Journal of Biosciences and Medicines, 2024, 12, 221-232
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Molecular Detection of Extended-Spectrum Beta Lactamase and Carbapenemase Genes in *Escherichia coli* and *Salmonella spp.* Isolated from Chickens Consumed Outdoors in Ouagadougou, Burkina Faso

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How to cite this paper: Mètuor Dabiré, A., Bénao, S.L.C.S., Ouédraogo, N., Tiemtoré, R.Y.W., Badini, R.O., Bambara, L.E.B. and Simporé, J. (2024) Molecular Detection of Extended-Spectrum Beta Lactamase and Carbapenemase Genes in *Escherichia coli* and *Salmonella spp.* Isolated from Chickens Consumed Outdoors in Ouagadougou, Burkina Faso. *Journal of Biosciences and Medicines*, **12**, 221-232.
https://doi.org/10.4236/jbm.2024.1211019

Received: September 7, 2024
Accepted: November 11, 2024
Published: November 14, 2024

Abstract

This study was carried out to assess the prevalence of resistance genes in strains of *Escherichia coli* and *Salmonella spp.* isolated from free-range chickens in Ouagadougou, where resistant bacteria can be transmitted to humans via faeces or contaminated meat. A total of 280 strains of *Escherichia coli* and 129 strains of *Salmonella spp.* resistant to at least one beta-lactam or carbapenem antibiotic were used in this study. PCR analyses revealed the presence of ESBL (extended spectrum beta lactamase) resistance genes in *Escherichia coli* isolates, with 3.21% (9/280) possessing the CTX-M (Cefotaximase) gene, 15.35% (43/280) had the SHV (Sulfhydril Variable) gene, and 11.42% (32/280) had carbapenemase resistance genes, more specifically IMP (Imipene mase metallo-beta-lactamase). As regards *Salmonella spp.* strains, only the presence of the SHV (Sulfhydril Variable) gene was identified in 2.32% (3/129) of isolates belonging to the ESBL family, while 26.35% (34/129) and 13.95% (18/129) of isolates respectively possessed the IMP (Imipenemase metallo-beta-lactamase) and NDM (New Delhi metallo-β-lactamase) genes, both of the carbapenemase type. The significant prevalence of resistance genes in bacterial strains isolated from chickens sold outdoors in Ouagadougou raises

major 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

Bacteria, Resistance, Extended-Spectrum Beta Lactamases, Carbapenemases, Ouagadougou

1. Introduction

Bacterial infections remain a significant global health challenge, contributing to substantial morbidity and mortality. These infections can range from mild illnesses, such as strep throat or urinary tract infections, to severe and life-threatening conditions like pneumonia, meningitis, and sepsis. Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, and *Neisseria meningitidis* are common causes of such infections [1].

One of the most pressing concerns in managing bacterial infections is the rise of antibiotic-resistant bacteria, or "superbugs". The overuse and misuse of antibiotics in human medicine and agriculture have accelerated the emergence of resistant strains [2]. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli*, for example, are becoming increasingly difficult to treat with standard antibiotics, leading to higher healthcare costs, prolonged hospital stays, and increased mortality rates [3] [4].

Enterobacteriaceae, a family of bacteria that includes pathogens such as *Salmonella spp.* and *Escherichia coli*, have garnered significant scientific interest due to their involvement in human and animal infections, as well as their role in the growing issue of antibiotic resistance [5]. The consumption of food products of animal origin, particularly chicken meat that may be contaminated with *Salmonella* or *E. coli*, has been identified as a major source of resistant bacteria transmission to humans [6].

In Burkina Faso, local weather conditions further exacerbate the spread of pathogenic Enterobacteria. The country's tropical climate, characterized by distinct wet and dry seasons, plays a pivotal role in shaping the transmission dynamics of these bacteria. During the hot season, water scarcity often forces communities to rely on potentially contaminated water sources, increasing the likelihood of waterborne diseases such as those caused by *Escherichia coli*, *Salmonella*, *Shigella*, and *Klebsiella* [7] [8]. On the other hand, heavy rainfall during the wet season leads to water contamination and heightens the risk of outbreaks.

