Antimicrobial resistance of *Escherichia coli* and *Klebsiella* spp. isolated from poultry in the commune of Mont-Ngafula in Kinshasa, Democratic Republic of the Congo

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Summary

Background: In poultry farming, the widespread use of antibiotics to prevent and treat infections and promote growth has led to the emergence of resistant bacteria. As a result, bacterial infections can spread in the environment, contaminating humans and other animals. ***Aim***: To assess the sensitivity of enterobacteria to antibiotics on poultry farms in the peri-urban area of Kinshasa, by analyzing fecal samples collected from poultry, including laying hens, broilers and ducks in the commune of Mont-Ngafula. ***Methods***: Antibiotic-resistant enterobacteria were identified with a classic gallery and sensitivity tests were conducted on the isolated strains using the disk diffusion method with Mueller Hinton agar. ***Results***: Sample analyses identified 64 bacterial isolates, including 60 *Escherichia coli* isolates (94%) and 4 *Klebsiella* spp. isolates (6%). Antibiotic sensitivity tests revealed resistance to amoxicillin (83.3%), ampicillin (83.3%) and sulfadimidine (83.3%), while all isolates were sensitive to gentamicin and cefuroxime (100%). ***Conclusions***: The high bacterial resistance to a wide range of antibiotics in poultry constitutes a serious risk, which must be addressed by the structures responsible for animal and public health. A more in-depth study would improve our understanding of the antimicrobial resistance observed in poultry in Kinshasa.

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■ INTRODUCTION

Enterobacteriaceae form a family of gram-negative bacteria, comprising around sixty genera and more than 290 species have been identified so far (Janda & Abbott, 2021). In general, they are present in the normal gut flora in the digestive tract of humans and animals. They are also found in the environment (soil, water, plants), following contamination by fecal matter (Karib *et al*., 2021). Some species cause severe infections in humans (typhoid fever, bacillary dysentery, etc.). Similarly, some species can cause very deadly infections, leading to epizootics in domestic animals (Egbule, 2022). The enterobacteria spp. most often implicated in human infections are *Salmonella*, *Shigella*, *Campylobacter*, *Enterobacter*, *Escherichia coli (E. coli)* and *Klebsiella* (Khorsand et al., s. d.). Several species, including *E. coli* (Taggar *et al*., 2020), cause infections in animals (Fu *et al*., 2024). In poultry, a number of enterobacteria are naturally present in the digestive ecosystem. By contaminating poultry products (meat and eggs) intended for human consumption, they can be transmitted to humans. This constitutes a major public health issue (Ayinla & Mateus, 2023).

E. coli, which is considered a commensal microorganism, is a bacterial species with pathogenic potential. Indeed, it can acquire virulence factors via plasmids or other mobile genetic elements by

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horizontal gene transfer. This phenomenon enables *E. coli* strains to cause intestinal or extra-intestinal diseases. In poultry, the most common pathotype is avian pathogenic *E. coli* (APEC), which causes avian colibacillosis (Khong *et al*., 2023). *E. coli* strains are zoonotic and also considered to be virulent in humans (Khairullah *et al*., 2024). Several studies have revealed the genetic similarity between *E. coli* in humans and animals, which causes urinary tract infections, meningitis and septicemia in poultry and its by-products intended for human consumption. This supports the hypothesis that poultry could act as a reservoir of *E. coli* that is pathogenic to humans (Leinyuy *et al*., 2023).

Klebsiella species cause lower respiratory tract infections and liver abscesses. They are commonly found in a variety of ecological niches, including soil, food, skin, intestines and mammalian feces. Some studies have highlighted the role of food as a vector for the transmission of *Klebsiella* spp. in humans (Ramatla *et al*., 2024). Although *Klebsiella* spp. are not recognized as a foodborne pathogen, they have been identified in samples of fresh vegetables, goat and sheep skin samples, prawns and farmed chicken (Darniati *et al*., 2024).

