



Antibiotic Residues in Broiler Chicken Liver Tissues and Resistance Profiles of Isolated Bacteria: Findings and Implications on One Health

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ABSTRACT

Antibiotic residues may be found in food of animal origin and pose a risk to human health. This study detected antibiotic residues in chicken liver tissues and assessed the resistance profiles of bacteria isolated from the chicken liver tissues in Ishaka-Bushenyi municipality, Uganda. A cross-sectional study was conducted from March 2023 to November 2023 in which 20 broiler liver samples were collected from Ishaka-Bushenyi municipality markets in Western Uganda. The liver samples underwent a microbiological analysis to test for the presence of antibiotic residues, isolation of bacteria and determination of their resistance profiles to antibiotics. Overall, 20% of the samples tested against *E. coli* ATCC 25922 from Ishaka were positive for antibiotic residues while 10% from Bushenyi were positive for antibiotic residues. All the samples (100%) tested against *S. aureus* ATCC 25923 were positive for the presence of antibiotic residues from both Ishaka and Bushenyi. We isolated *E. coli* (45%), *Klebsiella spp* (35%) and *S. aureus* (20%). All the three bacteria were 100% resistant to metronidazole. Among the detected isolates, 88.9% of *E. coli*, 100% of *S. aureus* isolates, and 85.7% of *Klebsiella spp.* were susceptible to meropenem. This study revealed that broiler chicken samples collected from Ishaka and Bushenyi markets contained antibiotic residues. Consequently, the isolated pathogens from the broiler chicken samples showed high resistance to antibiotics that are commonly used in humans. There is a need to restrict the use of antibiotics in food-producing animals like broiler chickens because humans end up consuming antibiotic residues.

HIGHLIGHTS

- Antibiotic residues present in food of animal origin pose a threat to human health.
- There is a need to restrict the use of antibiotics in food-producing animals.

Keywords: Antibiotic residues, Chicken Liver tissues, *Escherichia coli*, *Staphylococcus aureus*, One Health, Uganda

Poultry farming is an emerging industry in the livestock sector in Uganda employing thousands of farmers (Pius, Strausz and Kusza, 2021; Samuel *et al.*, 2023). Livestock contributes approximately 5% to the national gross domestic product (GDP) under which poultry farming falls (Behnke and Nakirya, 2012; Byaruhanga *et al.*, 2017). The increasing market for poultry products (meat

and eggs) has increased their demand and hence finding various means of increasing productivity. Antibiotics are

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used for growth promotion, prophylactic and treatment of diseases under veterinary medicine (Hind, Adil and Rade, 2014; Mikecz *et al.*, 2020). However, there have also been reports of the use of human antibiotics in poultry farming in recent times (Donoghue, 2003; Nayiga *et al.*, 2020). The use of antibiotics by farmers in livestock including poultry to enhance growth has been reported in various countries including Uganda (Donoghue, 2003; Nayiga *et al.*, 2020; Azabo *et al.*, 2022; Mudenda *et al.*, 2022).

The use of antibiotics has improved the health and well-being of poultry by decreasing the occurrence of disease (Agyare *et al.*, 2018; Mehdi *et al.*, 2018). The most commonly reported antibiotics in poultry products are tetracyclines, penicillin, aminoglycosides, macrolides, and sulphonamides (Azabo *et al.*, 2022; Mukosha, *et al.*, 2022; Chilawa *et al.*, 2023). The increasing number of farmers in poultry farming also increases the risk of use of these antibiotics for prophylaxis, growth promotion, and treatment of infections (Boamah and Agyare, 2016; Agyare *et al.*, 2018; Nkansa *et al.*, 2020; Koirala *et al.*, 2021; Chowdhury *et al.*, 2022). However, if withdrawal periods of antibiotics are not observed, antibiotic residues may appear in different animal products such as milk, eggs, and meat as reported by various studies (Hind, Adil and Rade, 2014; Ribeiro *et al.*, 2023). Since humans are the primary consumers of animal products, it has been discovered that prolonged exposure to antibiotics causes major health issues in people (Mughini-Gras *et al.*, 2014; Rasmussen *et al.*, 2015).

