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Environmental Reservoirs of *bla, qnr* and *mcr*-1 Resistance Genes in *Enterobacteriaceae* from Animal and Wastewater Sources in Daloa, Côte d'Ivoire

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Abstract The environmental spread of antibiotic resistance genes is an increasing public health concern, particularly in resource-limited countries where waste management systems are often inadequate. This study aimed to identify Enterobacteriaceae in different ecosystems of Daloa city (Côte d'Ivoire) and to assess the occurrence of critical resistance genes, including *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*S, and *mcr*-1. Forty samples were collected from poultry and pig feces, wastewater from gutters, and a water body receiving domestic waste. Bacteria were isolated on selective media, identified using the API 20E system, and subjected to multiplex PCR for resistance gene detection. A total of 60 isolates were recovered, primarily *Escherichia coli* (73.3%), followed by *Salmonella* spp. (13.3%), *Enterobacter cloacae* (8.3%), and *Klebsiella oxytoca* (5%). The most frequently detected genes were *qnr*B (30%) and *bla*-SHV (15%), whereas *mcr*-1 was not detected in any isolate. Analysis of gene distribution across environments revealed that quinolone resistance genes were generally more widespread and prevalent than β-lactam resistance genes. These findings underscore the role of environmental ecosystems in the dissemination of antibiotic resistance and highlight the urgent need to strengthen surveillance and waste management strategies.

Keywords: *Antibiotic resistance genes, β-lactamases, Enterobacteriaceae, Environmental reservoirs, Quinolones*

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1. Introduction

Antibiotic resistance is a major global public health problem. It is steadily increasing due to the irrational and inappropriate use of antibiotics [1,2,3]. This phenomenon results in the growing inefficacy of antibiotic treatments against bacterial infections, leading to therapeutic complications, increased morbidity and mortality rates, as well as high economic costs [4]. In 2019, antibiotic resistance was estimated to be responsible for approximately 27 deaths per 100,000 inhabitants and associated with nearly 115 deaths per 100,000 inhabitants in West Sub-Saharan Africa. These figures are higher than those observed in other regions of the world [3]. Bacteria increasingly showing resistance to antibiotics are Enterobacteriaceae [5,6]. Indeed, Enterobacteriaceae are etiological agents of numerous nosocomial infections worldwide [7]. In these bacteria, genes such as *bla*-TEM, *bla*-SHV, *bla*-CTX-M (responsible for extended-spectrum β-lactamase production), *qnr*A, *qnr*B, *qnr*S (conferring resistance to quinolones), as well as *mcr*-1 (associated with colistin resistance), are frequently implicated in antibiotic resistance [8,9]. The detection of these genes in bacteria isolated from non-clinical sources highlights the environmental dissemination of the phenomenon, promoted by domestic and agricultural discharges [10]. Thus, the emergence and spread of multidrug-resistant bacteria are no longer restricted to hospitals. Domestic and agricultural environments, such as wastewater, animal droppings, and drainage canals, serve as important reservoirs of multidrug-resistant bacteria [3,11]. In developing countries, where waste treatment systems are often inadequate, the contamination of water and soil by resistant bacteria can pose a significant risk to human

health [3,12]. Côte d'Ivoire, like many other African countries, is not exempt from this issue. It is therefore crucial to monitor potential sources of dissemination. Indeed, Enterobacteriaceae from animal excreta, wastewater, and polluted aquatic environments represent potential reservoirs of antibiotic resistance genes that can be transmitted to human pathogenic strains. The present study aims to evaluate the occurrence and distribution of selected antibiotic resistance genes in Enterobacteriaceae isolates obtained from different environmental ecosystems in the city of Daloa. Specifically, the study focuses on the isolation and identification of Enterobacteriaceae from environmental samples, including poultry and pig droppings, a domestic wastewater-receiving lake, and drainage canals. The presence of resistance genes such as *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*S, and *mcr*-1 will be assessed, while their frequency among isolates and distribution across the different environmental sources will also be analyzed.