Some of these bacteria have become resistant to certain families of antibiotics due to the extensive misuse of antibacterials. In poultry farming, infections that used to be commonplace are now fatal due to the widespread use of antibacterial drugs as prophylactics or growth promoters (Rahman *et al*., 2022). In their classic resistance mechanism, Enterobacteriaceae have the ability to produce beta-lactamases, enzymes that inactivate beta-lactam antibiotics, responsible for resistance to penicillins and cephalosporins (Worku *et al*., 2022). These bacterial enzymes can be found in different genera and strains of pathogenic or non-pathogenic bacteria, such as *E. coli*, *Salmonella* spp., *Klebsiella* spp. and other Enterobacteriaceae (Bertelloni *et al*., 2023). Many of the antibiotics commonly used in veterinary medicine belong to the same families as those used in human medicine (Olaru *et al*., 2023). Therefore, the use of antimicrobials in veterinary medicine could impact human health. Indeed, there is growing concern that resistant or multi-resistant bacteria could develop and be transmitted from animals to humans through the food chain or the environment (Kiskó *et al*., 2025). The misuse and overuse of antimicrobials for prophylactic and therapeutic purposes is a major factor in the emergence of, and the increase in antibiotic resistance. The use of antimicrobials as feed additives exacerbates the problem. As a result, microbes resistant to antibiotics and resistance genes are present in animal excreta, and antimicrobial residues are found in food of animal origin and animal waste, (Endale *et al*., 2023). The uncontrolled use of antibiotics in livestock production can increase the risk of human disease, as well as the costs of healthcare, with little economic benefit (Amine Alhadj *et al*., 2022).

The emergence and spread of antibiotic-resistant Enterobacteriaceae, therefore, represent a major animal and public health threat in both developed and developing countries (Esemu *et al*., 2022). In resource-limited countries, including many African countries, the threat is exacerbated by specific socioeconomic and behavioral factors (Sana *et al*., 2023). Diverse environmental factors may also make the situation worse, such as: naturally occurring resistance genes; the inadequate disposal of unused antimicrobials; contamination from waste in public settings, livestock farms, and pharmaceutical industries; and the use of chemicals in agriculture and sanitation (Abia Akebe *et al*., 2022).

In Kinshasa, the capital of the Democratic Republic of Congo (DRC), health monitoring on poultry farms depends mainly on the number of animals (Katunga *et al*., 2020). On farms with fewer than 100 animals, health monitoring is carried out by the farmers themselves. They give their animals antibiotics without seeking veterinary advice. They rarely use the correct dosage or follow recommendations for the duration or frequency of treatment. On farms with more than 100 animals, a vaccination schedule is applied. This is often combined with chemoprevention, which involves using (and often misusing) medicated feed to minimize the risk of disease. The abusive use of antibiotics tends to be carried out by agricultural engineers and assistants (98.4% of cases), rather than veterinarians and veterinary technicians (Moula *et al*., 2012). In addition, antibiotics are used as growth promoters in poultry farming. This unregulated practice poses a risk with regard to the emergence of antibiotic-resistant strains (Kiiti *et al*., 2021). The aim of this study was to determine the antibiotic susceptibility of *Escherichia coli* and *Klebsiella* spp. in poultry in a peri-urban area of Kinshasa.

■ MATERIALS AND METHODS

Study area

The study was carried out on poultry farms in the Lutendele, Kimwenza and Matadi Mayo neighborhoods in the commune of Mont-Ngafula, west of the city of Kinshasa. The choice of sites was determined by the number of poultry farms, the diversity of production (commercial broilers and layers) and the presence of traditional poultry farms. The commune is also home to a number of large industrial units with around 80,000 hens (commercial layers and broilers), which are the biggest suppliers of eggs and broilers in Kinshasa (Kinkela *et al*., 2017).

Sampling method

Our cross-sectional descriptive study examined poultry of different ages from various categories (local chickens, commercial layers, broilers and ducks), and from small, medium and large farms.

The sampling method was non-probabilistic and based on the 'snowball' sampling technique, i.e. the use of a resource person to identify additional units. Six farmers agreed to our visit and allowed us to investigate. Ten samples (consisting exclusively of droppings), were collected from the hen houses on each farm, giving us a total of 60 samples.

Sample collection

Before a farm visit, the protocol involved thorough hand washing and putting on personal protective equipment. Droppings were randomly collected directly from the cloaca using a sterile swab. Each swab was coded and kept in a cool box containing cold accumulators. Information about the samples was then filled in on the survey form. As soon as they were collected, the samples were sent to the Laboratoire Vétérinaire Central (LVC) in Kinshasa for bacteriological analysis. Analyses were conducted within two hours of receiving the samples.

Laboratory analyses

All the analyses carried out on the samples collected were performed in the bacteriology department at the LVC.