Increased presence and exposure to these antibiotics in poultry can lead to many pathological effects or even be risk factors for their occurrence which may include; allergies, spreading of drug-resistant microorganisms, potential harmful effects on human intestinal microflora, immunopathological effects, autoimmunity, carcinogenicity (sulfamethazine, oxytetracycline, furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol), allergy (penicillin) (Nisha, 2008; Hind, Adil and Rade, 2014). Furthermore, *Salmonella* spp., avian influenza virus, and bacteriophages that can lyse *Salmonella* have all been found in these production systems, according to recent investigations conducted in Chile which may lead to an apparent higher risk of presenting infectious diseases such as Highly Pathogenic Avian Influenza (HPAI), Newcastle Disease

(ND), Salmonellosis, Gumboro disease, among others (Cornejo *et al.*, 2020).

Research also shows that the use of antibiotics to promote growth in livestock has led to the production of resistant strains of microorganisms which can transmit this resistance to other bacteria (Xu *et al.*, 2020; Kebirungi *et al.*, 2022; Mudenda *et al.*, 2023). Maximum residue limits (MRLs) in foods of animal origin have been established by the European Commission and are stated in the Commission Regulation to prevent end consumers from being exposed to risks from antibiotic residues (Bellisai *et al.*, 2023; European Medicines Agency, 2023). In Uganda, there is some evidence of antimicrobial-resistant bacteria isolated from poultry (Odoch *et al.*, 2017; Kakooza *et al.*, 2021, 2023; Samuel *et al.*, 2023). However, there is a paucity of information on the presence of antibiotic residues in chicken products and the resistance patterns of pathogens isolated from broiler chickens, especially the Bushenyi district. It is against this background that this study evaluated the antibiotic residues in the chicken liver tissues and antibiotic resistance profiles of *E. coli*, *S. aureus* and *Klebsiella* spp isolated from the same chicken in Ishaka-Bushenyi municipality.

MATERIALS AND METHODS

Study design, area, and population

The study employed a cross-sectional study that involved the collection of broiler chicken samples that were analyzed in the laboratory to assess antibiotic residues in chicken tissues as well as testing the resistance patterns of isolated bacteria from the tissues. The study was conducted from March 2023 to November 2023 in Ishaka-Bushenyi municipality in Bushenyi district. The study area is located in the South-Western part of Uganda, approximately 280 km away from the capital city Kampala (Sanchez *et al.*, 2020). The study samples were collected in two markets; Ishaka town and the other in Bushenyi town. Sample and data analysis were conducted at Kampala International University in the Chemistry and Microbiology Laboratories both located along Mbarara-Kasese highway, Ishaka, Ishaka-Bushenyi municipality, Bushenyi District. This study was conducted on chicken tissue samples (liver) of slaughtered chicken sold in Ishaka-Bushenyi municipality markets. This study included chicken liver samples

collected from freshly slaughtered chickens in the market places in Ishaka and Bushenyi markets and chicken tissue samples from consented sellers. Conversely, samples from chickens that were slaughtered two or more days before the day of sample collection were excluded from this study.

Sample collection

20 fresh chicken liver tissue samples from both local (indigenous) broilers slaughtered on site and commercially reared broilers were collected randomly from different market areas using a simple random sampling technique according to (Ya *et al.*, 2016) in Ishaka-Bushenyi Municipality (Ramatla *et al.*, 2017). Dates and the name of the market were recorded. After collection, samples were packed in well labelled sterile polythene bags assigned a code; ISH1, ISH2, ISH3, ISH4, ISH5, ISH6, ISH7, ISH8, ISH9, ISH10, BUS1, BUS2, BUS3, BUS4, BUS5, BUS6, BUS7, BUS8, BUS9, and BUS10 and transported under aseptic conditions in an icebox to the Kampala International University Chemistry for extraction process. The samples were stored at 2-8°C and analyzed within 48 hours.

