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Prevalence of *Escherichia coli* and *Salmonella spp* Strains Isolated from Chicken Feces and Their Resistance to Antibiotics by Cefotaximase (CTX-M) Enzyme Production

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Abstract: The Increase in antibiotic resistance is a threat to the world health. Some resistant bacteria have the ability to transfer from animals to humans either through their stool or through their flesh. The spread and emergence of antibiotic resistance is therefore a public health problem. For this study, a total of 101 chickens were randomly selected from the busiest recreation areas of Ouagadougou and fresh chicken droppings taken directly from the animal's intestines were collected. Of the samples analyzed, 78.21% and 9.90% respectively contained *Escherichia coli* and *Salmonella spp*. For the 10 isolates of *Salmonella spp*, a resistance to CTX-M of 10%, 10% to CAZ, 10% to CRO and finally 70% resistant to AMC was observed. For the 79 strains of *Escherichia coli*, it was observed an absence of resistance to CTX-M, 3.79% to CAS, 3.79% to CRO and 84.81% of strain resistant to AMC. Four (4) mutil-resistant strains were identified and resistance genes were observed in 2 of these strains. The study revealed the presence of extended spectrum beta-lactamase genes in Enterobacteriaceae contained in chicken feces. A high consumption of flambé chickens infected or contaminated by these germs is then likely to increase the risk of development and spread of bacteria resistant to antibiotics in humans.

Keywords: Extended Spectrum Beta-Lactamases (ESBL), CTX-M Gene, PCR

1. Introduction

Enterobacteriaceae are optional Gram-negative aero-anaerobic bacteria found throughout the soil, in the air, in water and also in the intestines of humans and animals. They include a very high number of genera and species that may be responsible for bacterial infections that could endanger human life. In response to these infections, antibiotics are used for therapeutic purposes [1]. Today, these bacteria have the ability to mutate to be resistant to these antibiotics [2]. Several factors may be responsible for human contamination, but fecal contamination is the most common. The infection strategy is colonization of mucous membranes [3]. Two enterobacteriaceae are mainly found in fecal bacterial infections, namely *Escherichia Coli* and *Salmonella spp* [3]. These enterobacteriaceae can be found in the environment, contributing to contamination of food crops, water sources and food animals [4]. Gastroenteritis due to these bacteria is one of the main causes of food poisoning in developed countries, and infant mortality in developing countries [5]. Infections caused by *E. coli* and *salmonella spp*

are becoming a major public health problem that is growing daily. Poultry and poultry products, including meat and eggs, have long been recognized as a major source of food infections. Overall, it has been estimated that the majority of salmonellosis outbreaks are caused by chicken, which is considered a means of transmission [6]. Hence the interest of our work on the study of strains of *Escherichia coli* and *salmonella spp* found in poultry and infecting humans. Burkinabè population most often consumes poultry on site, in leisure spaces or outdoor restaurants. In local restaurants, chickens are killed, plucked, cleaned and cooked in the same place and in most cases by the same person. This is the basis of a risk of contamination of the chicken flared by its droppings. Knowing that these droppings contain a wide variety of enterobacteriaceae including *E. coli* and *Salmonella spp*, it could have a risk of contamination of enterobacteriaceae pathogens to humans through the consumption of contaminated chicken. In the face of enterobacteria, the main families of antibiotics of therapeutic interest. are β-lactamines, aminosides and quinolones. β-lactamines are the first-line antibiotics in the treatment of infections [7]. However, from the beginning of their mass use in the 1940s, their efficacy was confronted with the production of inactivating enzymes: β-lactamases (Ruppé, 2010). Gastroenteritis strains are increasingly multi-resistant to antibiotics, they use a resistance mechanism which is the production of enzyme; β-lactamases, among which β-Lactamases with Extended Spectrum (BLSE) [8]. They are induced either by plasmids or by mutation. This mechanism gives the affected bacteria the ability to hydrolyze many antibiotics. In the early 1980s, only plasma enzymes TEM (Temoneira) and SHV (Sulfhydril variable) were known. The majority of BLSE derives from point mutation in the genetic sequence coding for the active site of the β-lactamases TEM-1 and SHV-1. More than 300 SLCOs have been described to date. Our study focused on Cefotaximase (CTX-M) found in E. coli and *Salmonella spp*. Our goal was to determine the presence of this resistance gene in E. coli and *Salmonella spp* in chicken droppings consumed outdoors.