Cultural practices in Burkina Faso, particularly in Ouagadougou, also contribute to the spread of resistant bacteria. Poultry consumption is widespread, with chickens often slaughtered, cleaned, and cooked in open-air environments like leisure areas or restaurants. This practice, especially when performed by a single person handling all stages, poses a risk of contamination, as chicken droppings

that harbor *Escherichia coli* and *Salmonella spp.* can contaminate the food [9] [10]. Such practices raise public health concerns and emphasize the need for preventive measures to reduce contamination risks.

Enterobacteriaceae are also of significant therapeutic interest due to their susceptibility to certain families of antibiotics, including β-lactams, aminoglycosides, and quinolones. However, the increasing prevalence of antibiotic resistance in these bacteria, especially resistance genes like extended-spectrum beta-lactamase (ESBL) and carbapenemase, has compromised treatment efficacy [11]-[13].

In light of this public health threat, the present study focuses on bacterial strains isolated from free-range chickens in Ouagadougou. The primary goal is to identify and characterize resistance genes, particularly ESBL and carbapenemase genes, in order to better understand their frequency and assess the potential impact of antibiotic resistance on public health.

2. Materials and Method

2.1. Sample Preparation

The bacterial strains used in this study (280 strains of *Escherichia coli* and 129 strains of *Salmonella spp.* resistant to at least one beta-lactam or carbapenem antibiotic) were isolated from the intestinal faeces of 400 chickens sold outdoors in the city of Ouagadougou as previously described by Bénao and *et al.* [10].

2.2. DNA Extraction and Quantification

Chromosomal DNA was extracted using the heat shock method [14]. A pure colony of bacteria was suspended in 500 µl of sterile distilled water. This suspension was then subjected to heat treatment by placing it in a water bath at a temperature of 95˚C for 10 minutes. After this step, the suspension was immediately transferred to ice and kept there for 10 minutes. This heat shock process is repeated 2 to 3 times to lyse the bacteria and release the DNA. After heat treatment, a 10-minute centrifugation at 13,000 rpm is carried out to separate cellular debris and unwanted cellular components from the extracted DNA [15]. The supernatant, containing the extracted DNA, is recovered and used for applications such as PCR amplification. The supernatant is also stored at −20˚C for future use.

2.3. Amplification by Conventional PCR

Table 1. PCR program by gene type.

	Temperature/Duration			
Parameters	*blaSHV*	*BlaCTX-M*	*BlaIMP*	*BlaNDM*
Initiale Denaturation	96˚C/5 mn	96˚C /5 mn	96˚C/5 mn	96˚C/5 mn
Denaturation	96˚C/1 mn	96˚C/1 mn	96˚C/30 s	96˚C/30 s
Pairing	60˚C/1 mn	50˚C/1 mn	48˚C/1 mn	55˚C/30 s
Elongation	72˚C/1 mn	72˚C/1 mn	72˚C/30 s	72˚C/30 mn

Continued

Finale Elongation	72˚C/10 mn	72˚C/10 mn	72˚C/7 mn	72˚C/10 mn
Number of Cycles	35	35	30	30

Conventional PCR was carried out in a 25 µL reaction volume. The composition of this reaction volume was prepared as follows: 12.5 µL of master mix, 0.5 µL of F primer, 0.5 µL of R primer, 10.5 µL of PCR water and 1 µL of DNA extract. Detection was performed using the GeneAmp System PCR 9700 Thermal Cycler (Applied Biosystems, California, USA). Primers were also supplied by A Biosystems. Four amplification programmes were used to search for the four genes (Table 1).

Table 2 shows the primer sequences for the different ESBL and EPC genes detected [16]-[18]. These sequences play a crucial role in the identification and characterization of bacterial antibiotic resistance mechanisms, providing essential information on the prevalence and diversity of these genes within the bacterial strains studied.

Table 2. Primer sequences for the different ESBL and EPC genes detected.

