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(RESEARCH ARTICLE)

Antibiotic resistance and the search for resistance and virulence genes in *Escherichia coli* strains isolated from agropastoral farms (Poultry, Cattle, and Fish) In Southern Benin

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Abstract

Resistance of animal-origin enterobacteria to antibiotics is experiencing a concerning global increase, with an escalating impact of extended-spectrum beta-lactamases (ESBL). Finding solutions to this problem requires reliable and diverse data. It is in this context that the present study was initiated to combat infections caused by *Escherichia coli* in agropastoral farms (poultry, cattle, and fish) in southern Benin. Samples consisting of cattle dung, poultry droppings, as well as water and sludge from fish farms were aseptically collected for various laboratory analyses. A total of 714 *E. coli* strains were isolated from 1,376 collected samples, with 434 strains from the Atlantique department and 280 strains from the Zou department. The isolation of strains was carried out according to the NF V08-050: 2009 standard. The identified strains were tested against various antibiotics using the Bauer and Kirby disk diffusion method on agar, as recommended by the WHO. Strains resistant to one or more antibiotics were selected for the search for resistance and virulence genes. Furthermore, multidrug resistance of the strains to antibiotics was also observed, particularly in the Beta-lactam class (100% resistance to AMC) and tetracyclines (90% resistance to tetracyclines). Additionally, genotypic detection revealed a total absence of virulence genes, whereas resistance genes such as *beta-lactamase Sulfhydryl Variable* (*bla-SHV*), *beta-lactamase Temoniera* (*bla-TEM*), and *quinolone resistance gene B* (*qnrB*) were more frequently detected at respectively 58%, 82% and 60%.

Keywords: Antimicrobial resistance; *Escherichia. coli*; Agropastoral farms; Genotypic detection

1. Introduction

Livestock farming is crucial for food and livelihoods, but the consumption of animal products can lead to diseases caused by pathogens, such as *Salmonella spp.* and *Escherichia coli*, which are present in the intestines of animals [1]. These bacteria cause severe foodborne infections, posing public health problems and leading to significant economic losses in the agropastoral sector [2]. The lack of information on livestock management and the transmission of pathogens to humans exacerbates the situation [3,4].

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The prevalence of pathogenic *E. coli* strains is a major issue in agropastoral farms in Africa, particularly in Benin. In Sub-Saharan Africa, this prevalence can reach up to 25% in farms where hygiene is insufficient [5]. In West Africa, prevalence rates range from 20% to 28% [6]. In Benin, it is estimated between 18% and 22%, with a growing concern for toxigenic strains like *E. coli* O157 [7].

Antimicrobial resistance (AMR) in foodborne pathogens is a growing threat, worsened by zoonotic transmission and the overuse of antimicrobials in agriculture [8,9]. The 'One Health' approach is essential to address this issue by considering the interconnectedness of human, animal, and environmental health [10]. The pathogenicity of these strains, due to their invasive ability and toxin production, leads to increased antibiotic use, thus raising the risk of resistance [11].

Thousands of antibiotics are produced worldwide to treat and prevent infectious diseases in humans and animals [12,13]. However, the excessive and inappropriate use of antibiotics in veterinary and human medicine is widely recognized as the main driver of the emergence and spread of antibiotic-resistant bacteria, both among pathogens and commensal bacteria [14, 15, 16, 17].

Antimicrobial resistance (AMR) is a critical issue in Benin, affecting human health and animal production. [18] examined the prevalence of resistant bacterial strains from food-producing animals and humans. [19] documented AMR in clinical and environmental isolates, while [20] explored AMR in pathogens from hospital environments, revealing challenges in managing resistant infections. [7] studied the impact of AMR on animal health in smallholder systems, and [21] highlighted the need to strengthen AMR surveillance in zoonotic bacteria from poultry.

Despite extensive research on AMR in Benin, studies on bacteria, particularly *E. coli*, from agropastoral environments remain limited, especially in the Atlantique and Zou departments in southern Benin. This study aims to combat *E. coli* infections in poultry, cattle, and fish farms in southern Benin.