Bacterial isolation

Pre-enrichment was performed on all samples from fecal swabs placed in 1 ml of buffered peptone water, then incubated at 37°C for 18 h. Each pre-enriched suspension was transferred to methylene blue eosin gelose (EBM, Oxoid, UK) in petri dishes. This culture medium has the advantage of growing several enterobacteria. The plates were then incubated at 37°C and read after 24 hours. The colonies measuring 2 to 3 mm in diameter, which were flat, with dark violet coloration and a metallic sheen (beetle back), and sometimes appeared to confluence, were suspected of being *E. coli*. The large convex colonies of a pinkish color, with or without mucous, were suspected of being *Klebsiella* spp. The various characteristic colonies were re-incubated on blood agar at 37°C for 24 hours for purification.

Biochemical identification

A series of biochemical tests were used on Kligler-Hajna, Simmons citrate, urea-indole and mannitol media. One to two purified colonies were identified in the above-mentioned classical galleries. After 24 h of incubation, the Kligler-Hajna, Simmons citrate and Mannitol media were read by turning the medium. Kovac's reagent was added to the urea-indole medium to observe the presence of the red ring. By reading and interpreting the results, we were able to identify the colonies that showed biochemical reactions specific to each of the bacterial species isolated.

Antibiotic susceptibility testing

The disk diffusion method was used to determine the antibiotic susceptibility of the Enterobacteriaceae. A 0.5 McFarland inoculum – corresponding to approximately 1.5 × 108 CFU/mL (colony-forming units per milliliter) – was prepared using a densitometer. Using a swab, a spread was made and the antibiotics were placed 20 mm apart on a Mueller-Hinton agar plate. The following antibiotic discs were used (disc loading in µg): amoxicillin (25 µg), ampicillin (10 µg), cefuroxime (30 µg), gentamicin (10 µg), sulfadimidine (0.5 µg). After incubation at 37°C for 24 hours, the zone of inhibition was measured using a ruler and the results were interpreted as resistant (R), sensitive to standard dose (I) and sensitive to high exposure (S) according to the recommendations of the French Microbiology Society (Amara *et al*., 2024). *E. coli* ATCC 25922 was used as a quality control strain.

Statistical analysis

All data were checked for completeness, then encoded and entered using Epi-data version 4.6 (Lauritsen & Bruus, 2005) before being exported to Stata software for analysis. A frequency analysis was carried out to determine the frequency of antibiotic resistance.

■ RESULTS

Bacterial isolation and identification

Inoculation of these samples revealed 64 isolates, 60 of which (93.7%) were identified as *E. coli* and 4 (6.3%) as *Klebsiella* spp. *E. coli* species were found in all the samples analyzed in this study, whereas *Klebsiella* spp. were only present in 9% of layers, 9% of the local hens and 17% of ducks. The results for the enterobacterial strains identified by animal category are shown in Table I.

Table I: *E. coli* and *Klebsiella* spp. strains identified in poultry /// *Souches d'*E. coli *et de* Klebsiella *spp. identifiées chez les volailles*

Poultry category	Total of isolates	Frequency (%) *E. coli*	Frequency (%) *Klebsiella* spp.
Layers (3 months old)	10	10 (100%)	0
Broilers	10	10 (100%)	0
Pre-lay chickens	10	10 (100%)	0
Laying hens (over 18 weeks)	11	10 (91%)	1 (9%)
Local chickens	11	10 (91%)	1 (9%)
Ducks	12	10 (83%)	2 (17%)
Total	**64 (100%)**	**60 (93.7%)**	**4 (6.3%)**

Susceptibility of Enterobacteriaceae strains to antibiotics

In this study, we tested the sensitivity of isolated strains to five antibiotics: amoxicillin, ampicillin, sulfadimidine, gentamicin and cefuroxime. Overall, the results showed that all *E. coli* and *Klebsiella* spp. isolates were susceptible to gentamicin and cefuroxime, while a high level of resistance (83.3%) was observed for ampicillin, amoxicillin and sulfadimidine (Figure 1). Taken separately, *E. coli* strains showed higher resistance than *Klebsiella* spp. to ampicillin (85% versus 50%), amoxicillin (87% versus 25%) and sulfadimidine (83% versus 75%) (Figures 2 and 3).