Gene Detected	Primer	Primer Sequences (5'-3')	Amplicons	Reference
Bla CTX-M	*CTX-M* fwd	*GTT-ACA-ATG-TGT-GAG-AAG-CAG*	1000 pb	[17]
	CTX-M rev	*CCG-TTT-CCG-CTA-TTA-CAA-AC*		
Bla SHV	*SHV* fwd	ATG-CGT-TAT-ATT-CGC-CTG-TG	875 pb	[17]
	SHV rev	TTA-GCG-TTG-CCA-GTG-CTC		
BlaIMP	*IMP* fwd	CATGGTTTGGTGCTTGT	500 pb	[18]
	IMP rev	ATAATTTGGCGGACTTTGGC		
BlaNDM	*NDM* fwd	GGAATAGAGTGGCTTAATTCTC	521 pb	[16]
	NDM rev	CGGAATGGCTCATCACGATC		

2.4. Electrophoresis

DNA fragments amplified by conventional PCR were subjected to electrophoresis on a 1.5% agarose gel prepared in a 0.5X tris base-borate-EDTA solution containing ethidium bromide [19]. DNA fragments were migrated at a voltage of 110 millivolts (mV). The migration buffer used was Tris-EDTA-Acetate (TEA), composed of Tris-HCL at a concentration of 1.6 mM with a pH of 8, EDTA at a concentration of 4 µM and sodium acetate at a concentration of 1.6 mM [19]. Once migration was complete, the migration products, *i.e.* DNA fragments separated according to size, were visualised under UV light. The presence of ethidium bromide in the gel stained the DNA, which appeared as fluorescent bands when exposed to UV light [19]. This visualisation step enabled the PCR results to be analysed and documented by identifying the amplified DNA fragments according to their respective sizes.

3. Results

3.1. Resistance Genes

The resistance genes encoding the production of ESBLs and carbapenemases are key genetic elements involved in the resistance of bacteria to antibiotics. These genes give bacteria the ability to degrade a wide range of beta-lactam and carbapenem antibiotics, rendering certain treatments with commonly used antibiotics ineffective. In our study, the DNAs of all bacterial strains showing resistance to at least one antibiotic were subjected to conventional PCR to search for the ESBL and EPC genes using specific primers. Analysis of the PCR products obtained after agarose gel electrophoresis revealed that 44 isolates of *Salmonella spp.* and 67 isolates of *Escherichia coli* tested carried at least one resistance gene studied.

3.2. Genes Identified by Zone

Analysis of the genes identified according to the collection sites has enabled us to gain a better understanding of the distribution and diversity of antibiotic resistance among the different bacterial strains collected from the sites studied. Table 3 and Table 4 provide a summary of the resistance genes for all sites and antibiotic resistances.

Table 3. Distribution of *E. coli* samples with antibiotic resistance genes by site.

Collection Site	Number of Strains with a Resistance Gene	Percentages
KOULOUBA	4	5.98%
OUAGA 2000	8	11.94%
CITÉ AN 2	11	16.41%
MOOGO NAABA	9	13.43%
NAGRIN	2	2.98%
KARPALA	8	11.94%
BENOGO	4	5.98%
1200 LOGEMENT	1	1.49%
ZAD	0	0%
SOMGHANDE	2	2.98%
TAMPOUI	12	17.91%
HAMDALAY	6	8.95%
TOTAL	67	100%

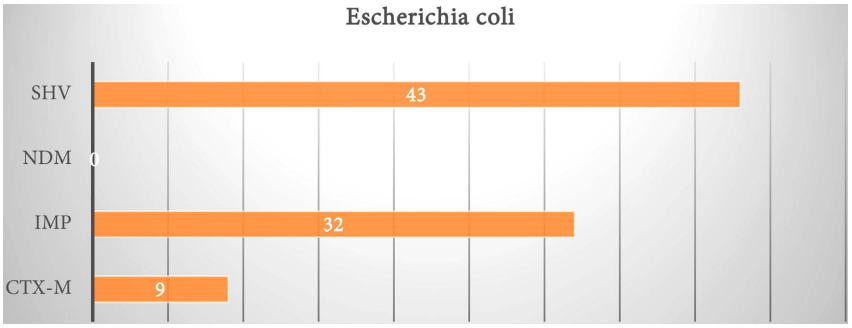
Table 4. Distribution of *Salmonella spp.* samples with antibiotic resistance genes by site.