2. Material and methods

2.1. Study area

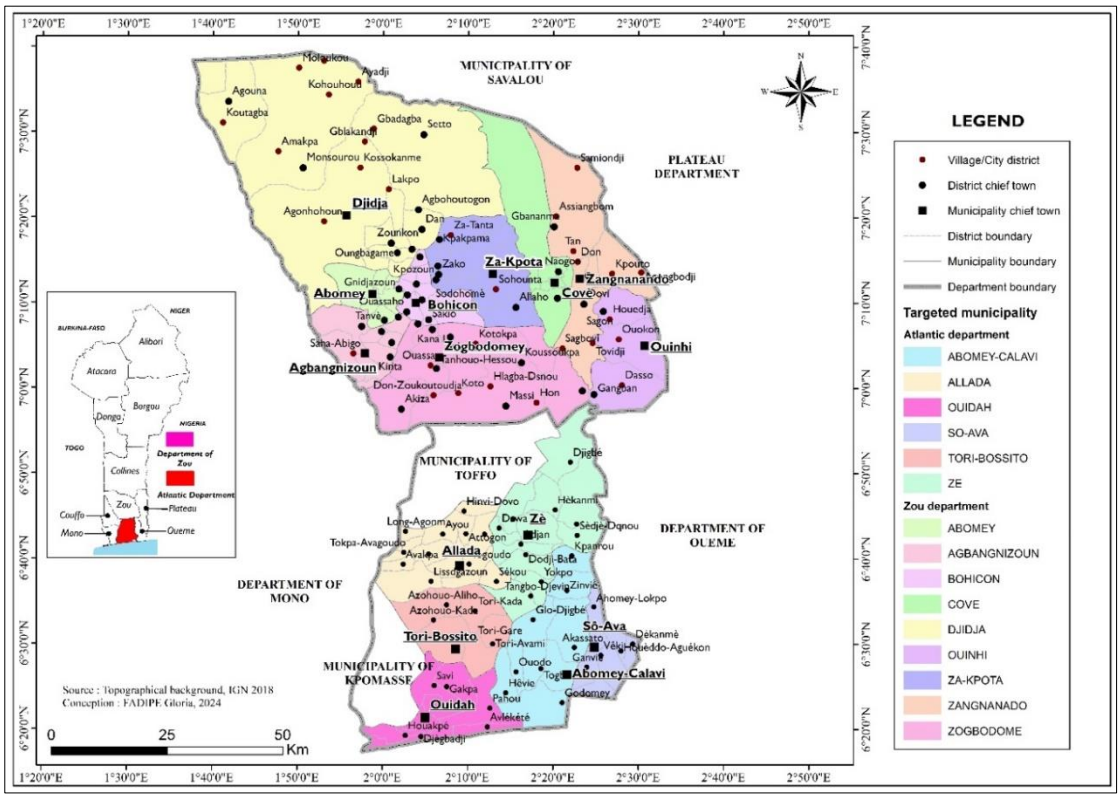


Figure 1 Sampled Study Area

The fieldwork was conducted in two departments in southern Benin, namely the Atlantique and Zou departments, due to the high concentration of producers and consumers in the poultry, cattle, and fish farming sectors. Six (06) municipalities in the Atlantique department and nine (09) in the Zou department were selected as study sites for the prospecting activities. In Atlantique (6° 39' 31.82" N 2° 13' 25.201" E), the selected municipalities are Abomey-Calavi, Ouidah, Allada, Tori-Bossito, Sô-Ava, and Zè. In the Zou department (7° 20' 48.937" N 2° 3' 59.472" E), the selected municipalities are Bohicon, Abomey, Agbangnizoun, Za-kpota, Zogbodomey, Ouinhi, Zangnanado, Covè, and Djidja. Figure 1 showed the sampled study area.

2.2. Materials

The antibiotic discs used and their families are summarized in Table 1 below:

Table 1 Summary of Antibiotic Discs Used

Families	Antibiotics	Charge
Bêta-lactams	Ceftriaxone (Oxoid, United Kingdom)	30 µg
	Amoxicillin + Clavulanic acid (Oxoid, United Kingdom)	30 µg
Aminoxides	Netilmicin (Oxoid, United Kingdom)	10 µg
Macrolides	Erythromycin (Oxoid, United Kingdom)	5 µg
Quinolones	Ciprofloxacin (Oxoid, United Kingdom)	5 µg
Nitrofurans	Nitrofurantoin (Oxoid, United Kingdom)	300 µg
Cyclins	Tetracycline (Oxoid, United Kingdom)	30 µg.

2.3. Sampling

Photos: (A) Sample of fresh stools emitted on the ground; (B) Sample at the anal orifice of a bovine; (C) Sample at the anal orifice of a poultry; (D) Sample at a fish pond. (Source: FADIPE Gloria, 2024)

Figure 2 Images of the Different Samples Taken

A total of 1,372 samples were collected by swabbing fresh feces from poultry and cattle using sterile swabs to reach the second layer between the surface and the part not in contact with the ground (Figure 2A). Samples were also taken from the anal openings of cattle and poultry (Figures 2B and 2C). In fish farms, the swabs were dipped in water collected by the fish farmer and then placed in a sterile bottle containing the same solution (Figure 2D). The swabs were immediately placed in a sterile bottle containing buffered peptone water (Hi Media, India).

The various samples of chicken droppings, cattle dung, water, and pond sludge collected were transported in insulated bags containing dry ice to the Laboratory of Microbiology, Food Technology, and Phytopathology (LAMITAP) of the Department of Plant Biology at the Faculty of Sciences and Techniques of the University of Abomey-Calavi for microbiological analyses. Once at the laboratory, the microbiological analyses focused on the isolation, antibiotic resistance of bacterial strains, and biochemical characterizations of *E. coli* strains. The genotypic detection of resistance and virulence genes was carried out at the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM) of the Department of Biochemistry and Cell Biology at the Faculty of Sciences and Techniques of the University of Abomey-Calavi.

