

Multidrug-Resistant of *Escherichia coli* and *Salmonella* spp. Strains in Chicken Feces Intended for Consumption in Open Spaces of Ouagadougou, Burkina Faso

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Abstract

Resistant bacteria can be transmitted to humans through feces or contaminated meat from local chickens. Bacterial strains were isolated from the intestinal contents of 400 local chicken samples from various sales sites. These strains were then characterized using bacteriological and biochemical methods to identify resistant strains. In a study conducted in Ouagadougou, we systematically collected chicken fecal samples from 20 locations across the city, followed by isolation and identification of Salmonella spp. using specific enrichment and culture methods, as well as Escherichia coli. Bacterial strains were characterized using antibiotic resistance profiles were determined through agar diffusion tests, revealing sensitivity or resistance to a range of antibiotics based on established scientific criteria. The results showed that out of the 400 samples collected, 81.25% and 63.5% were contaminated by Escherichia coli and Salmonella spp., respectively. Among these, 86.15% of identified Escherichia coli and 50.78% of Salmonella spp. displayed resistance to at least one tested antibiotic. Among 280 Escherichia coli isolates identified resistant to at least one antibiotic, 31.07% were resistant to cefotaxime (CTX), 20.35% to ceftazidime (CAZ), 21.07% to ceftriaxone (CTR), 75% to amoxicillin + clavulanic acid (AMC), 23.57% aztreoname (ATM) and 27.14% were resistant to imipenem (IMP). In the case of the 129 Salmonella spp. isolates resistant to at least one tested antibiotic, 34.88% were resistant to CTX; 41.08% to CAZ; 35.65% to CTR, 92% to AMC, 39.53% to ATM and finally 47.28% were resistant to IMP. Our study revealed high prevalence of resistance in bacterial strains isolated from local chickens sold outdoors in Ouagadougou. These findings raise significant public health concerns, due to the possible transmission of these resistant strains to humans through the consumption of contaminated meat, thus complicating the treatment of bacterial infections.

Keywords

Multidrug-Resistant, Chicken, Ouagadougou, *Escherichia coli, Salmonella* spp., Antibiotic

1. Introduction

Enterobacteria is a widespread group of bacteria, including notable pathogens such as *Salmonella* spp. and *Escherichia coli*. They are of scientific interest because of their key role in human and animal infections and their involvement in antibiotic resistance [1]. Increasing their resistance to antibiotics has become a major global public health problem [2]. The consumption of animal-derived food products, such as chicken meat, can significantly contribute to the spread of antibiotic-resistant bacteria in humans [3]. However, a common practice in local food places is to kill, pluck, clean and cook chickens in the same place, often by the same person, which creates a potential risk of contamination of the flaming chicken by its own droppings. Because these droppings contain a wide variety of enterobacteria, including *Escherichia coli* and *Salmonella* spp., there is a risk of transmission of these pathogenic enterobacteria to humans through the consumption of contaminated chicken. This raises public health concerns and the need for preventive measures to avoid such contamination risks.

Enterobacteria is of therapeutic interest to some families of antibiotics, including β -lactams, aminoglycosides and quinolones. However, due to the increasing prevalence of antibiotic resistance in these bacteria, the effectiveness of treatments is compromised [4]. In this study, we focused on *Escherichia coli* and *Salmonella* spp. strains isolated from local chickens sold outdoors in Ouagadougou. The objective is to better understand the prevalence of resistant bacterial strains and to determine their potential impact on public health. The results of this study will help to assess the scope of antibiotic resistance within the food chain and guide bacterial resistance management strategies, crucial for preserving the efficacy of antibiotic treatments and safeguarding public health.

2. Material and Method

2.1. Sample Collection

In order to identify multi-resistant bacterial strains capable of infecting both animals and humans, we collected biological samples by taking fecal samples directly from the chicken intestine immediately after slaughter. Sample collection was carried out at 20 highly frequented sites, ensuring even distribution across the city of Ouagadougou, to guarantee geographical representativeness of the sampling. Samples were aseptically placed in tubes containing Luria bertani (LB) liquid medium to ensure the viability and multiplication of the bacteria collected.