Collection Site	Number of Strains with a Resistance Gene	Percentages
KOULOUBA	6	13.3%
OUAGA 2000	5	11.36%

Continued

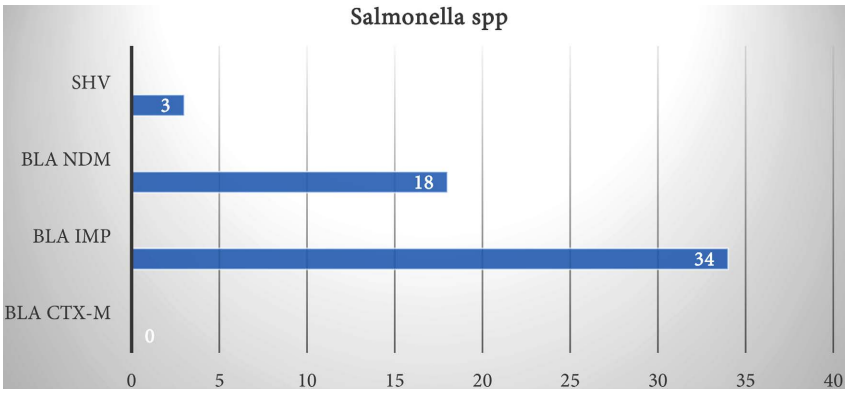
CITÉ AN 2	3	6.68%
MOOGO NAABA	2	4.54%
NAGRIN	0	0%
KARPALA	1	2.27%
BENOGO	0	0%
1200 LOGEMENT	7	15.91%
ZAD	9	20.45%
SOMGHANDE	8	18.18%
TAMPOUI	3	6.68%
HAMDALAY	0	0%
TOTAL	44	100%

3.3. Genes Identified per Bacterium



Legend of Figure 1: BlaSHV: Sulfhydril Variable, BlaNDM: New Delhi metallo-β-lactamase, BlaIMP: Imipenemase metallo-beta-lactamase, BlaCTX-M: Cefotaximase.

Figure 1. Strain with resistance genes coding for the production of *E. coli* ESBLs and EPCs.



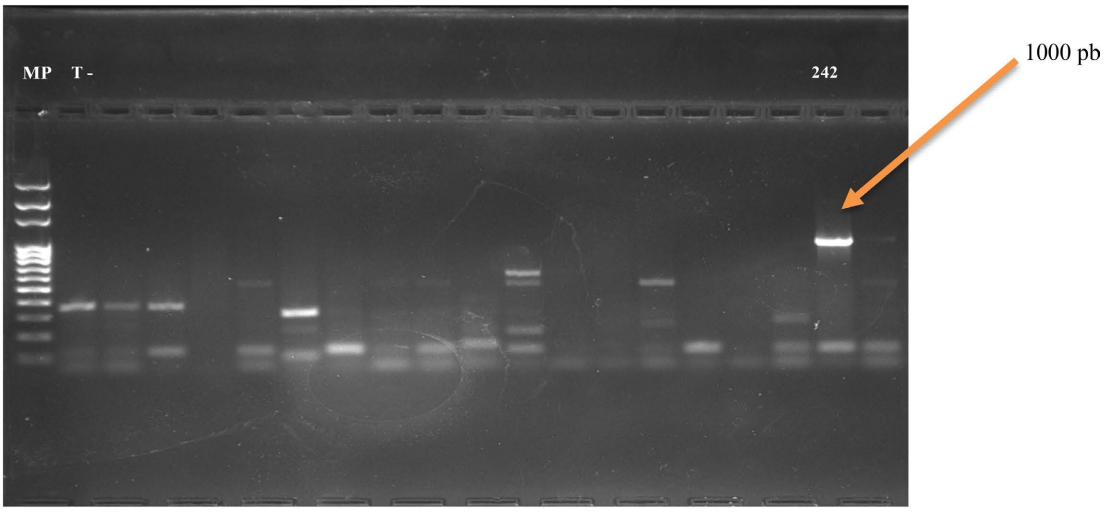
Legend of Figure 2: BlaSHV: Sulfhydril Variable, BlaNDM: New Delhi metallo-β-lactamase, BlaIMP: Imipenemase metallo-beta-lactamase, BlaCTX-M: Cefotaximase.