2. Materials and Methods

2.1. Study Design

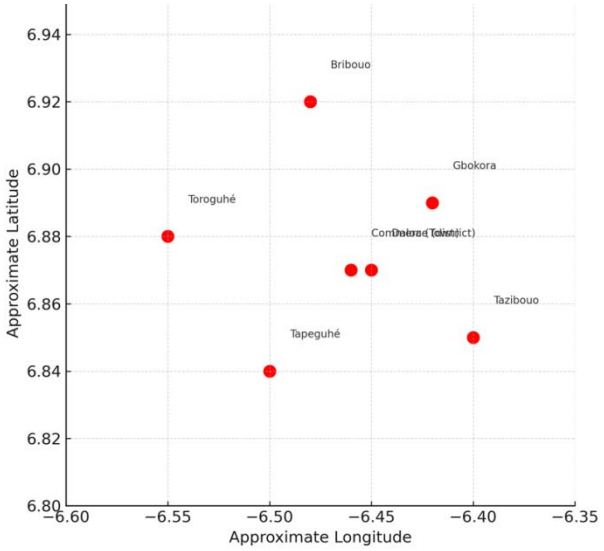

Figure 1. Schematic representation of sampling sites around Daloa. This schematic map illustrates the main sampling sites located in and around Daloa, Côte d'Ivoire. The sites include poultry and pig farms (Gbokora, Tazibouo, Bribouo, Tapeguhé, Toroguhé), as well as the urban district of Commerce and a collection point in the town of Daloa. The positions are approximate and intended for illustrative purposes only

Sampling was carried out in the city of Daloa and its surroundings (Figure 1), covering different types of environments. Chicken droppings were collected from five farms located in the neighborhoods of Gbokora and Tazibouo, with two samples per farm, totaling 10 samples. For pig droppings, five farms in the peripheral villages of Daloa (Gbokora, Tazibouo, Bribouo, Tapeguhé, and Toroguhé) were selected, also with two samples per farm, for a total of 10 samples. Wastewater samples were collected from the Commerce district (downtown Daloa) from two distinct gutters, with five samples collected per gutter. Finally, lake water samples (from a water body receiving domestic waste) were collected in the Gbokora neighborhood at five different points of the lake, with two samples per point, for a total of 10 samples. Overall, the study was based on 40 samples, including 10 chicken droppings, 10 pig droppings, 10 wastewater, and 10 lake water samples. Samples were collected in sterile bottles (500 mL for water and 200 g for droppings), labeled, placed in a cooler containing ice packs, and transported to the laboratory for further analyses.

2.2. Detection of Enterobacteriaceae in the Different Samples

Enterobacteriaceae were investigated from the collected samples by preparing a stock suspension in Buffered Peptone Water (BPW, BioRad, France) and performing decimal dilutions according to ISO 6887-2:2017 (ISO, 2017). The dilutions were plated on VRBL agar (Conda, Spain) for the enumeration of total coliforms and Rapid'E.coli 2 agar (Merck, Germany) for *E. coli* and other coliforms, followed by incubation at 37°C for 24 h. Characteristic colonies were subcultured on nutrient agar (BioRad, France) to obtain pure isolates. Preliminary bacterial identification was then carried out based on colony morphology, cell morphology determined by Gram staining, and biochemical tests including sugar fermentation [13]. Confirmed Enterobacteriaceae isolates were preserved in LB broth supplemented with 20% glycerol for long-term storage and further analyses. In total, 60 isolates were preserved, with 15 isolates obtained from each sample matrix.

2.3. Identification of Bacterial Isolates Using the Standardized API 20E System

The identification of Enterobacteriaceae isolates was carried out as in [13] using the standardized API 20E system (BioMérieux, France). Pure isolates were subcultured on EMB agar and incubated at 37 °C for 18-24 h. A homogeneous bacterial suspension was then prepared and inoculated into the tubes and wells of the API 20E strip, ensuring anaerobiosis for specific tests (arginine dihydrolase, decarboxylases, urease, and H_2S production) by overlaying with sterile mineral oil. The tests were incubated at 37 °C for 18–24 h, and identification was performed according to the numerical profile provided by the API 20E system.