Sample preparation

The samples were prepared according to procedures used in a previous study (Matubber *et al.*, 2021). A total of 5 grams of each chicken tissue sample (liver) were weighed, cut into small pieces with a knife, ground and blended. A total of 10 mL Phosphate Buffer Saline (pH-6.5) was added and mixed by vortexing. The sample was then mixed with 2 mL 30% TCA and then centrifuged at 60000 rpm for 20 minutes. The supernatant was collected and filtered with Whatman 1 filter paper and funneled into a measuring cylinder. To the filtrate, an equal amount of diethyl ether was added and left for 10 min at room temperature. The bottom layer was collected and supernatant extraction was repeated twice using diethyl ether. The final volume of the extracts was pooled carefully into a test tube, sealed and kept in a refrigerator for analysis.

Method for detecting the antibiotic residues

The Well diffusion method adopted from another study was used in this study (Balouiri, Sadiki and Ibnsouda,

2016). Mueller Hinton agar plate surfaces were inoculated by spreading a volume of the standard microbial inoculums (ATCC25922 and ATCC25923) over the entire agar surface. Holes with a diameter of 8 mm were punched aseptically with a sterile 8 mm cork borer. A volume (20–100 µL) of the sample solution was introduced into the wells (holes made) in triplicates. The agar plates were incubated at 35-37 °C for 24 hours. The diameters of the zones of inhibition were then measured, the average zones were calculated and recorded and compared to the United States Department of Agriculture Laboratory Guidebook for Bioassay for the Detection, Identification and Quantization of Antimicrobial Residues in Meat and Poultry Tissue.

Method for determining the resistance pattern of the bacteria

Disk diffusion method (apparatus) sample preparation and the procedure was carried out according a previous study (Ramatla *et al.*, 2017). A total of 15 grams of each chicken tissue sample were cut into small pieces with a sterile knife and transferred aseptically into 200 ml of nutrient broth and mixed properly for about 5 minutes. The sample was enriched with nutrient broth and incubated for 24 hours at 37 °C. From the broth, a sterile wire loop was inserted to pick microorganisms which were sub-cultured on different agar (mannitol and MacConkey). From the different subcultures, organisms were picked with a sterile wire loop and put into normal saline to obtain Macfarlane turbidity (0.5 McFarland, corresponding to $\sim 1.5 \times 10^8$ CFU/mL) and from the suspensions, microorganisms were picked with a sterile cotton swab and spread onto plates with Mueller Hinton agar through the entire surface of the agar plates. The inoculum was allowed to dry for about 5 minutes. Using the Kirby- Bauer disk diffusion technique, four standard antibiotic disks each containing the specific concentration of antibiotics (gentamicin 10µg, metronidazole 10 µg, ceftriaxone 30 µg and meropenem 10 µg) were inserted per plate in triplicates. The agar plates were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured; the average zones were calculated and recorded in millimetres for interpretation with the guidelines of the Clinical Laboratory Institute Standards 2020. The results were recorded as sensitive, intermediate and resistant.

Quality control

To ensure quality, reproducibility and uniformity of the techniques and results of this study: Generally, proper hygiene and labelling of the samples to avoid any form of mix-ups in either method was maintained. Sterilization of equipment was carefully done using an autoclave to ensure sterility.

Data analysis plan

The data obtained from this study was run into Microsoft Excel 2016, GraphPad Prism, and IBM Statistical Package for Social Sciences (SPSS) version 25.0 and results were presented in the form of tables and graphs.

Ethical considerations

Permission was sought from the School of Pharmacy and the Research and Ethics Committee of Kampala International University. Recommendation letters from the School of Pharmacy and the Research and Ethics Committee of Kampala International University were obtained and used as introductory letters in the market places during sample collection for the laboratory study. A consent form explaining the benefit of the study was given to the market chicken vendors and consent signatures were obtained.

RESULTS

The findings of the present study showed a 20% (2 of 10 samples) presence of antibiotic residues tested against standard *E. coli* from samples obtained in Ishaka and a 10% (1 of 10 samples) presence of antibiotic residues tested against standard *E. coli* from Bushenyi (Table 1). All the 20 chicken liver samples (10 from each site) inhibited *S. aureus* ATCC 25923, indicating detectable antibiotic residues in 100 % of specimens (Table 2).

The findings revealed that *E. coli* showed the lowest sensitivity against the antibiotic residues in the chicken from both Ishaka and Bushenyi and *S. aureus* showed the highest sensitivity to the antibiotic residues in the samples (Fig. 1). Among the 10 isolates recovered from Ishaka, *E. coli* accounted for 5 (50 %), *Klebsiella* spp. for 4 (40 %), and *S. aureus* for 1 (10 %) of the total (Table 3).