2.4. Isolation of *Escherichia coli*

The isolation of *Escherichia coli* strains was conducted according to the ISO 16649-1: 2018 standard. Tryptone Bile X-glucuronide (TBX) agar (Biokar Diagnostics, France) was used. It involved aseptically spreading 0.1 mL of inoculum onto the TBX medium. The plates were incubated at 44°C for 24 hours. Colonies showing a blue-green color with a metallic sheen and a diameter of 0.5 mm or larger were considered characteristic of *E. coli*. The obtained strains were subjected to both morphological identification through wet mount tests and Gram staining (Kit Gram's stain pack tcs Biosciences) and biochemical identification through various tests (Identification on Triple Sugar Iron (TSI) agar [22, 23] catalase test [24], oxidase test [25], and identification using the API 20 E gallery).

2.5. Antibiogram test

The [26] method, recommended by the WHO, was used to evaluate antibiotic resistance. This method relies on the diffusion of antibiotics from discs on Mueller-Hinton agar inoculated with a bacterial suspension of 10^6 bacteria/mL. After 24 hours of incubation at 37°C, the inhibition zones around the discs are measured. Results are interpreted according to CASFM 2020 standards. The antibiotics tested include: Ceftriaxone (30 µg), Amoxicillin + Clavulanic Acid (30 µg), Netilmicin (10 µg), Erythromycin (5 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), and Tetracycline (30 µg).

2.6. Detection of extended spectrum ß-lactamases (ESBL)

The detection of ESBLs was performed using the double disc synergy method [27] for all enterobacterial strains. Mueller-Hinton agar (Bio Rad, France) was inoculated with a bacterial suspension of 10^6 bacteria/mL. The test used third-generation cephalosporins, namely cefotaxime (CTX) and ceftriaxone (CRO), with an amoxicillin + clavulanic acid (AMC) disc placed at the center of the two cephalosporin discs, 30 mm apart. After incubation at 37°C for 24 hours, a positive result is indicated by a corkscrew-shaped halo between the CTX and AMC discs and between the AMC and CRO discs, demonstrating the synergy of beta-lactams with clavulanic acid [28].

2.7. Genotypic detection of β-lactamases

2.7.1. Choice of strains

Quinolone-resistant *E. coli* BLSE strains were selected for molecular typing of antibiotic resistance genes.

2.7.2. Extraction and purification of DNA

The DNA extraction was performed using the method adapted from [29]. It consisted of inoculating the strain to be tested on Muller-Hinton (MH) agar medium poured into Petri dishes. The dishes were incubated at a temperature of 37°C for 18 hours. The next day, a pre-culture of a few young colonies was performed in 1 mL of liquid MH in each Eppendorf tube. The Eppendorf tubes were incubated in an incubator at a temperature of 37°C for 15 hours. After incubation, the tubes containing the pre-cultures were centrifuged using a centrifuge (Sigma 202 MK) at 12,000 rpm for 5 min. Then the supernatant was poured. Then 500 µL of EDS were added to the pellet. The tubes previously homogenized using a vortex mixer were heated in a dry bath (Bioblock Scientific code: 92617) at a temperature of 95°C for 15 min. After the bacterial cells burst, a second centrifugation was performed at 12,000 rpm for 5 min. The supernatant of each tube was collected in another Eppendorf tube and 500 µL of 70% (v/v) alcohol was added. A third centrifugation was performed at 12,000 rpm for 5 min. The supernatant was emptied and 50 µL of EDS was added to

the tubes containing the DNA pellets previously air-dried under the hood. The DNAs are stored at 4°C for immediate use or at -20°C for long-term use.

2.7.3. PCR amplification

Table 2 Primer Sequences of Resistance Genes of Strains

Genes	Primer sequences (5'–3')	Sizes (bp)	Reference
bla-SHV	F: CGCCGGGTTATTCTTATTTGTCGC R: TCTTTCCGATGCCGCCGCCAGTCA	1017	[30]
bla-TEM	F: ATTGGGTGCACGAGTGGGTTAC R: ATAATTGTTGCCGGGAAGCTAG	465	[30]
qnrA	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	[31]
qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	[31]
qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	416	[31]

The PCR reaction was performed in a 5 Prime thermal cycler (5 PRIME/05; Serial No: 51730-2 Made in UK). The different primer sequences (Forward and Reverse) used for the detection of multidrug-resistant strains producing β-lactamases and for the virulence of *E. coli* are listed in Tables 2 and 3. The 20 µL reaction medium contained in the Eppendorf microtubes consists of 12 µL of Master Mix (Taq polymerase + MgCl2 + dNTP + Buffer), 1 µL of each of the primers (sense and antisense), 1 µL of DNA-free PCR water and 5 µL of DNA. The amplifications were performed according to the following program: an initial denaturation; a number of cycles consisting of a denaturation step, an annealing, an elongation and a final elongation. Then, the thermocycler was set to store the PCR products at a temperature of 4°C before migration.