2.4. Molecular Detection of Beta-lactamase, Quinolone and Colistine Resistance Producing Genes

The detection of resistance genes to β-lactams, quinolones, and colistin was performed according to the method as in [14], with slight modifications. Under a Bunsen burner, a well-developed bacterial colony previously grown on LB agar was collected and suspended in 50 µL of sterile distilled water. The suspension was then diluted 50-fold and vortexed to lyse the bacterial cells. The supernatant containing the extracted DNA was transferred into sterile 1.5 mL Eppendorf tubes and stored at –5 °C. A multiplex PCR was subsequently carried out to amplify different resistance genes, with the primer

sequences listed in Table 1. The amplification products were subjected to agarose gel electrophoresis, with gel concentration adjusted according to the size of the DNA fragments.

Table 1. List of primers employed for PCR amplification of the resistance genes *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*S, and *mcr*-1

Genes	Primers	Sequences	Size (bp)	References
bla-TEM	TEM	F ATGAGTATTCAACATTTCCGTG R TTACCAATGCTTAATCAGTGAG	840	[15]
bla-SHV	SHV	F TTTATGGCGTTACCTTTGACC R ATTTGTCGCTTCTTTACTCGC	1051	[15]
bla-CTX-M	CTX-M	F GGTTAAAAAATCACTGCGTC R TTGGTGACGATTTTAGCCGC	863	[16]
qnrA	QnrA	F GATAAAGTTTTTCAGCAAGAGG R ATCCAGATCGGCAAAGGTTA	543	[17]
qnrB	QnrB	F GACAGAAACAGGTTCACCGGT R CAAGACGTTCCAGGAGCAACG	469	[8]
*qnr*S	QnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCAACCTGTAGGC	417	[18]
mcr-1	CLR	F : CGGTCAGTCCGTTTGTTC R : CTTGGTCGGTCTGTAGGG	309	[19]

2.5. Analysis of the Distribution of Resistance Genes by Environmental Matrix

The distribution of resistance genes by environmental source was performed as described in [20]. To assess the distribution of resistance genes across different environments, each identified Enterobacteriaceae isolate was assigned to its original matrix: chicken droppings, pig feces, wastewater from gutters, and water bodies used as disposal sites for household and rainwater waste. The presence of the genes *bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*S, and mcr-1 was determined for each isolate by PCR. The distribution of genes within each matrix was evaluated by counting the number of detected genes among the isolates from that matrix. The results were then expressed as percentages and as the number of positive isolates relative to the total number of isolates from the matrix (n/N), allowing visualization of the contribution of each environment to the spread of resistance genes according to the formula below. This approach enabled the identification of the matrices most critical in terms of diversity and abundance of resistance genes.

$$Percentage\ of\ dispersion(\%) = \frac{n}{N} X100$$

n:Number of positive isolates for each gene
N**:** Total number of isolates in the source (matrix)

2.6. Statistical Analysis

The collected data were analyzed using descriptive statistics. Means and standard deviations were calculated for quantitative variables, while absolute and relative frequencies (percentages) were determined for qualitative variables. The analysis was performed using Excel 2016.

3. Results

3.1. Diversity of Enterobacteria in the Studied Ecosystems

Table 2 presents the results of bacterial isolation from four ecosystems: chicken droppings, pig droppings, a domestic wastewater-receiving water body (lake), and sewage drains. Four (4) bacterial genera were identified: *Escherichia*, *Klebsiella*, *Salmonella* sp., and *Enterobacter*, totaling 60 isolates. *E. coli* was the most frequently isolated bacterium (73.33% of the isolates) and was present in all ecosystems. *Klebsiella oxytoca* was found in lower proportions (5%). Regarding ecosystems, animal droppings (chicken and pig) showed a high prevalence of *E. coli* (26 isolates out of 42 in total). Lake and sewage water samples harbored all four (4) bacterial species, unlike animal droppings.