2. Methods

2.1. Bacteriological Analysis

2.1.1. Sampling Strategy and Escherichia Coli and Salmonella spp Isolation

Samples of chicken feces were taken in sterile Eppendorf tubes directly from the animal's intestine in order to avoid any risk of contamination. these Eppendorf tubes contained a preservation medium (LB) and was stored in a cooler in order to maximize the amount of strain to collect. After sampling, bacterial isolation was performed on the selective EMB medium for *Escherichia coli* and on the SS medium for *Salmonella spp*. The sowing was done by the technique of streaks of exhaustion where a drop of saddle is sown on a medium of choice. Petri dishes were incubated at 37°C for 18 to 24 hours. After incubation, the dishes were examined for the presence of characteristic colonies of salmonella and *Escherichia coli*. *Salmonella spp* grows on the SS medium giving colorless colonies with a black center. *Escherichia coli* on selective medium EMB, are semi-domed purple colonies 2-3 mm in diameter with greenish metallic luster of dark center. Once the bacteria were isolated in the respective selective media, an identification by the API 20E gallery allowed us to confirm the presence of the strains sought.

2.1.2. Antibiotic Sensitivity

The isolated and identified bacteria were reseeded on solid medium MH and incubated for 18-24 hours to obtain pure colonies that were used for the antibiogram. The sensitivity of the strains to the different antibacterial agents was determined by the agar diffusion method. The pure cultures obtained on the MH medium were placed in a 5 ml suspension of saline solution (0.9% NaCl) equivalent to the standard McFarland 0.5 (108CFU). From this bacterial suspension, a 1/10 dilution in physiological water (0.9% NaCl) is carried out and well homogenized; then the whole was seeded by swabbing on Mueller-Hinton Petri dishes. The antibiotic discs Amoxicillin + Clavulanic Acid (AMC) 30µg, CTX 30µg, CAZ 30µg, and CRO 30µg were deposited at a distance of 20 to 30 mm using a clamp on the petri dish. The inhibition zones were measured and compared to the critical values according to the recommendations of the antibiogram committee of the French microbiology society (CASFM, 2020) and interpreted as sensitive (S), resistant (R), or intermediate (I).

2.2. Molecular Analysis

2.2.1. Extraction of DNA

A pure colony was collected from Petrie MH boxes and suspended in 200ul of distilled water previously aliquoted in labeled eppendorf tubes. The suspension was then soaked in a water bath at 100°C for 15 minutes to release the genetic material from the bacteria. After a 10 min centrifugation at 12000 rpm was performed and the supernatant containing the released DNA was transferred into a new eppendorf tube. After quantification and verification of DNA purity with Nanodrop, part of the supernatant was used for amplification and the remainder was retained at -20°C.

2.2.2. Detection of BLSE Genes

(i). Amplification by Classic PCR

The classic Polymerase Chain Reaction (PCR) was performed in a 25 µL reaction volume. This reactive volume was prepared as follows: 12.5 µl of green master mix + 0.5 µl of F primer + 0.5 µl of R primer + 10.5 µl of PCR water + 1 µl of DNA extract. An amplification program was used to investigate the BlaCTX-M gene (Table 1).

Table 1. *BlaCTX-M PCR Program.*

Parameters	***BlaCTX-M***		
Initial denaturing	96°C/5 mn		
Denaturing	96°C/1mn	number of cycles	35
Matches	50°C/1mn		
Elongation	72°C /1 mn		
Final elongation	72°C /10 mn		

The 1000 pb DNA fragments were obtained with the presence of blaCTX-M.