Table 3 Primer Sequences of Virulence Genes of Strains

Genes	Primer sequences (5'–3')	Sizes (bp)	Reference
Int1	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	280	[32]
Int2	F: TTATTGCTGGGATTAGGC R: ACGGCTACCCTCTGTTATC	233	[32]
Int3	F: AGTGGGTGGCGAATGAGTG R: TGTTCTTGTATCGGCAGGTG	600	[32]
invA	F: GTG AAA TTA TCG CCA CGT TCG GGC AA R: TCA TCG CAC CGT CAA AGG AAC CCG TT	284	[33]
spvR	F: CAGGTTCCTTCAGTATCGCA R: TTTGGCCGGAAATGGTCAGT	310	[34]
lt	F: GCGACAAATTATACCGTGCT R: CCGAATTCTGTTATATATGT	315	[35]

The program for the amplification of resistance and virulence genes is summarized in the following tables 4 and 5:

Table 4 Resistance Genes Amplification Program

Amplification stage	Temperature / Duration condition		
	CTX-M, TEM, SHV	***qnrA*, q*nrB*, *qnrS***	***tetA***
Initial Denaturation	94°C/5min	95°C/5min	95°C/5min
Cyclic Denaturation	94°C/1min	95°C/30s	95°C/30s
Hybridization	60°C/1min	60°C/30s	56°C/30s
Cyclic Elongation	72°C/1min	72°C/1min	72°C/1min
Final Elongation	72°C/7min	72°C/10min	72°C/10min
Number of cycles	30	35	35

Table 5 Virulence Genes Amplification Program

Amplification stage	Temperature / Duration condition			
	Int 1,2,3	***SpvR***	***InvA***	***lt***
Initial Denaturation	94°C/5min	94°C/3min	94°C/5min	94°C/3min
Cyclic Denaturation	94°C/1min	94°C/30s	94°C/1min	94°C/30s
Hybridization	60°C/30s	50°C/30s	60°C/30s	50°C/30s
Cyclic Elongation	72°C/2min	72°C/30s	72°C/2min	72°C/30s
Final Elongation	72°C/10min	72°C/5min	72°C/10min	72°C/5min
Number of cycles	30	35	30	35

2.8. Electrophoresis

The amplification products were subjected to electrophoresis on a 1.5% agarose gel stained with ethidium bromide and buffered with 1X TBE. The migration was carried out under a constant voltage of 120 V for 30 minutes. The gel was then observed under UV light, and the presence of a fluorescent band indicating the expected fragment size confirmed the presence of the target gene. A 100 bp molecular weight marker (Promega, USA) was used to estimate the size of the DNA fragments.

2.9. Data processing

The laboratory data were recorded in an Excel 2021 database. Graphs were created using GraphPad Prism 8.0, and all statistical analyses were performed with R software 3.6.1. Proportion comparisons were conducted using Pearson's chi-square test and Fisher's exact test, with statistical significance set at $p<0.05$.

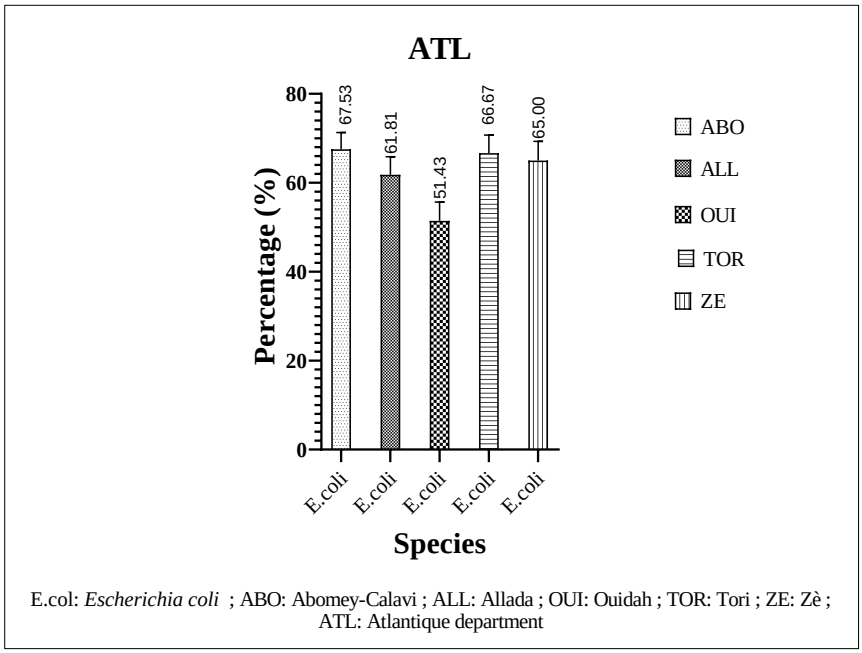
3. Results and discussion

3.1. Isolation frequency

Morphological and biochemical identification confirmed 714 strains of *E. coli* isolated from the 1,376 samples collected from various agropastoral farms. A high prevalence of *Escherichia coli* strains was observed in the municipalities of Atlantique (Figure 3). The highest isolation rates were recorded in Abomey-Calavi (67.53%), Tori (66.61%), Zè (65.00%), and Allada (61.81%). Conversely, the lowest isolation rate for *E. coli* was found in Ouidah, at 51.43%. In the Zou department (Figure 4), *E. coli* strains were most frequent in the municipality of Zangnanado (56.66%). In contrast, the municipality of Agbangnizoun had the lowest rate for these strains, at 25%. These results are consistent with those obtained by [36], who also found a high presence of *E. coli* strains (75%) compared to *Salmonella spp.* strains (47.82%) in poultry and fish farms in southern Benin. However, these results slightly differ from those reported by [37], who observed a prevalence of 20.2% for *E. coli* strains isolated from cattle in Abidjan. This discrepancy may be due to [37]'s

results involving strains isolated from cattle farms, while this study addresses strains isolated from poultry, cattle, and fish farms.