Figure 2. Strain with resistance genes coding for the production of *Salmonella spp.* ESBLs and EPCs.

The genes identified in each type of bacterium were analysed in detail to

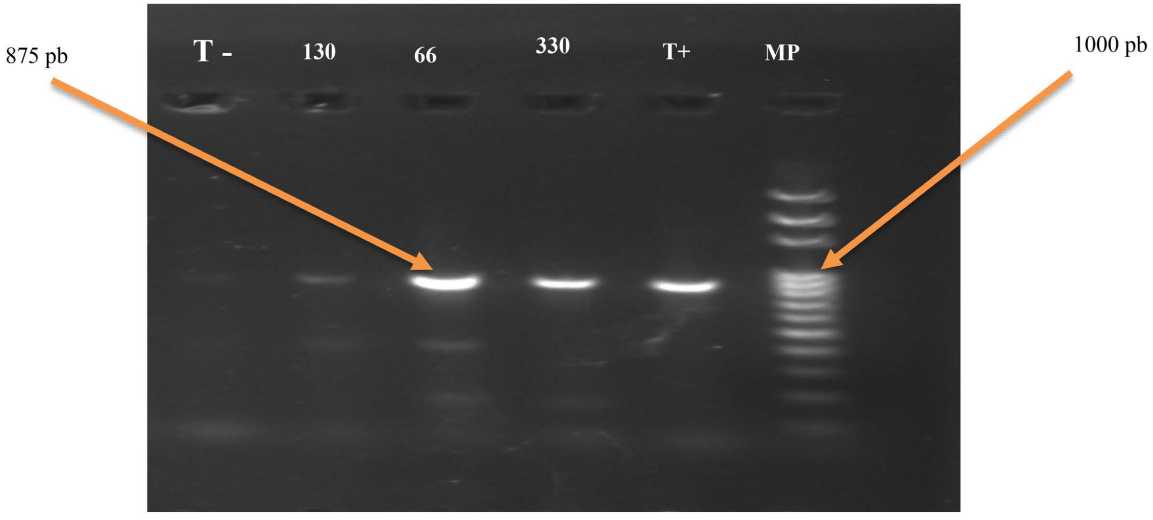
determine their antibiotic resistance profile, thus contributing to a better understanding of the molecular mechanisms of bacterial resistance in the different strains studied. Figure 1 and Figure 2 reveal the presence of resistance genes in at least 67 isolates of *Escherichia coli* and 44 isolates of *Salmonella spp.* These results highlight the importance of antibiotic resistance in the bacterial strains studied.

3.4. Characteristics of the Resistance Genes Coding for the Production of BLSES and Carbapenemases (BlaSHV, BlaCTX-M, BlaNDM, BlaIMP)



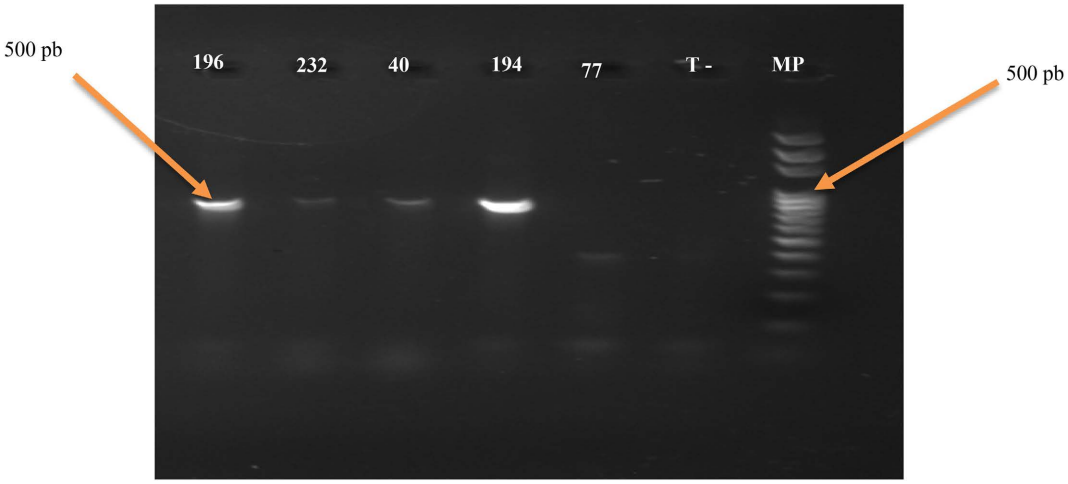
Legend of Figure 3: MP = Molecular weight marker (GeneRuler 1 Kb DNA Ladder), T– = Negative control. The number 242 represents the samples of PCR products carrying blaCTX-M type genes used for amplification. The direction of migration of the electrophoresis is from top to bottom.