The following primer sequences were used: CTX-M fwd: GTT-ACA-ATG-TGT-GAG-AAG-CAG et CTX-M rev: *CCG-TTT-CCG-CTA-TTA-CAA-AC*

(ii). Electrophoresis on Agarose Gel

The amplified DNA fragments were separated by electrophoresis on a 1.5% agarose gel prepared in tris base solution - borate – EDTA 0.5X and containing ethidium bromide with a molecular weight marker of 1 kb as a reference to assess expected band sizes. The migration was carried out at a voltage of 120 millivolts (mV) for 45 minutes. The resulting migration products were viewed under UV light with the GENE FLASH device and the photos were recorded.

3. Results

3.1. Distribution of Samples by Source

A total of 101 stool samples were collected from several chicken-eating locations in Ouagadougou, specifically from open-air restaurants cooking "sin mooré. Our study classified the collection points by neighborhood. The majority of the samples came from Kamboinssin with a total of 25 samples (Figure 1).

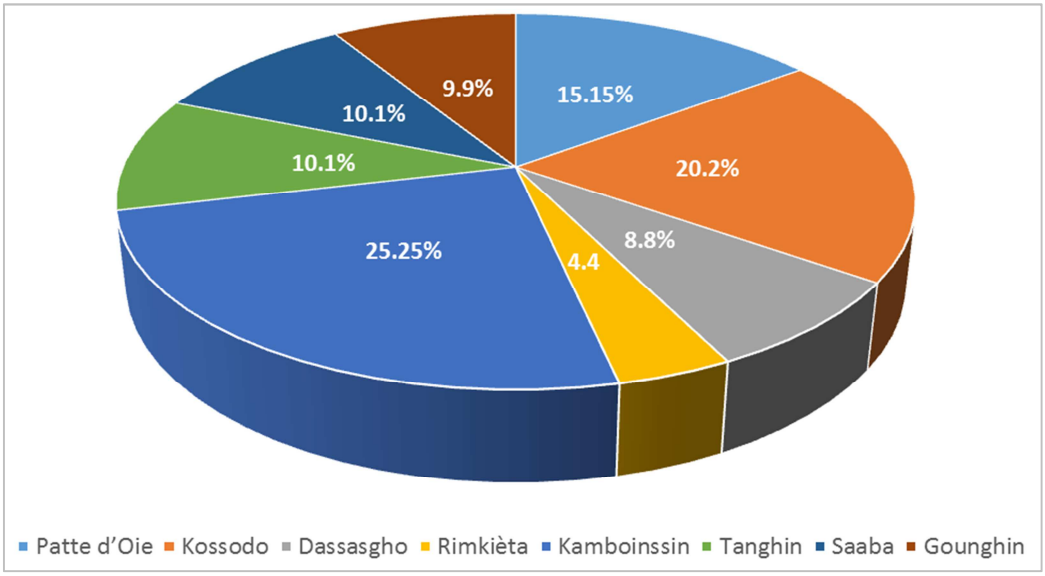

Figure 1. *Distribution of samples by location.*

3.1.1. Identification of Strains of Salmonella spp and Escherichia Coli

The 101 fecal samples were cultured on the selective EMB medium for *Escherichia coli* and on the SS medium for *Salmonella spp*. These cultures allowed us to isolate and identify the strains to be studied. Using the Api galleries, the identification of these strains was confirmed.

Seventy-nine (79) *Escherichia coli* strains (78.21%) out of 101 EMB dung samples were identified and ten (10) strains for *Salmonella spp* (9.90%) out of 101.