Figure 5 shows the prevalence of strains in different types of farming in the Atlantique and Zou departments. It appears that *E. coli* strains are more frequent in cattle, with a rate of 82.7%, compared to 82.3% and 42.5% observed in poultry and fish farms, respectively, in the Atlantique department. In contrast, the highest proportion of *E. coli* in the Zou department is recorded in fish farms, with a rate of 54.34%, compared to cattle and poultry, where proportions of 48.97% and 42.13% were observed. Similar results were obtained by [36], who observed a high presence of *Salmonella spp.* strains in fish farms compared to *E. coli* strains, which were more prevalent in poultry farms. The presence of these strains in farms can be explained by the fact that *Salmonella* and *E. coli* are enterobacteria whose main reservoir is the digestive tract of vertebrates [38, 39].



E.col: *Escherichia coli* ; ABO: Abomey-Calavi ; ALL: Allada ; OUI: Ouidah ; TOR: Tori ; ZE: Zè ; ATL: Atlantique department

Figure 3 Prevalence of Strains in the Municipalities of the Atlantique Department

Colonies with a blue-green color and a metallic sheen, with a diameter of 0.5 mm or more, are characteristic colonies of *E. coli* on TBX medium. The serotyping of *E. coli* strains is divided into 2 classes: enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC). Microscopic observation after Gram staining of *E. coli* strains showed a red or pink coloration with rod-shaped, non-spore-forming cells. All isolated *E. coli* strains tested positive for the catalase test. This result differs from that of [37], who observed the predominance of *E. coli* O127 among his serogroups. These differences might be attributed to the variation in samples from which these germs were isolated. These results could be due to poor management of the farms as well as failures in hygiene or biosafety measures. Regarding *E. coli* strains, based on their specific virulence properties and serotypes, they are among the most frequent causes of acute diarrhea. These findings are concerning and necessitate the adoption of preventive measures for better consumer health assurance.