2.2. Isolation and Identification

Chicken feces are collected aseptically from the intestine and stored in sterile 1.5 ml Eppendorf tubes containing Luria bertani (LB) medium. This versatile medium promotes rapid bacterial growth, facilitating the transfer of samples from the collection site to the laboratory [5]. Once in the laboratory, buffered peptone water was utilized to prepare bacterial samples for isolation, facilitating the retrieval of pathogenic microorganisms from clinical or environmental samples. Samples originally contained in LB were individually inoculated into 9 ml of peptone water, homogenized for 2 minutes, and subsequently incubated at 37 degrees Celsius for 16 to 20 hours [6].

After incubation in peptone water, samples were cultured in Eosin Methylene Blue (EMB) selective culture medium using a 10-microliter calibrated loop, then incubated at 37°C for 18 to 24 hours to promote *Escherichia coli* growth and obtain characteristic colonies [7].

Before isolating *Salmonella spp* strains, we stimulated their proliferation while inhibiting the growth of other bacterial strains. To achieve this, 0.1 ml of peptone water from each sample, containing a variety of chicken gut bacteria, was transferred to 10 ml of selenite-cystine broth. The mixture was then incubated at 42°C for 18 to 24 hours, as described by Nair *et al.* [8]. Then, the samples were grown on selective culture media specific to *Salmonella* spp (Agar *Salmonella Shigella* The seeding technique used was streak spreading, where a small amount of selenite-cystine broth containing the enriched strains was deposited and spread on each culture medium. Petri dishes containing the selective media were then incubated at 37°C for 18 to 24 hours [8]. After this incubation period, they were visually examined for characteristic *Salmonella* spp. and *Escherichia coli* colonies. This approach of selective cultivation and observation of colony characteristics enables us to identify and distinguish the bacterial strains of *Escherichia coli* and *Salmonella* spp that were present in the chicken feces samples.

2.3. Antibiotic Resistance Profile

Isolated and identified bacteria were plated on Mueller-Hinton (MH) agar and incubated for 18 to 24 hours to obtain pure bacterial colonies. These colonies were then used for susceptibility testing to detect antibiotic resistance. For this purpose, pure cultures on MH agar were suspended in a 0.9% NaCl solution to obtain a concentration equivalent to McFarland 0.5 (108 CFU/ml). This dilution was then inoculated onto Petri plates containing Mueller-Hinton agar.

Of these seeded petri dishes, antibiotics discs, such as Amoxicilline + acide clavulanique (AMC) discs and céfotaxime (CTX), Ceftazidime (CAZ), Ceftriax-one (CTR), aztréoname (AZT) and imipenem (IMP) discs, were placed at 20 to

30 mm using sterile antibiotic forceps [9]. The inhibition zones that formed around the discs were measured and compared to the critical values established by the French Society of Microbiology's Antibiogram Committee (CASFM, 2020) [9]. These critical values enable the interpretation of results as sensitive (S), resistant (R) or intermediate (I) based on the size of the inhibition zones. Therefore, this agar-based antibiogram method enabled the determination of the sensitivity of the studied bacterial strains to various tested antibiotics, following standardized criteria established by scientific recommendations. We selected these specific antibiotics for our study because of their relevance in the treatment of common bacterial infections, as well as their frequency of use in clinical practice [10] [11]. In addition, we considered the reported prevalence of antibiotic resistance in the study region. In selecting these antibiotics, we aim to assess the susceptibility of isolated bacterial strains to commonly used drugs, as well as to detect any emerging or prevalent resistance that could have an impact on public health.

3. Results

3.1. Isolated Strains Identified by Collection Site

Isolated and identified bacterial strains are the focus of this study, and their characteristics will play an essential role in the analysis and interpretation of the results obtained. Twenty different locations were sampled, with 25 chicken intestines collected from each site for analysis. The sites were chosen according to their attendance. **Figure 1** shows the different collection sites circled in red [12].

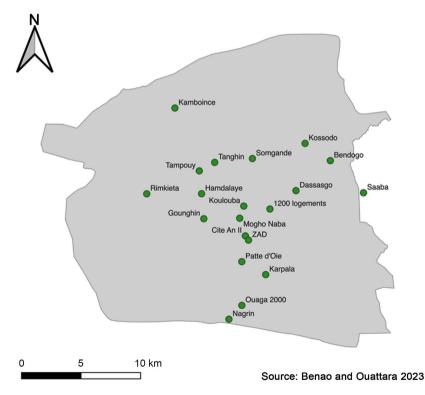


Figure 1. Collection sites in Ouagadougou, Burkina Faso.

Table 1 shows the number of bacterial strains identified according to their collection site.