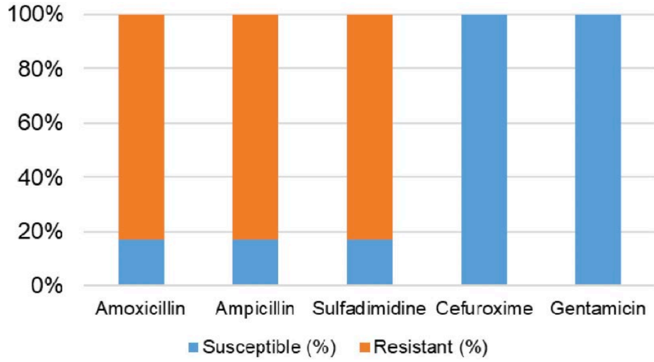

Figure 1: Sensitivity of *E. coli* and *Klebsiella* spp. isolates to antibiotics /// *Sensibilité des isolats d'*E. coli *et de* Klebsiella *spp. aux antibiotiques*

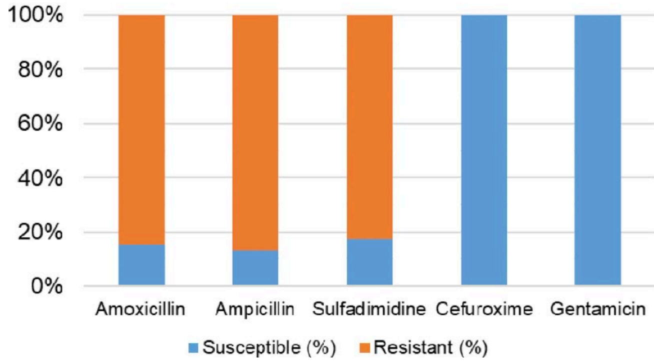

Figure 2: Antibiotic susceptibility of *E. coli* isolated from poultry in Kinshasa /// *Sensibilité des volailles à Kinshasa aux antibiotiques des* E. coli *isolés*

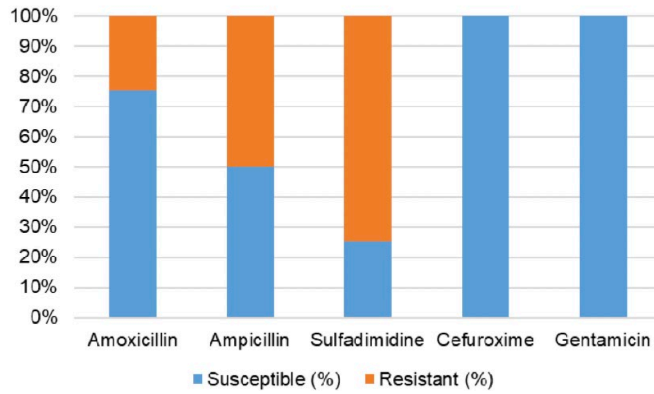

Figure 3: Antibiotic susceptibility of *Klebsiella* spp. isolated from poultry in Kinshasa /// *Sensibilité aux antibiotiques de* Klebsiella *spp. isolés de volailles à Kinshasa*

■ DISCUSSION

The results of this study showed that *E. coli* isolates were present in samples from all poultry samples (100%). Our findings are in line with a study conducted in Tanzania (Kiiti *et al*., 2021), where *E. coli* was detected in all broilers and layers, and with a study in Egypt (Ahmed, 2022), involving several hundred small poultry flocks. This can be explained by the fact that *E. coli* is naturally present in the gastrointestinal tract of poultry, a phenomenon that can lead to environmental contamination (through poultry droppings) and cross-contamination between animals of different species (Ugbo *et al*., 2023).

In addition, four isolates (6.3%) of *Klebsiella* spp. were identified in laying hens (9%), local hens (9%) and ducks (17%). Several studies have indicated the presence of these enterobacteria in poultry farms in both middle- (Munim *et al*., 2023) and low-income countries (Kimera *et al*., 2022). It has also been found in chicken meat (Projahn *et al*., 2019). Studies in Kenya (Ndukui *et al*., 2022), Nigeria (Jesumirhewe *et al*., 2023) and Indonesia (Permatasari *et al*., 2020) have identified the same strains in domestic poultry. These commensal bacteria, which are found in the digestive tract of humans and many animal species, are of crucial importance in human and veterinary medicine (Olaru *et al*., 2023).