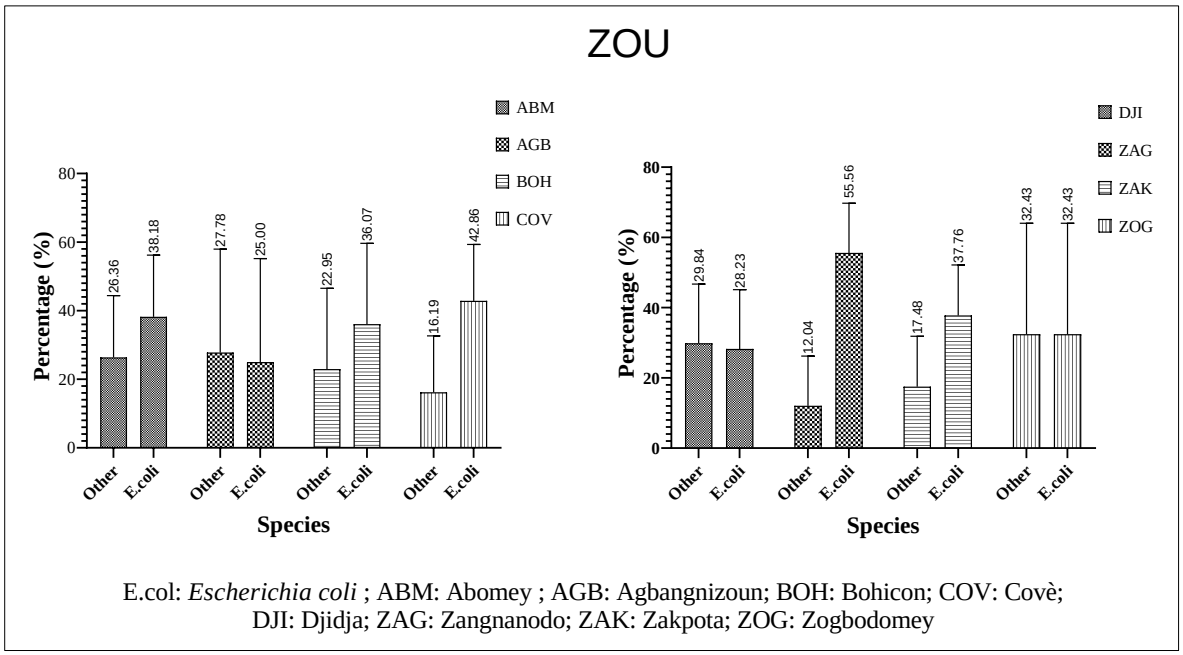


Figure 4 Prevalence of Strains in the Municipalities of the Zou Department

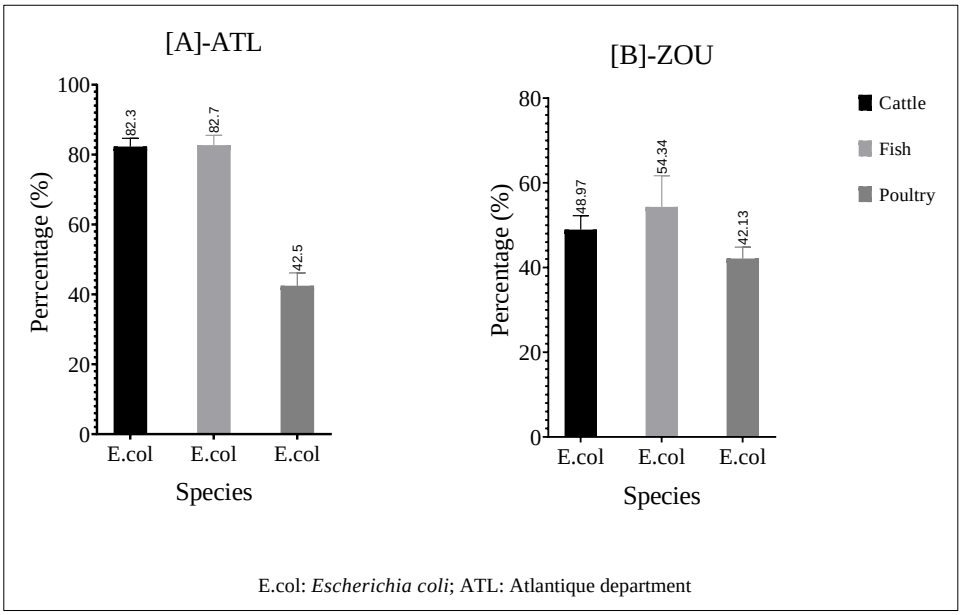


Figure 5 Prevalence of Strains in Different Types of Farms in the Atlantique Department [A] and Zou Department [B]

3.2. Antibiotic sensitivity of *Escherichia coli* strains

Among the 434 *E. coli* strains isolated in the Atlantique department, all were resistant to Amoxicillin + Clavulanic acid. Moderate resistance was observed to Ceftriaxone (15-19 mm inhibition zone, 50%) and Netilmicin (51.56%). High resistance rates were noted for Ciprofloxacin (65.88%) and Tetracycline (67.21%) (Figure 6). In the Zou department, all 280 *E. coli* strains were resistant to Amoxicillin + Clavulanic acid (100%). Very high resistance levels were observed to Netilmicin (85.57%) and Tetracycline (90%). Resistance rates to Ceftriaxone and Ciprofloxacin were 40% and 51.78%, respectively (Figure 7).

Additionally, *E. coli* strains from poultry farms showed higher resistance to Ciprofloxacin compared to those from cattle farms. Resistance levels varied across different types of farms, with *E. coli* from cattle farms showing lower resistance to Ceftriaxone (2.02%) compared to fish (25.71%) and poultry farms (50%). Similar patterns were observed with lower resistance to Netilmicin (10.13%), Ciprofloxacin (26.35%), and Tetracycline (36.48%) in cattle farms, compared to

higher resistance in fish (Netilmicin 50.81%, Ciprofloxacin 44.77%, Tetracycline 67.21%) and poultry farms (Netilmicin 51.56%, Ciprofloxacin 65.88%, Tetracycline 51.56%).

In both departments, the *E. coli* strains show complete (100%) resistance to beta-lactams, particularly Amoxicillin + Clavulanic Acid, which aligns with the findings of [36] and recent studies. This high resistance level exceeds that reported by [40] and [41] and may result from excessive use of these antibiotics due to their broad spectrum and low cost.

A high resistance to tetracyclines was also observed, reaching 67.21% in fish farms in the Atlantique department and 98.59% in poultry farms in the Zou department. This phenomenon is consistent with the observations of [42] and [43] and is attributed to the high use of tetracyclines, particularly Oxytetracycline, in treating bacterial infections in livestock. The experience of farmers also influences this usage, with more experienced ones prescribing more tetracycline. This situation is concerning as it may lead to therapeutic failures and increased antibiotic resistance.

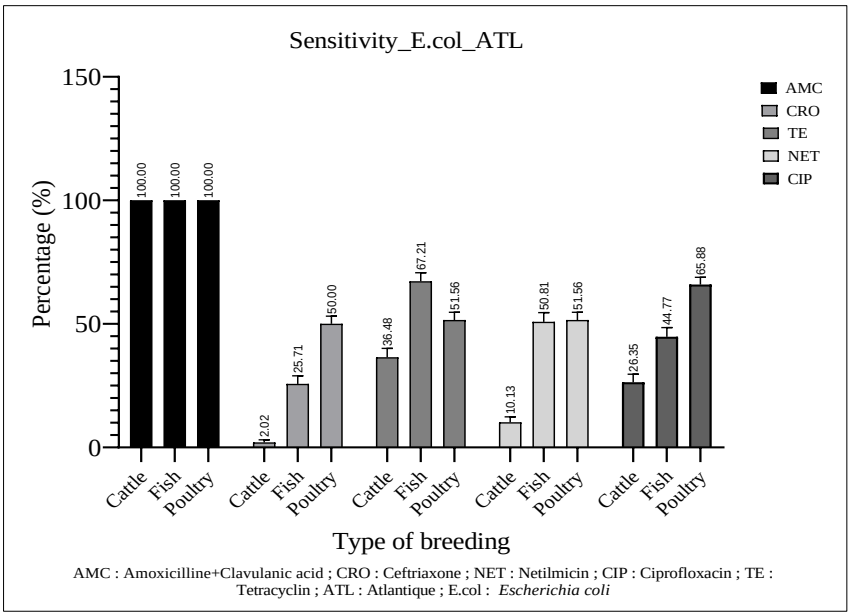


Figure 6 Sensitivity of *E. coli* Strains to Antibiotics in the Atlantique Department

