoring the influence of animal origin food on the development of antibiotic resistant bacteria in the environment can be developed.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Author contributions

DKN and LMK conceived the idea for the study and designed the experiment. DKN and PKK collected data, performed the experiments and analyzed data. DKN and HAM prepared and reviewed the manuscript. All authors read and approved the final manuscript.

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