The *E. coli* and *Klebsiella* spp. isolates identified in our study had high levels of resistance to ampicillin, amoxicillin and sulfadimidine regardless of origin (small family farms and semi-intensive poultry farms). This could be due to the inappropriate use of antibiotics, which are sold freely on the market at a relatively low cost. The resulting selective pressure could, therefore, lead commensal bacteria to develop cross-resistance between several classes of antibiotics (Egbule, 2022). These alarming findings underline the importance of a more rational approach to the use of antibiotics in veterinary medicine. Indeed, the misuse of antimicrobial agents to treat livestock, especially animals for food production, increases the risk of human exposure to antibiotic-resistant bacteria.

In this study, gentamicin and cefuroxime were the only two molecules effective on all the strains isolated. Comparable results were reported in a study in Nigeria (Ojja *et al*., 2024), where *E. coli* and *Klebsiella* pneumoniae were sensitive to gentamicin. Similarly, the studies carried out in Tanzania (Kiiti *et al*., 2021) and Ethiopia (Sarba *et al*., 2019) also demonstrated low enterobacterial resistance to gentamicin in poultry. This could be explained by the fact that both gentamicin and cefuroxime are expensive and consequently less widely used for poultry (Deng *et al*., 2024).

The high rate of enterobacterial resistance observed in this study is cause for concern. It could undermine the therapeutic value of a wide range of antibiotics for treating colibacillosis and possibly other avian or even human diseases of bacterial origin. Conducting further studies to identify the factors influencing the emergence of this resistance on poultry farms is essential. The regular use of antibiotics to control bacterial infections in poultry is a major public health risk and may contribute to the emergence of antibiotic resistance in *E. coli* bacteria (Ojja *et al*., 2024). The increase in antimicrobial resistance (AMR) affects both animal and human health sectors.

The emergence of colibacillosis due to strains of *E. coli* on poultry farms in Kinshasa has a considerable impact on animal production and welfare. The overuse of antibiotics may also affect the development of AMR bacteria that can potentially be transmitted to humans (Ebwa *et al*., 2020) Here, there is a risk of spreading resistance to pathogenic bacteria, such as Salmonella or Campylobacter (Chavan & Vashishth, 2025). Between December 2010 and February 2015, a rise in bird mortality on five broiler chicken farms located in the peri-urban areas of Kinshasa was observed, ranging from 3.9% to 25.6%. Mortality was attributed to *E. coli*, 86.2% of which was multi-resistant (Moula *et al*., 2012). Despite antibiotic treatment, no clinical improvement was observed. Bacteria can be transmitted to human populations through the consumption of contaminated foods. In Kinshasa, food sold in the open air is exposed to *Proteus*, *E. coli*, *Klebsiella*, *Salmonella* spp. and *Shigella*, which have been found on vegetables, breads and salted fish, respectively, by Mukeba *et al*. (2021), Umba *et al*. (2018) and Bumona Zayukua *et al*. (2019).

This study was limited to assessing the sensitivity of Enterobacteriaceae to a few antibiotic discs available on local markets (beta-lactams, aminoglycosides, sulfonamides). Further research will be carried out to determine the status of other antibiotics used on poultry farms for both the treatment and prevention of infections.

■ CONCLUSION

Results of this preliminary study showed the presence of *E. coli* in all the poultry examined (commercial broilers, laying hens, local hens and ducks), and of *Klebsiella* in ducks, local hens and commercial laying hens, respectively. The enterobacteria isolated showed high levels of resistance to several antibiotics, including amoxicillin, ampicillin and sulfadimidine. In addition, a substantial level of sensitivity was observed to gentamicin and cefuroxime. This study shows that poultry are a potential reservoir of antibiotic-resistant Enterobacteriaceae. This situation could cause treatment failures, facilitate the spread of antibiotic resistance, vertically and horizontally, and lead to the contamination of other bacterial species in the environment (through fecal matter). The results of this study suggest that larger studies, with more samples and a wider range of antibiotics, should be conducted to determine the extent of resistance on farms in Kinshasa.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Authors' contributions

DBK and TM participated in the design; TB, TM, DBK and BKM carried out the laboratory work and data analysis and interpretation. DBK, BKM, TM, JM and TB contributed to the interpretation and drafting of the manuscript. All authors read and approved the final manuscript.

Ethics Statement

To conduct this study, we obtained ethical approval from the Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine, Université Pédagogique Nationale in Kinshasa, DRC (REF. CE 205/FMV-UPN/2018).