Susceptibility pattern profiles of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* spp are shown in Fig. 2, 3, and 4. This study found that *S. aureus* was 100% resistant to metronidazole. However, *S. aureus* was 100% susceptible to meropenem, 75% susceptible to ceftriaxone and 75% susceptible to gentamicin (Fig. 2).

Escherichia coli isolates were 100% resistant to metronidazole and 55.6% resistant to gentamicin. Consequently, *E. coli* isolates were 88.9% susceptible to meropenem and 55.6% susceptible to ceftriaxone (Fig. 3).

This study found that *Klebsiella* spp were 100% resistant to metronidazole. Conversely, *Klebsiella* spp were 85.7% susceptible to meropenem, 85.7% susceptible to gentamicin, and 71.4% susceptible to ceftriaxone (Fig. 4).

Table 1: Presence of antibiotic residues in chicken liver with activity on *E. coli* ATCC 25922

Sampling site	Antibiotic residues with activity on <i>E. coli</i> ATCC 25922		P-value
	Absent	Present	
Ishaka	8 (80%)	2 (20%)	0.391
Bushenyi	9 (90%)	1 (10%)	
Total	17 (85%)	3 (15%)	

Table 2: Presence of antibiotic residues in chicken liver with activity on *S. aureus* ATCC 25923

Sampling site	Antibiotic residues with activity on <i>S. aureus</i> ATCC 25923		P-value
	Absent	Present	
Ishaka	0	10(100%)	—
Bushenyi	0	10 (100%)	
Total	0	20 (100%)	

Table 3: Prevalence of bacteria isolated from Ishaka and Bushenyi municipality, Uganda

Sampling site	<i>Klebsiella</i> spp	<i>E. coli</i>	<i>S. aureus</i>
Ishaka	4 (40%)	5 (50%)	1 (10%)
Bushenyi	3 (30%)	4 (40%)	3 (30%)
Total	7 (35%)	9 (45%)	(20%)

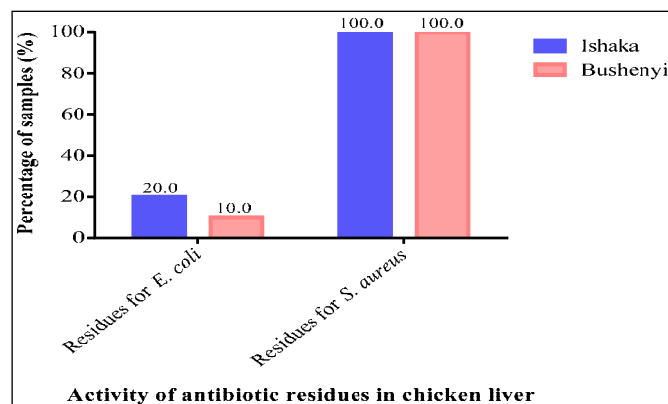


Fig. 1: Activity of Antibiotic residues detected in chicken liver on *E. coli* and *S. aureus*