Table 2. Distribution of bacterial isolates identified across the different studied ecosystems

Sources of isolation	Presumtptive microorganisms identified in samples				
	E.coli	*K. oxycota*	*Salmonella* spp	*E. cloacae*	Total isolates
Chicken feces	14	0	1	0	15
Pig feces	12	0	2	1	15
Wastewater discharge gutters	9	2	2	2	15
Lake	9	1	3	2	15
Total	44	3	8	5	60
Percentage (%)	73.33	5	13.33	8.33	100

3.2. Prevalence of Resistance Genes (*bla*-TEM, *bla*-SHV, *bla*-CTX-M, *qnr*A, *qnr*B, *qnr*S, *mcr*-1) among the Identified Isolates

The results in Table 3 reveal that the distribution of resistance genes varies according to Enterobacteriaceae species. In *E. coli*, the genes *qnrB* (34.1%) and *bla-SHV* (15.9%) were the most frequent, while *mcr-1* was absent. *Klebsiella oxytoca* mainly carried *bla-CTX-M* (66.7%), and *Enterobacter cloacae* showed a notable prevalence of

qnrA (40%), with lower frequencies for the other genes. In *Salmonella* spp., *bla-TEM* was the most detected (50%), followed by *bla-CTX-M* (25%) and the quinolone resistance genes *qnrB* and *qnrS* (12.5% each). Overall, β-lactam and quinolone resistance genes were the most widespread, while *mcr-1* was not detected in any isolate.

3.3. Environmental Dissemination of Antibiotic Resistance Genes

Environmental dissemination of antibiotic resistance genes is illustrated in Figure 2 below. The analysis of resistance gene prevalence across environmental matrices shows that all studied environments contained isolates carrying critical genes. In chicken droppings, the *qnrB* gene was the most frequent, with 33% of positive isolates (5/15), followed by *bla*-TEM and *bla*-SHV (14% and 13%, i.e., 2/15 each). Pig droppings exhibited a similar distribution, with *qnrB* detected in 33% of isolates (5/15) and *bla*-TEM and *bla*-SHV in 13% (2/15) each. In wastewater, *qnr*B was present in 27% of isolates (4/15), *bla*-SHV in 20% (3/15), and *bla*-CTX-M in 13% (2/15). Finally, in the lake, *qnrB* and *bla*-TEM were detected in 27% (4/15) and 20% (3/15) of isolates, respectively, while the other genes were found at similar levels (7-13%, i.e., 1-2/15 isolates). The *qnr*A and *qnr*S genes occurred at low to moderate levels across all matrices, and no isolate tested positive for *mcr*-1. These findings suggest that chicken droppings and wastewater represent the most critical matrices for the dissemination and concentration of antibiotic resistance genes in the environment in Daloa.

To support these prevalence data, representative agarose gel electrophoresis profiles are presented in Figures 3. Figure 3a shows the amplification of the *bla*-TEM gene in lake water isolates (band at 840 bp), while Figure 3b illustrates the same gene in gutter water isolates. Figures 3c and 3d display the amplification of qnr genes: *qnr*A (543 bp), *qnr*B (469 bp), and *qnr*S (417 bp) in chicken fecal isolates (3c), and *qnr*A (543 bp) in pig fecal isolates (3d). The presence of clear bands at expected sizes confirms the detection of these resistance genes and corroborates the molecular data reported above.

Table 3. Frequency of β-lactam, quinolone, and colistin resistance genes in *Enterobacteriaceae* from environmental samples in Daloa, Côte d'Ivoire

Isolates (species)	Total isolates tested	Prevalence of resistance genes (%)						
		bla-TEM	*bla*-SHV	*bla*-CTX-M	*qnrA*	*qnr*B	*qnr*S	*mcr*-1
E.coli	44	5 (11.4%)	7 (15.9%)	1 (2.3%)	2 (4.5%)	15 (34%)	7 (15.9%)	0 (0%)
K.oxytoca	3	0%	1 (33.3%)	2 (66.7)	0%	1 (33.3%)	0 (0%)	0 (0%)
E.cloacae	5	0%	1 (20%)	1 (20%)	2 (40%)	1 (20%)	1 (20%)	0 (0%)
Salmonella spp	8	4(50%)	0 (0%)	2(25%)	0%	1 (12.5%)	1 (12.5%)	0 (0%)
Total	60	9(15%)	9 (15%)	6 (10)	4(6.7%)	18(30%)	9 (15%)	0 (0%)