Figure 3. Agarose gel of PCR products of blaCTX-M identified in *E coli.*



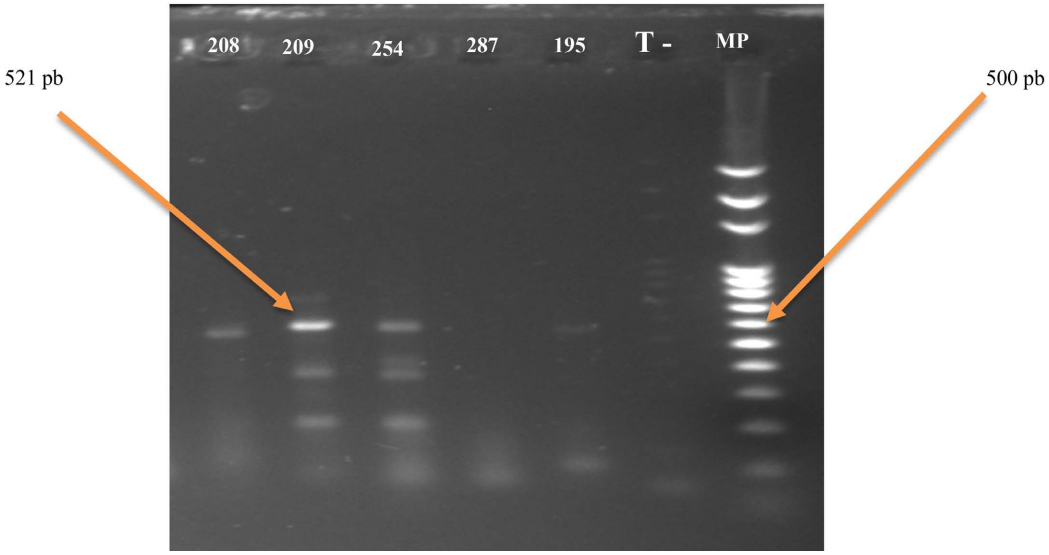
Legend of Figure 4: MP = Molecular weight marker (GeneRuler 1 Kb DNA Ladder), T– = Negative control, T+ = Positive control. Numbers 330, 66, 130 represent samples of PCR products carrying blaSHV-type genes used for amplification. The direction of migration of the electrophoresis is from top to bottom.

Figure 4. Agarose gel of PCR products of blaSHV identified in *E coli.*



Legend of Figure 5**:** MP = Molecular weight marker (GeneRuler 1 Kb DNA Ladder), T– = Negative control. Numbers 194, 40, 232 and 196 represent the samples of PCR products carrying blaIMP-type genes used for amplification. The direction of electrophoresis migration is from top to bottom.

Figure 5. Agarose gel of PCR products of blaIMP identified in *Salmonella spp.*



Legend of Figure 6: MP = Molecular weight marker (GeneRuler 1 Kb DNA Ladder), T– = Negative control. Numbers 208, 209 and 254 represent the samples of PCR products carrying blaNDM-type genes used for amplification. The direction of electrophoresis migration is from top to bottom.