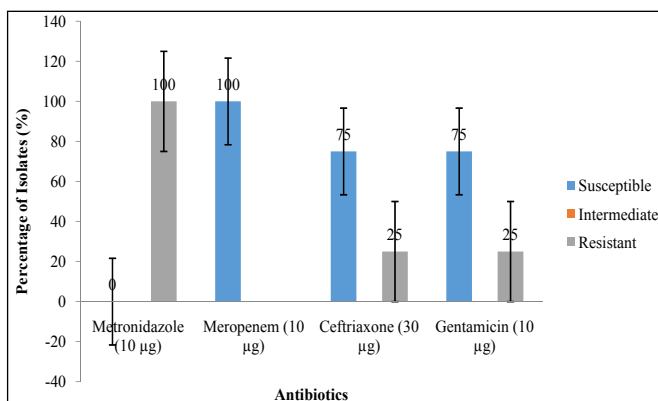


Fig. 2: Antibiotic susceptibility profiles of *Staphylococcus aureus*

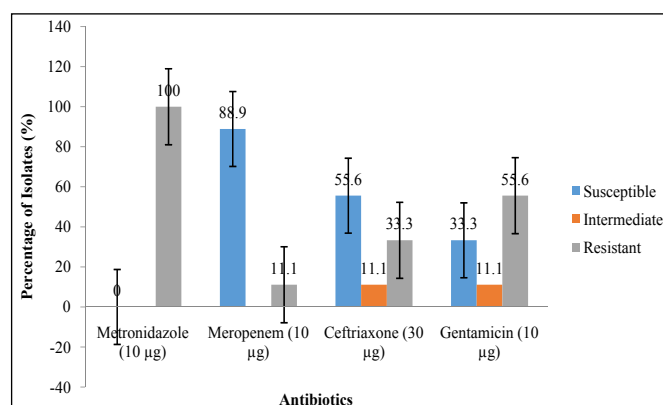


Fig. 3: Antibiotic susceptibility profiles of *Escherichia coli*

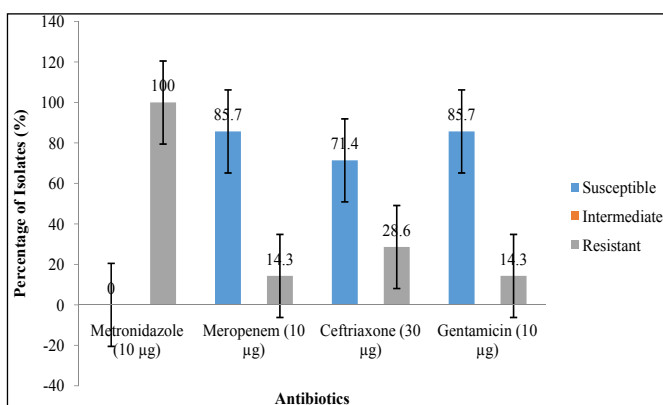


Fig. 4: Antibiotic susceptibility profiles of *Klebsiella spp.*

DISCUSSION

This study was conducted to detect the antibiotic residues in chicken liver tissues and how the presence of antibiotic residues in chicken tissues influenced the susceptibility patterns of the bacteria subsequently isolated from similar tissues in Ishaka- Bushenyi municipality in Kampala, Uganda. 20% (2/10) of Ishaka samples and 10 % (1/10) of Bushenyi samples showed inhibitory activity against *E. coli* ATCC 25922, corresponding to overall 15 % (3/20) positive samples. Additionally, this study found a 100% presence of antibiotic residues tested against standard *S. aureus* from both Ishaka and Bushenyi. The results obtained are comparable to the results obtained from a study conducted by Adla (2019) in Lebanon which reported the presence of antibiotic residues (Jammoul and Darra, 2019). This could be due to the increased use of antibiotics in the poultry sector for growth enhancement and prophylaxis in Uganda (Kebirungi *et al.*, 2022; Samuel

et al., 2023). The presence of antibiotic residues from chicken samples have also been reported in other studies and evident enough of the increased usage of antibiotics in the poultry sector (Jammoul and Darra, 2019; Sani *et al.*, 2023). Consequently, chicken meat that is contaminated with antibiotic residues can have harmful effects to humans and contribute to the ever growing problem of AMR (Mund *et al.*, 2017).

The findings revealed that *E. coli* showed the lowest sensitivity against the antibiotic residues in the chicken from both Ishaka and Bushenyi and *S. aureus* showed the highest sensitivity to the antibiotic residues in the samples. This could be because the antibiotic residues present in the samples have a lower activity against the gram-negative bacteria *E. coli* and a higher activity against gram-positive *S. aureus*. A similar study conducted in Algeria by Hakem (2013) showed similar results where there was a lower



sensitivity to antibiotic residues by gram-negative bacteria compared to gram-positive bacteria (Hakem *et al.*, 2013).

The present study findings showed that *E. coli* was the most bacteria isolated 9 (45%), followed by *Klebsiella spp* 7 (35%) then *S. aureus* was the least 4 (20%). The results are comparable to the study conducted in Egypt in 2021 where *E. coli* were the most isolated bacteria (Desouky *et al.*, 2021). Other studies have also shown high isolation of *E. coli* from chickens (Phiri *et al.*, 2020; Mudenda *et al.*, 2023). The difference could be due to the changes in area and environment.