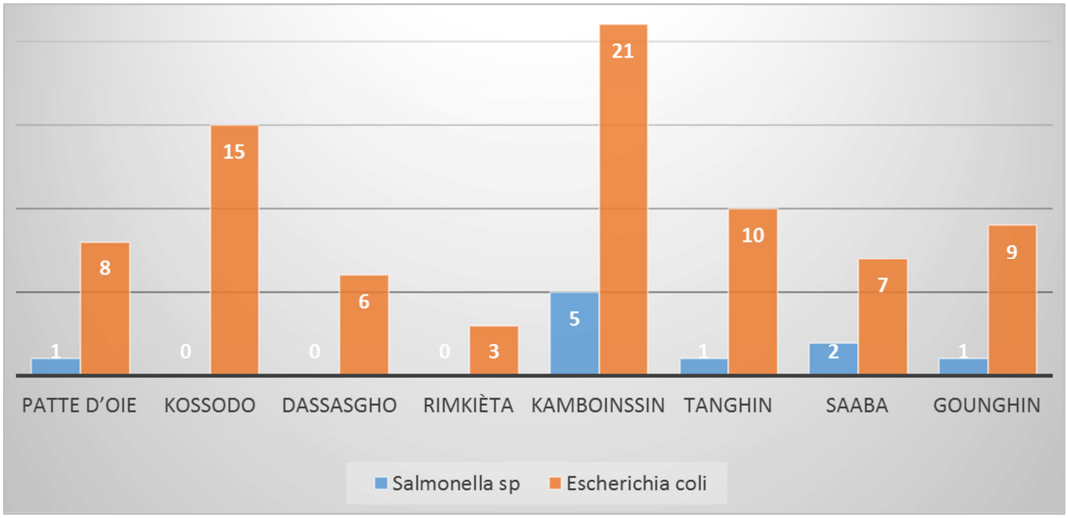

Figure 2. *Identification of enterobacteriaceae strains according to localities.*

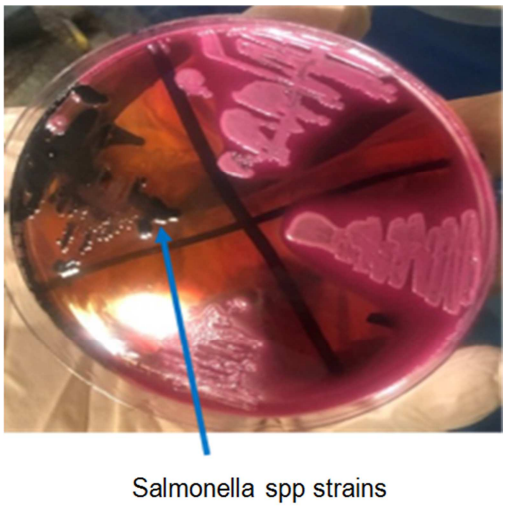

Figure 3. *Salmonella spp strains on SS medium.*

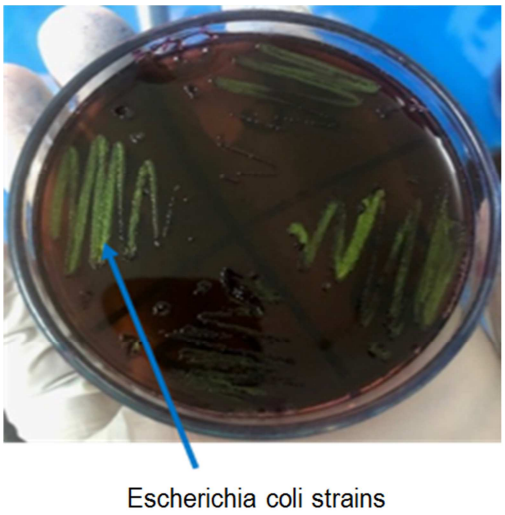

Figure 4. *Escherichia coli strains on EMB medium.*

3.1.2. Antibiotic Resistance Phenotype

Antibiogram was performed on 79 strains of *Escherichia coli* and 10 strains of *Salmonella spp*. We obtained seventy (70) strains all together resistant to at least one antibiotic (CAZ, CTX, CRO, AMC) (Figure 5).