A high rate of resistance to tetracycline was noted in poultry farms, surpassing the 46.24% rate reported by [44] in Senegal. Results for other antibiotics are similar to those of [45], who observed underuse of Aminoglycosides (100%), Macrolides (90.6%), and Tetracyclines (74.5%), likely due to their common preventive use. Recent studies, such as those by [46] in Central Europe and [47] in Australia, also reported increased resistance to tetracycline in poultry and cattle farms. In contrast, Nitrofurans, quinolones, and sulphonamides are better dosed due to a better cost/dosage ratio. These under dosing practices increase the risk of antibiotic resistance, representing a serious threat to public health [48].

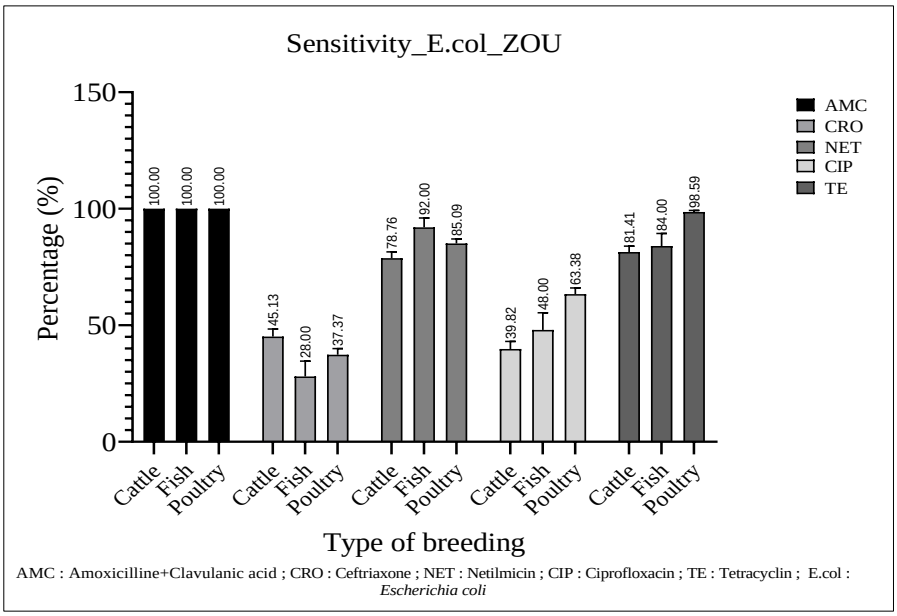


Figure 7 Sensitivity of *E. coli* Strains to Antibiotics in the Zou Department

Figure 8 shows how the resistance or sensitivity of a strain to different antibiotics is been appreciated.