3.2. Resistance Profile of Isolated Bacteria

The resistance profile of isolated bacteria is an essential element in the study. It will determine the susceptibility or resistance of *Salmonella* spp and *Escherichia coli* to target antibiotics. Figure 2 shows the total number of bacteria identified that demonstrate additional resistance to at least one antibiotic.

The distribution of resistant bacterial strains was analyzed according to the bacterial species and the specific antibiotic. **Figure 3** shows the distribution of *Escherichia coli* strains by resistance. Resistance to amoxicillin + clavulanic acid is the most observed.

Figure 4 shows the distribution of *Salmonella* spp strains by resistance to target antibiotics. Resistance to amoxicillin + clavulanic acid is always the most observed.

| Collection sites | Salmonella spp. | Escherichia coli | |
|------------------|-----------------|------------------|--|
| KOULOUBA | 19 | 21 | |
| OUAGA 2000 | 24 | 22 | |
| CITÉ AN 2 | 23 | 24 | |
| MOOGO NAABA | 21 | 0 | |
| NAGRIN | 22 | 25 | |
| KARPALA | 15 | 16 | |
| BENOGO | 21 | 23 | |
| 1200 LOGEMENTS | 20 | 24 | |
| ZAD | 19 | 14 | |
| SOMGANDE | 21 | 15 | |
| TAMPOUY | 20 | 22 | |
| HAMDALAYE | 15 | 14 | |
| PATTE D'OIE | 3 | 8 | |
| KOSSODO | 0 | 15 | |
| DASSASGO | 0 | 6 | |
| RIMKIETA | 1 | 2 | |
| KAMBOINSSIN | 11 | 21 | |
| TANGHIN | 6 | 9 | |
| SAABA | 5 | 6 | |
| GOUNGHIN | 7 | 9 | |

Table 1. Distribution of samples by source.

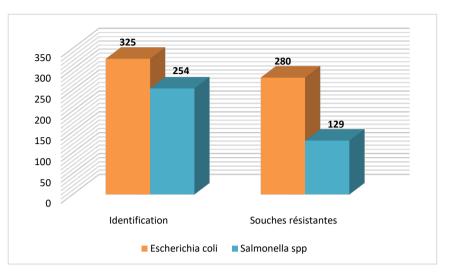


Figure 2. Strains identified and resistance profile.

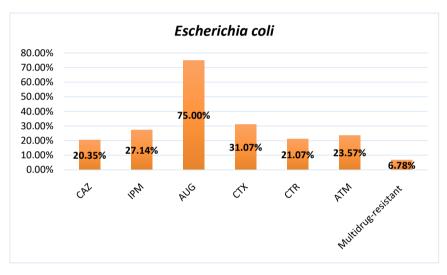
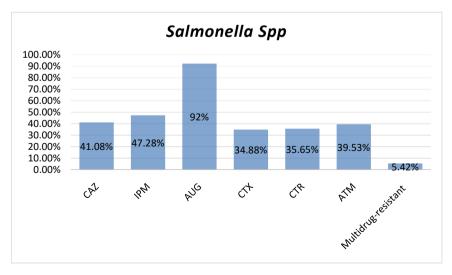
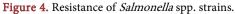


Figure 3. Resistance of *Escherichia coli* strains.





3.3. Bacterial Resistance Identify by Area

Bacteriological analysis according to collection sites allowed us to better understand the distribution and diversity of antibiotic resistance among the different bacterial strains collected from the sites studied. **Table 2** and **Table 3** are summaries of all resistant strains by site.

Bacterial Multidrug-resistant refers to the ability of a bacterial strain to resist several different classes of antibiotics. This ability increases the complexity of bacterial infection management. In our studies several strains have been identified as Multidrug-resistant to different types of antibiotics. A summary of Multidrug-resistant strains is presented in **Figure 5**.

4. Discussion

The aim of our study was to identify the prevalence of *Salmonella* spp. and *Escherichia coli* strains present in the feces of chickens sold outdoors in Ouaga-dougou.