Klebsiella spp showed 100% resistance to metronidazole, 85.7% were susceptible and only 14.3% showed resistance to meropenem, 71.4% were susceptible against ceftriaxone and 28.6% being resistant, 85.7% were susceptible and 14.3% showed resistance to gentamicin. These findings are comparable to a study by Gamal in Egypt where there was increased resistance against cephalosporins (Younis *et al.*, 2016). Meropenem and gentamicin showed the best activity against *Klebsiella spp* isolated and hence could be the best drugs for treating infections caused by *Klebsiella spp* acquired zoonotically from the chicken consumed in Ishaka-Bushenyi municipality which findings are comparable to a study conducted in Egypt by Ashraf (2021) where *Klebsiella* isolated from chicken showed a low resistance to meropenem and gentamicin (Abd El-Tawab *et al.*, 2022).

E. coli also showed 100% resistance against metronidazole, however, 88.9% of *E. coli* was susceptible to meropenem leaving only 11.1% resistant. For ceftriaxone, 55.6% was susceptible, 11.1% was intermediate and 33.3% was resistant. The isolated *E. coli* showed 33.3% susceptibility and 55.6% resistance against gentamicin. These results are comparable to those obtained in a study carried out in Saudi Arabia by Abdullah (2010) where *E. coli* isolates from Chicken, however, showed a much lower resistance against gentamicin (Altalhi *et al.*, 2010).

Our study found that *S. aureus* isolates were 100% resistant to metronidazole but 100% susceptibility to meropenem, 75% susceptibility and 25% resistance against ceftriaxone and gentamicin. The results obtained show a similar susceptibility pattern reported by a study by Hamed *et al.* (2021) where gram-negative bacteria showed a higher resistance to antibiotics (Hamed *et al.*, 2021). Similar

results were reported by a study conducted in Ethiopia in 2020 (Jemal *et al.*, 2020).

Meropenem however, showed better activity against all the bacteria across which makes it a better antibiotic of choice followed by ceftriaxone and gentamicin according to the study a study conducted by Elmanama (2021) in Gaza which showed good activity of carbapenems against bacteria isolated from poultry (Elmanama *et al.*, 2019). This could be because meropenem is one of the least used drugs reserved for resistant strains of bacteria and treatment of multidrug-resistant pathogen infections (World Health Organization, 2022; Mudenda *et al.*, 2023). Hence, exposure of chickens to meropenem is very low thereby maintaining its good activity against bacteria isolated from poultry.

Our study highlight the significance of a One Health approach in addressing AMR as discussed in several studies (Gray *et al.*, 2021; Alzahrani *et al.*, 2022; Habiba *et al.*, 2023; Mudenda, Chabalenge, *et al.*, 2023). This is because humans consume poultry products as a source of nutrition and end up consuming antibiotic residues.

CONCLUSION

This investigation indicates that there were antibiotic residues in chicken liver tissues in Ishaka and Bushenyi municipality in Western Uganda. This highlights the potential danger to consumers on risks of consuming antibiotic-contaminated chicken food products. It is therefore imperative to implement surveillance of antibiotic residues in food of animal origin which could be a preventive means that fully guarantees the safety of these food products. The results from this study also indicate an increased antibiotic resistance of bacteria against the used antibiotics hence emphasizing the importance of antibiotic stewardship and responsible use of antibiotics to prevent the emergence of antibiotic-resistant bacteria. It is also important to continue monitoring antibiotic resistance patterns in bacterial isolates to inform appropriate treatment strategies and prevent the spread of resistant strains.

Recommendations

Further analytical research needs to be conducted to identify and quantify the antibiotics to calculate the

risks and this requires reliable and specific detection and confirmation methods with appropriate detection and quantification thresholds as well as an official definition of an acceptable level in food as compared to the Maximum Residue limits by Europe.

Limitations of the study

Only 20 chicken liver samples were analyzed (10 from Ishaka and 10 from Bushenyi), which somewhat limited the generalizability of our findings to the wider poultry population in other regions.

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