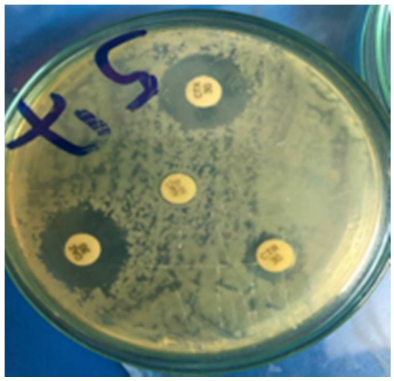

Figure 5. *Petri dish with a strain of Salmonella spp resistant to CRO, CAZ, CTX and AMC.*

3.1.3. Distribution of Resistant Bacterial Strains by Bacterial Species and Antibiotic

Out of the 10 strains of *Salmonella spp* we observed 7 strains resistant to at least one antibiotic. We therefore obtained a resistance to CTX of 10%, 10% resistance to CAZ, 10% resistance to CRO and finally 70% of strain resistant to AMC. We then have a total of 70% of antibiotic-resistant *Salmonella spp*.

For the 79 strains of *Escherichia coli,* we observe 67 strains resistant to at least one antibiotic. No resistance to CTX was observed. Three strains of resistant *Escherichia coli* (3.79%) to CAZ, three strains (3.79%) to CRO and 67 strains (84.81%) to AMC were identified. We then have a total of 84% of *Escherichia coli* resistant to at least one antibiotic.

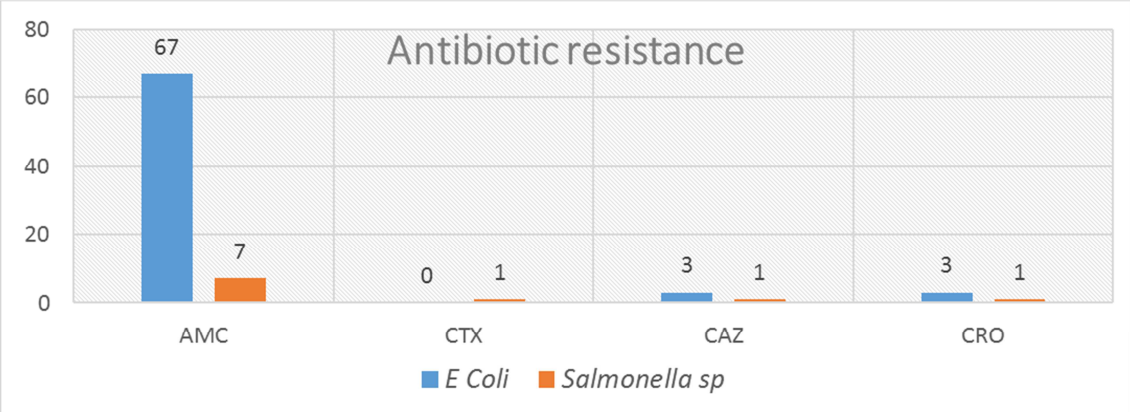

Figure 6. *Resistant bacterial strains by bacterial species and by antibiotic.*

3.2. Identification of the Resistance Gene Coding for CTX-M Type BLSE Production

At least one C3G resistant 04-strain DNAs were subjected to PCR for BLSE gene type research using specific primers for blaCTX-M. Examination of the PCR products obtained after agarose gel electrophoresis revealed that 2 isolates were carriers of CTX-M-type BLSE genes (Table 2 and (Figure 7).