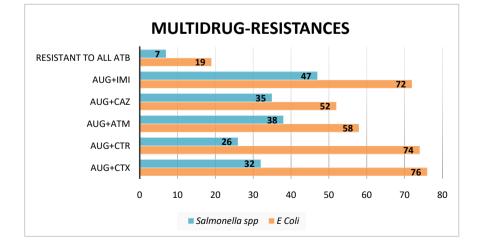
| | ATB RESISTANCE | | | | | | |
|------------------|----------------|-----|-----|-----|-----|-----|--|
| Collection sites | AUG | CTX | CTR | ATM | CAS | IPM | |
| KOULOUBA | 5 | 4 | 4 | 0 | 2 | 0 | |
| OUAGA 2000 | 14 | 6 | 8 | 10 | 7 | 2 | |
| CITÉ AN 2 | 6 | 5 | 4 | 1 | 1 | 2 | |
| MOOGO NAABA | 7 | 4 | 6 | 3 | 2 | 3 | |
| NAGRIN | 18 | 16 | 14 | 12 | 9 | 3 | |
| KARPALA | 14 | 8 | 6 | 5 | 3 | 7 | |
| BENOGO | 16 | 8 | 8 | 6 | 6 | 9 | |
| 1200 LOGEMENT | 23 | 17 | 15 | 13 | 14 | 20 | |
| ZAD | 12 | 6 | 8 | 4 | 4 | 8 | |
| SOMGHANDE | 13 | 7 | 5 | 8 | 4 | 9 | |
| TAMPOYI | 15 | 3 | 3 | 4 | 3 | 9 | |
| HAMDALAYE | 7 | 3 | 3 | 0 | 2 | 4 | |
| PATTE D'OIE | 0 | 0 | 0 | 0 | 0 | 0 | |
| KOSSODO | 15 | 0 | 0 | 0 | 0 | 0 | |
| DASSASGHO | 6 | 0 | 1 | 0 | 0 | 0 | |
| RIMKIETA | 2 | 0 | 0 | 0 | 0 | 0 | |
| KAMBOINSSIN | 19 | 0 | 2 | 0 | 0 | 0 | |
| TANGHIN | 6 | 0 | 0 | 0 | 0 | 0 | |
| SAABA | 5 | 0 | 0 | 0 | 0 | 0 | |
| GOUNGHIN | 7 | 0 | 0 | 0 | 0 | 0 | |
| TOTAL | 210 | 87 | 87 | 66 | 57 | 76 | |

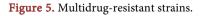
Table 2. Distribution of antibiotic-resistant strains of *E*. by site.

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| | RESISTANCE AUX ATB | | | | | | |
|------------------|--------------------|-----|-----|---------|-----|-----|--|
| Collection sites | AUG | CTX | CTR | AUX AIB | CAS | IPM | |
| KOULOUBA | 3 | 2 | 5 | 4 | 4 | 4 | |
| OUAGA 2000 | 10 | 6 | 6 | 6 | 5 | 5 | |
| CITÉ AN 2 | 7 | 0 | 1 | 1 | 1 | 2 | |
| MOOGO NAABA | 4 | 4 | 4 | 5 | 4 | 2 | |
| NAGRIN | 15 | 7 | 8 | 6 | 8 | 14 | |
| KARPALA | 11 | 2 | 4 | 5 | 4 | 8 | |
| BENOGO | 10 | 3 | 1 | 3 | 3 | 6 | |
| 1200 LOGEMENT | 7 | 4 | 3 | 2 | 5 | 5 | |
| ZAD | 13 | 6 | 3 | 4 | 5 | 3 | |
| SOMGHANDE | 10 | 4 | 4 | 4 | 6 | 3 | |
| TAMPOYI | 7 | 4 | 3 | 5 | 3 | 4 | |
| HAMDALAY | 6 | 2 | 3 | 6 | 4 | 5 | |
| PATTE D'OIE | 0 | 0 | 0 | 0 | 0 | 0 | |
| KOSSODO | 0 | 0 | 0 | 0 | 0 | 0 | |
| DASSASGHO | 0 | 0 | 0 | 0 | 0 | 0 | |
| RIMKIETA | 0 | 0 | 0 | 0 | 0 | 0 | |
| KAMBOINSSIN | 8 | 1 | 1 | 0 | 1 | 0 | |
| TANGHIN | 2 | 0 | 0 | 0 | 0 | 0 | |
| SAABA | 2 | 0 | 0 | 0 | 0 | 0 | |
| GOUNGHIN | 4 | 0 | 0 | 0 | 0 | 0 | |
| TOTAL | 119 | 45 | 46 | 51 | 53 | 61 | |

Table 3. Distribution of antibiotic-resistant strains of *Salmonella* spp by site.