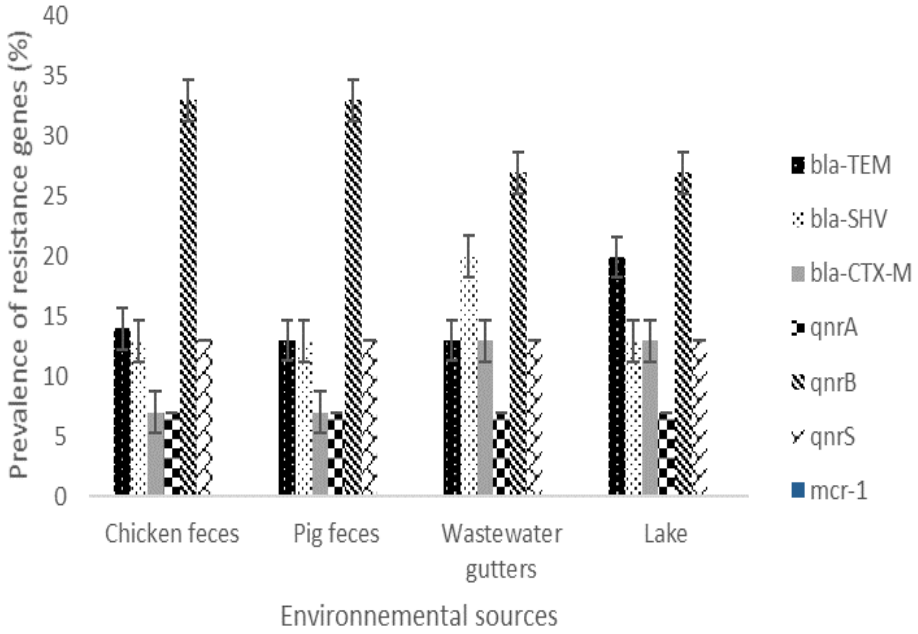

Figure 2. Distribution of antibiotic resistance genes across Environmental Sources

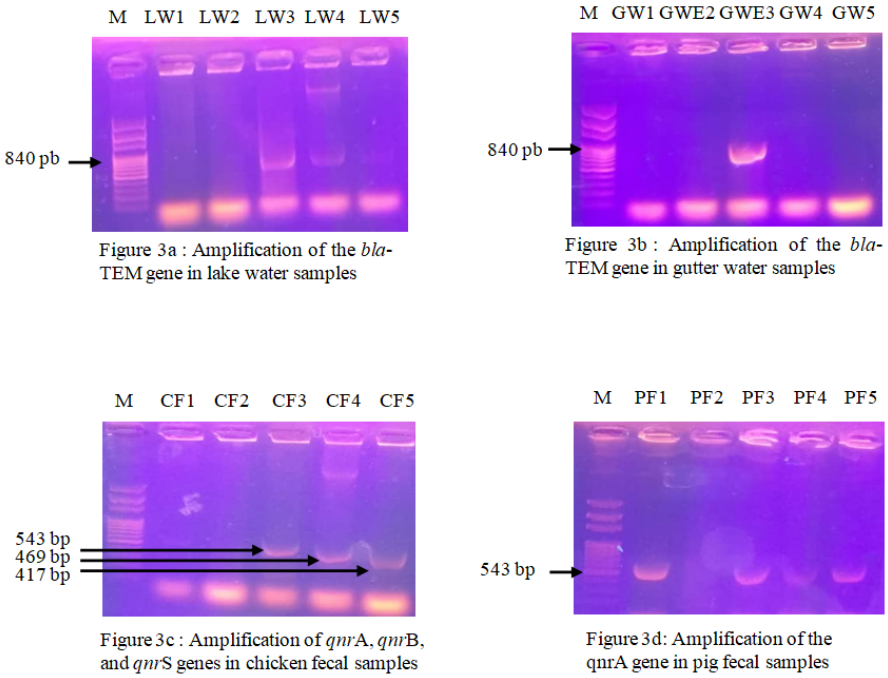

Figure 3a : Amplification of the *bla*-TEM gene in lake water samples

Figure 3b : Amplification of the *bla*-TEM gene in gutter water samples

Figure 3c : Amplification of *qnr*A, *qnr*B, and *qnr*S genes in chicken fecal samples