Table 2. *Resistance genes encoding the production of BLSEs obtained.*

species	N°	CTX M
	40	X
Escherichia coli	51	-
	59	-
Salmonella spp	57	X

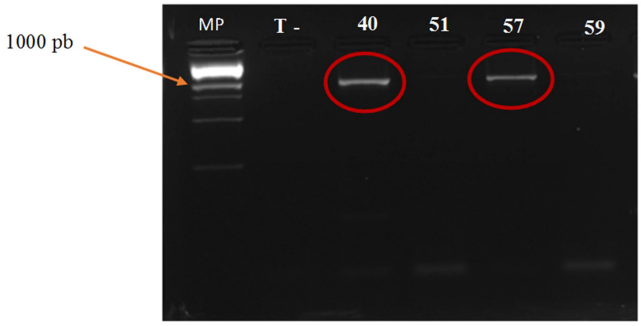

Figure 7. *Agarose gel from CTX-M PCR products.*

Legend: MP= Molecular weight marker (GeneRuler 1Kb DNA Ladder), T- = Negative control. Numbers 40, 51, 57, 59 represent the 4 samples used for amplification. The direction of electrophoresis migration is from top to bottom.

4. Discussion

The aim of our work has been to isolate strains of *Salmonella spp* and *Escherichia coli* in the feces of chickens eaten in the open area in the city of Ouagadougou. Second, this work allowed us to highlight the presence of antibiotic resistance genes by the production of cefotaximas in strains isolated from chicken droppings.

The choice of sampling sites was made randomly according to the places where high inflows were observed. The amount of sample taken per location was based on size and flow rate in order to have representative sampling. Just like Bako et al in 2017 who chose places where there is a high confluence with a large number of animals in order to have a qualitative sampling. We identified 78.21% of strains containing *Escherichia coli* isolates as well as 9.90% of *Salmonella spp* isolates from the 101 chicken droppings sampled. The characterization of the isolates was carried out by bacteriological and biochemical methods. According to a study conducted in Washington in 2001 on chicken samples a significant difference was observed: 38.7% of strains identified as *E. coli* and only 3.0% for *Salmonella* [9]. Moreover, these results corroborate our results to the extent that *E. coli* is also mostly isolated in relation to *salmonella spp*. These results are in line with studies on the prevalence of *Salmonella spp* in Italy, Lithuania and the Netherlands with 20% respectively, 29% and 11% while in Germany *Salmonella spp* was not detected due to lack of referenced data for limited sampling [10]. In Kenya, a similar study was carried out; following the bacteriological and biochemical methods used, they deduced that 57% of the total feces of sampled chickens contained strains of *Escherichia coli* and that 12% contained *Salmonella spp*. The results of this study are approximately equal to those reported in our study, especially since they worked on 150 samples in total [11]. The chickens in our study are local chickens and not broilers, so they are more likely to be in contact with enteric pathogens because they are grown outdoors. Variable rates were also recorded in Egypt, with 8.3% for *Salmonella spp* versus 11.7% for *Escherichia coli*.

In Burkina Faso, a study was conducted on the prevalence of *Salmonella spp* and *Escherichia coli* in outdoor retail meat [4]. They obtained a prevalence of *Salmonella spp* of 9.3% and 100% *E coli* [4]. The high percentages can be explained by the fact that *E. coli,* unlike *Salmonella spp*, is normally found in animals and the environment [4]. Identified *E. coli* isolates are part of the normal enteric flora present in animals [12]. Variation in prevalence of *Salmonella spp* and *E. coli* isolates may be related to differences in hygiene practices among chicken farmers.

We observed that 70 strains were all resistant to at least one antibiotic, 7 resistant strains were obtained for *Salmonella spp*. For these strains we observe a general resistance of 70% of which 10% with cefotaxime, 10% with ceftazidime, 10% also for ceftriaxone and finally 70% of strain resistant to amoxicillin+ clavulanic acid. Resistant strains of *Escherichia coli* amounted to 67 strains we observe a general resistance of 84.81% including 3.79% resistance to ceftazidime, 3.79% for ceftriaxone and 84.81% resistant to amoxicillin + clavulanic acid. These percentages are well below a study conducted in Egypt where resistance of *Escherichia coli* to cefotaxime (80.0%) predominated [13]. *Salmonella spp* isolated from animal droppings and feed of animal origin in Italy was also found to be multi-resistant [14].