The selection of sampling sites was carried out using a random sampling method, favouring places with high traffic. The amount of sample collected at each site was limited to 25 per site. This number was determined according to the size and flow rate of consumption, thus ensuring an adequate representativeness of the sampling. This approach is along the same lines as Bako *et al.* in order to have a representative and qualitative sampling [10] [13].

Of the 400 samples collected, 81.25% and 63.5% contained isolates of *Escherichia coli* and *Salmonella* spp., respectively, confirmed by bacteriological. These results are consistent with a study conducted in Bangladesh in 2014, which recorded a prevalence of 87.55% for *Escherichia coli* and 66.66% for *Salmonella* spp, although this was done on litter samples rather than directly in the intestines, increasing the risk of environmental contamination [14]. Similar observations were made in Côte d'Ivoire in 2021, where a high percentage of *Escherichia coli* isolates, 91.74%, was observed, but with a lower prevalence of 15.58% for *Salmonella* spp. [15]. In contrast, relatively lower results were reported in Thailand in 2021, with only 33.33% of samples containing *Escherichia coli* and 33.34% containing *Salmonella* spp. [16]. The fact that the chickens in our study are of local strain and not specific to meat production makes them more exposed to enteropathogens due to their outdoor breeding [16] [17].

Among the 325 isolates of *Escherichia coli* identified, 86.15% were resistant to at least one antibiotic including 31.78% to cefotaxime (CTX), 20.71% to ceftazidime (CAS), 29.64% to ceftriaxone (CTR), 76.42% to amoxicillin + clavulanic acid (AMC) ESBL type resistance against 23.92% to aztreonam (ATM) and 27.14% to imipenem (IMP) carbapenemases type resistance. The resistance rates observed in our study are considerably higher than those reported in Côte d'Ivoire for ESBLs, more specifically amoxicillin (25.94%) and ceftriaxone (2.36%) (11). A study carried out in Palestine obtained results roughly similar to ours regarding the prevalence of carbapenemases resistant strains, which was 12% [18].

Among the 254 isolates from *Salmonella* spp identified, 50.78% were resistant to at least one antibiotic. Namely a resistance to CTX of 35.64%, 41.08% to CAS, 34.10% to CTR, 92% to AMC ESBL type resistance, as well as 38.75% to ATM and 48.06% to IMI carbapenemases type resistor. Its results are as high in Ivory Coast in terms of ESBLs with 88.23% of strain resistant to AMC and 35.29% to CTX [19]. As for carbapenemase type resistance, no resistance was found in a study carried out in Cameroon in 2021 [20]. These results are in the same direction as a study carried out in Nigeria where only 1% of isolates had a resistance profile of 1% to IMI [21]. Very little convincing data is found in the literature in relation to the carbapenemase resistance profile of isolates of *Salmonella* spp. found in chicken feces. This great variability in increasing resistance to be-ta-lactams and carbapenems throughout the world can be explained by the administration of antibiotics in veterinary medicine, particularly in poultry farms. This can in turn affect the human intestinal flora and the sensitivity to antibiotics of the strains responsible for infections in humans.

5. Study Limitations

Sample collection for this study was not exhaustive, which could lead to an underestimation or overestimation of the true prevalence of resistant strains. In addition, the absence of chicken health records at the time of collection posed a major challenge. Some restaurateurs expressed fears of reprisals, while others simply didn't have carnets for various reasons. This limited our ability to establish a correlation between the antibiotic treatments undergone by the chickens and the percentages of resistance found in their feces.

6. Conclusion

This study identified and characterized resistant bacteria in *Escherichia coli* and *Salmonella* spp. The widespread presence of resistant bacteria raises serious public health concerns. Additionally, the presence of these resistant strains in the intestines of chickens sold outdoors in Ouagadougou raises concerns about their potential impact on human health. These resistant bacteria could be transmitted to humans through the consumption of these chickens, complicating the treatment of certain infections. Managing antibiotic resistance in animal products is crucial to preserve the effectiveness of medical treatments and safeguard public health. Implementing rigorous prevention and control measures are essential to limit the spread of these resistant strains and maintain the effectiveness of antibiotics in bacterial infection treatments. Faced with this threat, it is imperative to implement a comprehensive approach to surveillance, awareness, and management of antibiotic resistance. This approach is necessary to protect public health and ensure the efficacy of medical therapies in the future.

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Ethics Approval and Consent to Participate

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

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.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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