Figure 6. Agarose gel of PCR products of blaNDM identified in *Salmonella spp.*

Following PCR, amplified DNA fragments from samples carrying beta-lactamase resistance genes revealed amplicons of 1000 base pairs (bp) in the case of the presence of the blaCTX-M gene, as shown in **Figure 3**, and amplicons of 875 base pairs (bp) in the case of the presence of the blaSHV gene, as shown in **Figure 4** [17]. For samples carrying carbapenemase resistance genes, amplicons of 500 base pairs showed the presence of the blaIMP gene, as shown in **Figure 5**, and amplicons of 521 base pairs (bp) showed the presence of the blaNDM gene, as shown in **Figure 6** [16]-[18].

4. Discussion

The results of the PCR analyses revealed the presence of ESBL resistance genes in the *Escherichia Coli* isolates, with percentages of 3.21% for the CTX-M gene, 15.35% for the SHV gene, and 11.42% for carbapenemase resistance genes, more specifically IMP. In the case of *Salmonella spp.* strains, only the SHV gene was identified in 2.32% of isolates belonging to the ESBL family, while 26.35% and 13.95% of isolates possessed the IMP and NDM genes respectively, both of the carbapenemase type.

There is little data in the scientific literature to allow in-depth comparisons with the results obtained in this study. This may be due to the particular nature of this subject. Indeed, the majority of studies on the presence of extended-spectrum beta-lactamase and carbapenemase genes in *Escherichia coli* and *Salmonella spp.* often focus on target populations other than chicken faeces. However, a number of articles and reviews have provided an overview of the situation and relevant comparative contexts.

In Malaysia, a study of 320 cloacal samples from farm chickens revealed antibiotic-resistant isolates, 84.5% of which were positive for at least one ESBL gene, mainly blaCTX-M 62.9% [20]. In Nigeria, high levels of ESBL resistance were observed, with 100% blaSHV, 87.5% blaTEM and 54.2% blaTEM-SHV co-resistance [21]. In addition, a review of 137 articles conducted between 2009 and 2023 revealed variable prevalences ranging from 1.1% to 48.5% of carbapenem-resistant bacteria in animals, including chickens, throughout Africa (22). From Algeria to Cameroon via Zambia, the genes associated with this prevalence were blaNDM (40.5%), blaSHV (27.4%) and blaIMP (14.3%) [22]. These multiple resistance prevalences are in line with the present study and highlight a variable increase in antibiotic-resistant bacterial strains.

It is clear that a wide distribution of chickens carrying resistant bacteria can contribute to the spread of antibiotic resistance. Given the high consumption of chicken in the city of Ouagadougou, there is an increased likelihood of human infection with multi-resistant *Salmonella spp.* and *Escherichia coli.* This represents a major public health problem, likely to have an impact on the effectiveness of future treatments unless preventive measures are put in place. Also, results of this study show the scale of the problem of antibiotic resistance in the food chain. This could guide management strategies to maintain the effectiveness of antibiotic treatments and improve public health. Limiting the spread of these resistant strains and preserving the effectiveness of antibiotics in treating bacterial infections in the future would be effective and necessary preventive and control measures. Managing antibiotic resistance involves promoting rational antibiotic use, improving infection control, and reducing the use of antibiotics in agriculture. It also requires investing in research for new treatments and strengthening global surveillance systems to monitor resistance trends. Public education and international cooperation are essential for a coordinated response to this global health threat.

5. Conclusions

Betalactam and carbapenem resistance genes in various strains of *Escherichia coli* and *Salmonella spp.* were identified and characterised in this study. The presence of genes such as ESBLs and carbapenemases in many isolates is a major public health concern. Resistance genes from *Escherichia coli* and *Salmonella spp.* have been found in the intestines of free-range chickens in Ouagadougou. This situation raises concerns about their potential impact on human health, particularly because of transmission through consumption of these chickens, making it difficult to treat certain infections. To maintain the effectiveness of medical treatments, it is essential to manage antibiotic resistance in food products of animal origin.

To achieve this, we need ongoing surveillance and public health interventions to reduce the risks associated with antibiotic-resistant infections.

Acknowledgements

We thank all the local chicken sellers in the city of Ouagadougou (HOSCO), the Molecular Biology and Molecular Genetics Laboratory (Labiogene) and the Pietro Annigoni Biomolecular Research Center (CERBA) who actively participated in the realization of this work.

Conflicts of Interest

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

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