The use of antibiotics in animal health, in livestock, also has an impact on the endogenous flora of humans and on the susceptibility to antibiotics of strains responsible for human infections. Because BLSE resistance genes present in bacterial strains in farmed animals can be transmitted to the commensal enterobacteriaceae of the human organism knowing moreover that some antibiotics are common to animal health as well as human.

We see a multiresistance in Burkina Faso, but a very low multiresistance due to 4 multi-resistances compared to that observed in other countries. This considerable difference may be due first of all to the fact that our study is limited to a few areas in a short period of time, or to the fact that most of the sellers of flambé chicken buy from so-called traditional breeders. This low multi-resistance can be explained by the fact that the chickens used are local chickens that are not always in contact with antibiotics unlike broilers that are on antibiotics for prevention.

Our study indicates that outdoor consumption of flambé chicken may be a potential source of ESBF isolate acquisition for consumers. Resistance genes can then be used to confirm the presence of resistance. For example, a PCR identification of the CTX-M BLSE gene by PCR was performed. At PCR, 2 isolates were carriers of the CTX-M BLSE gene. BLSE were generally carried by large plasmids. The use of penicillins and cephalosporins, particularly C3G, has been shown to contribute to the selection of BLSE-

producing strains [15]. In North Africa, class A BLSE was found to be the most common, as in Egypt, where 42.9% of samples with class A BLSE genes were found [16]. Whereas in Guinea-Bissau and Libya it was BLSE class A and D [17]. In West Africa, Ghana, Mali and Burkina Faso, Class A BLSE was found in 49.4; 96% and 62% respectively [18]–[20]. In South Africa, class A and D BLSE were present, with prevalence ranging from 8.8% to 13.1% [21]. The results of all these studies come from human samples and are above the results obtained in our study on local chickens. But we can deduce that BLSE in general is expanding throughout Africa.

The percentages of bacterial resistance obtained in other studies on chickens are still higher than those in our study. This is the case of a study conducted in Kenya where some bacteria detected as multi-resistant had antibiotic resistance genes.

5. Conclusion

We can conclude that a large distribution of chickens that may contain resistant bacteria is likely to participate in the growth of antibiotic resistance. Given the high consumption of chickens in the city of Ouagadougou, the probability that *Salmonella spp* and multidrug-resistant *Escherichia coli* infect humans is even greater. We are therefore facing a major public health problem that will have an impact on the future of therapies if no preventive measures are taken.

List of Abbreviations

AMC: Amoxicilline + acide clavulanique, ATM: aztréoname, BET: Bromure d'Ethidium, BLSE: β-lactamases à spectre élargi, C3G: céphalosporines de troisième génération, CAZ: Ceftazidime, CERBA: Centre de Recherche Biomoléculaire Pietro Annigoni, CMB: Concentration minimale bactéricide, CMI: concentration minimale inhibitrice, CRO: Ceftriaxone, CTX: céfotaxime, CTX-M: céfotaximase, E. coli: *Escherichia coli*, EMB: Eosine bleu de méthylène, ESBL: Extented-spectral- β-lactamase, LB: Luria bertani, MH: Muller Hinton, OMS: Organisation Mondiale de la Santé, Pb: paire de base, PCR: polymerase chain reaction, SHV: Sulfhydril Variable, TEM: Temoneira.

Availability of Data and Materials

All the data sets used to support the findings of this study are available from the corresponding author upon request.

Author's Contributions

B.S.L.C.S, A.M.D and J.S designed the study; R.Y.T, B.S.L.C.S, A.M.D performed the experiments, analyzed the data and wrote the manuscript. R.Y.T, B.S.L.C.S, A.M.D, G.G.J, S.P.A.C, and J.S participated in the critical review of the manuscript. All authors have read and approved the final version.

Ethics Approval and Consent to Participate

The institutional ethic committee of CERBA/LABIOGENE reviewed and approved the study protocol.

Competing Interests

The authors declare that they have no competing interests.

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