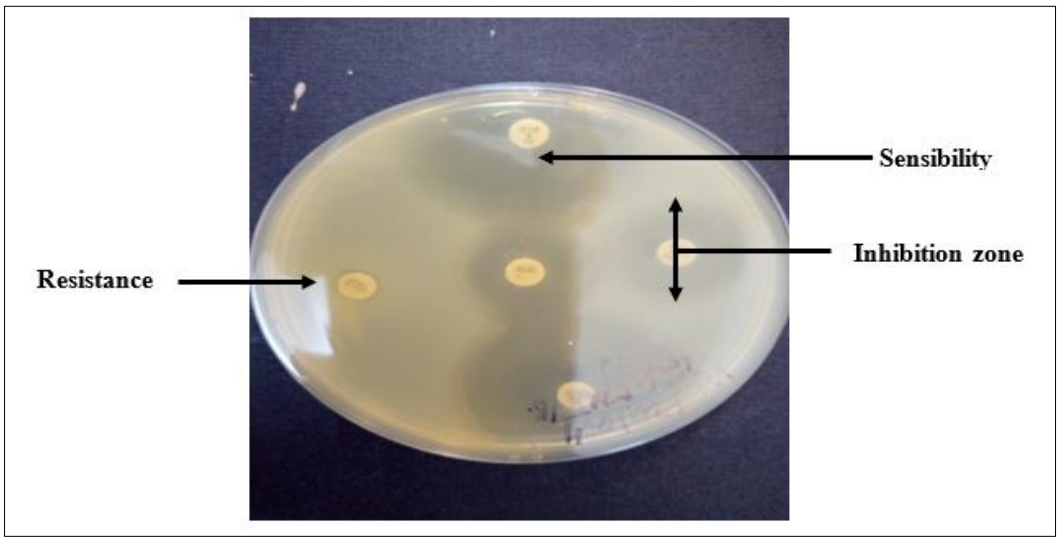


Figure 8 Image Showing the Resistance or Sensitivity of a Strain to Antibiotics

3.3. Genotypic detection of strains

A total of ten (10) *E. coli* strains were tested for resistance genes (*bla-SHV, bla-TEM, qnrA, qnrB, qnrS*) and virulence genes (*spvR, int1, int2, int3, invA, lt*). Electrophoretic analysis revealed DNA fragments of 1017 bp, 465 bp, and 469 bp corresponding to the *bla-SHV*, *bla-TEM*, and *qnrB* genes, respectively. Pathogenic *E. coli* strains carried the *bla-SHV*, *bla-TEM*, and *qnrB* genes with frequencies of 58%, 82%, and 60%, respectively. None of the remaining virulence or resistance genes were detected *int1*; *int2*; *int3*; *qnrA*; *qnrS*; *invA*; *spvR* and *lt*. Figure 9 shows an image of the gel after electrophoresis indicating the size of the *qnrB* gene.

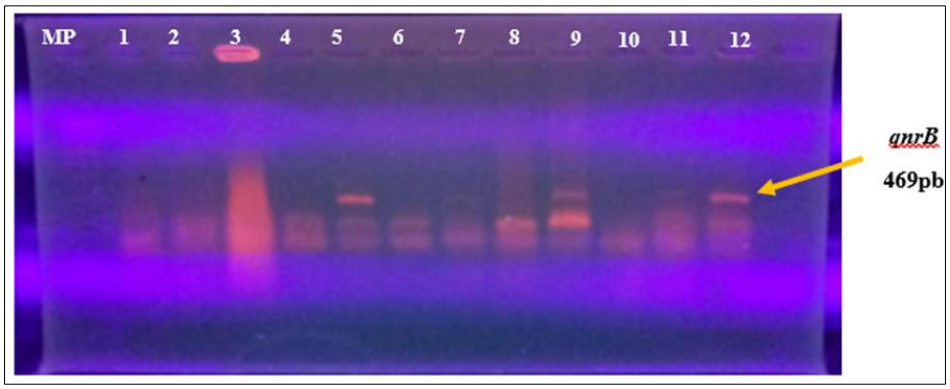


Figure 9 Gel Image After Electrophoresis Indicating the Size of the *qnrB* Gene

Table 6 shows the different results obtained after searching for resistance and virulence genes in *E. coli* strains.

Table 6 Results of the detected genes rates

Types of genes	Genes	Percentage of detection
Resistance genes	*bla-SHV*	58%
	bla-TEM	82%
	qnrA	0%
	qnrB	60%
	qnrS	0%
Virulence genes	*Int1*	0%
	Int2	0%
	Int3	0%
	invA	0%
	spvR	0%
	lt	0%

In Gram-negative pathogenic bacteria, the production of beta-lactamase is a key factor in resistance to beta-lactams [49]. Prolonged exposure to these antibiotics leads to overproduction and mutations of beta-lactamase genes, enhancing the bacteria's ability to hydrolyse broad-spectrum penicillin and cephalosporins [50]. In this study, *bla-TEM* genes were detected at a rate of 82%, while *bla-SHV* genes were detected at a moderate rate of 58%, which contrasts with other studies showing variable results [51, 52, 53].

In Ethiopia and Nigeria, a high prevalence of resistant bacteria was observed, particularly in agricultural environments and animal-derived foods, posing a significant public health risk [54, 55]. In this study, *qnrB* genes were found in 60% of *E. coli* strains, which aligns with [56] but contrasts with [57], who reported a 95% prevalence of the *qnrS* gene in multidrug-resistant enterobacteria. The presence of these genes may contribute to resistance to fluoroquinolones and increase the risk of spreading this resistance in the environment [58, 59].

Moreover, no virulence genes such as *int1*, *int2*, *int3*, *invA*, *spvR*, or *lt* were detected, which is consistent with the results of [60] but differs from [37], who detected the *invA* gene in all *Salmonella* strains

4. Conclusion

In Benin, animal production is the second most important economic activity after agriculture, supported by agricultural policies and the increasing use of veterinary medicines to treat diseases, stimulate growth, and improve yields.

However, the overuse of antibiotics in both veterinary and human medicine contributes to the emergence of resistant microorganisms, increasing the risk of therapeutic failures in farming and zoonotic diseases affecting human health. Analyses revealed that *E. coli* strains in different farms are predominantly found in poultry and cattle farms. These strains exhibit a diversity of serotypes, including pathogenic EHEC and ETEC strains. *E. coli* strains show multidrug resistance, particularly to beta-lactams and cyclins, with variable resistance levels across farms. Resistance genes such as *bla-SHV*, *bla-TEM*, and *qnrB* are common in these strains. Although virulence genes were not detected, animal feces are a major vector for the spread of pathogenic and resistant bacteria, posing a significant public health problem. Recommendations are made to authorities, veterinarians, farmers, and consumers to limit the misuse of antimicrobials in farming. These include implementing regulations, surveillance programs, awareness campaigns, and responsible practices in drug prescription, administration, and farm management to prevent the spread of infections and bacterial resistance.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflicts of interest relevant to this article.

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