Data availability

The data were not deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Résumé

Byakya Kikukama D., Kitoko Musala B., Banze T., Makoka T., Masumu J. Résistance aux antimicrobiens d'*Escherichia coli* et de *Klebsiella* spp. isolés de volailles dans la commune de Mont-Ngafula à Kinshasa, République démocratique du Congo

Contexte : En aviculture, l'utilisation généralisée d'antibiotiques pour prévenir et traiter les infections et favoriser la croissance a conduit à l'émergence de bactéries résistantes qui peuvent se propager dans l'environnement et contaminer l'homme et les autres animaux. ***Objectif*** : Afin d'évaluer la sensibilité des entérobactéries aux antibiotiques, notre étude s'est intéressée aux élevages de volailles dans la commune de Mont-Ngafula en zone périurbaine de Kinshasa. ***Méthodes*** : Des entérobactéries résistantes aux antibiotiques ont été isolées à partir d'échantillons fécaux prélevés sur des volailles, notamment des poules pondeuses, des poulets de chair et des canards. Elles ont été identifiées avec une galerie classique et des tests de sensibilité ont été réalisés à l'aide de la méthode de diffusion par disque sur gélose de Mueller Hinton. ***Résultats*** : L'analyse des échantillons a permis d'identifier 64 isolats bactériens, dont 60 isolats *d'Escherichia coli* (94%) et 4 isolats de *Klebsiella* spp. (6%). Les tests de sensibilité aux antibiotiques ont révélé une résistance à l'Amoxicilline (83,3%), à l'Ampicilline (83,3%) et à la Sulfadimidine (83,3%), tandis que tous les isolats étaient sensibles à la Gentamicine et à la Cefuroxime (100%). ***Conclusions*** : Cette forte résistance de bactéries à une large gamme d'antibiotiques chez les volailles constitue un sérieux risque, qui doit être pris en compte par les structures en charge de la santé animale et de la santé publique. Une étude plus approfondie permettrait d'améliorer notre compréhension de la résistance observée chez les volailles à Kinshasa.

Mots-clés : poulet, résistance aux antibiotiques, *Escherichia coli*, *Klebsiella*, République démocratique du Congo

Resumen

Byakya Kikukama D., Kitoko Musala B., Banze T., Makoka T., Masumu J. Resistencia a los antimicrobianos de las *Escherichia coli* y *Klebsiella* spp. aisladas de aves de corral en la comuna de Mont-Ngafula, en Kinsasa (República Democrática del Congo)

Contexto: En avicultura, la utilización generalizada de antibióticos para prevenir y tratar las infecciones y favorecer el crecimiento ha hecho emerger bacterias resistentes que pueden propagarse en el medio ambiente y contaminar al hombre y a los demás animales. ***Objetivo***: Para evaluar la sensibilidad de las enterobacterias a los antibióticos, nuestro estudio se interesa por la cría de aves de corral de la comuna de Mont-Ngafula, en la zona periurbana de Kinsasa. ***Métodos***: Se aislaron enterobacterias resistentes a los antibióticos a partir de muestras fecales tomadas en aves de corral, especialmente gallinas ponedoras, pollos para carne y patos. Se identificaron con una galería clásica y se realizaron pruebas de sensibilidad con la ayuda del método de difusión por disco sobre gelosa de Mueller Hinton. ***Resultados***: El análisis de las muestras permitió identificar 64 cepas bacterianas aisladas, entre las cuales había 60 cepas de *Escherichia coli* (94 %) y 4 cepas de *Klebsiella* spp. (6 %). Las pruebas de sensibilidad a los antibióticos revelaron una resistencia a la amoxicilina (83,3 %), a la ampicilina (83,3 %) y a la sulfadimidina (83,3 %), mientras que todas las cepas bacterianas aisladas eran sensibles a la gentamicina y a la cefuroxima (100 %). ***Conclusiones***: Esta fuerte resistencia de las bacterias a una amplia gama de antibióticos en las aves de corral constituye un serio riesgo, que deben tener en cuenta las estructuras encargadas de la salud animal y de la salud pública. Un estudio más profundo permitiría mejorar nuestra comprensión de la resistencia observada en las aves de corral de Kinsasa.

Palabras clave: pollo, resistencia a los antibióticos, *Escherichia coli*, *Klebsiella*, República Democrática del Congo