Figure 3d: Amplification of the qnrA gene in pig fecal samples

Figure 3. Representative agarose gel electrophoresis of PCR products confirming the presence of *bla* and *qnr* resistance genes in environmental Enterobacteriaceae isolates. Figure 3a : PCR amplification of the *bla*-TEM gene (840 bp) in lake water isolates. Lane M: molecular size marker; lanes LW1–LW5: lake water samples. Figure 3b: PCR amplification of the *bla*-TEM gene (840 bp) in gutter water isolates. Lane M: molecular size marker; lanes GW1-GW5: gutter water samples. Figure 3c: PCR amplification of *qnr*A (543 bp), *qnr*B (469 bp), and *qnr*S (417 bp) genes in chicken fecal isolates. Lane M: molecular size marker; lanes CF1–CF5: chicken fecal samples. Figure 3d: PCR amplification of the *qnr*A gene (543 bp) in pig fecal isolates. Lane M: molecular size marker; lanes PF1–PF5: pig fecal samples

4. Discussion

The present study aimed to assess the prevalence of enteric bacteria in various anthropized ecosystems and to characterize their antibiotic resistance profiles using a molecular approach. The results provide significant insights into the extent of bacterial resistance in the environmental context. Regarding bacterial diversity in the ecosystems studied, microbiological analysis led to the isolation of a total of 60 strains belonging to the genera *Escherichia coli*, *Salmonella* sp., *Klebsiella*, and *Enterobacter*. *E. coli* was largely predominant (73.33%), particularly in animal droppings, which confirms its well-established status as a classical indicator of fecal contamination [21,22]. Indeed, this bacterium represents the dominant species of the aerobic intestinal flora [23]. However, it is also found in gutter and lake waters.

Our results are consistent with those reported in [24]; according to these authors, the distribution of *E. coli* in nature is very broad, as it can be found in plants and in the environment (soil and water). The occurrence of *Salmonella*, *Enterobacter*, and *Klebsiella* also reflects the diversity of Enterobacteriaceae circulating in these environments, confirming their role as microbial reservoirs. The presence of *Salmonella* sp. (13.33%) in all environments is of concern, given its pathogenic potential in humans. Indeed, this pathogenic bacterium is responsible for typhoid fever [25]. The distribution of isolates across the different ecosystems reveals that animal droppings, particularly poultry feces, represent an important source of bacterial dissemination. This finding is consistent with the observations reported in [26]. Indeed, according to these authors, intensive farming has been identified as a hotspot for the spread of multidrug-resistant bacteria in the environment. In contrast, gutter and lake waters exhibited a higher diversity of bacterial species, reflecting the mixture of domestic and agricultural discharges that promotes cross-contamination. These aquatic environments, sometimes used for domestic purposes, may therefore represent a major route for the dissemination of resistant Enterobacteriaceae within human communities. As for the distribution of resistance genes, it varies according to the species found in the different environments. Indeed, molecular analysis revealed a high prevalence of resistance genes to β-lactams and quinolones. These observations are consistent with several studies conducted in West Africa, which confirm the widespread prevalence of these resistance genes [27]. In *E. coli*, the *qnr*B gene was the most frequently detected (34%), followed by *bla*-SHV (15.9%) and *bla*-TEM (11.4%). The presence of *bla*-CTX-M in *Klebsiella oxytoca* and *Enterobacter cloacae* confirms the rapid dissemination of this gene family, which is now considered the most widespread worldwide. Isolates of *Salmonella* spp., on the other hand, were characterized by a high prevalence of *bla*-TEM (50%), a gene frequently reported in zoonotic strains of this genus [28]. Overall, these results confirm the concurrent circulation of *bla* and *qnr* genes, reinforcing the threat of multidrug resistance in these environments. Regarding the dispersion of resistance genes, it also varies depending on the environments studied. In animal droppings, the *qnr*B and *bla*-SHV genes predominated, reflecting the selective pressure exerted by the frequent use of fluoroquinolones and β-lactams in livestock farming. In wastewater, a greater diversity of genes was observed, notably *bla*-CTX-M and *qnr*A, reflecting inputs from domestic and hospital

effluents. Lake samples revealed an intermediate situation, suggesting that receiving water bodies serve as convergence points and secondary hubs for the dissemination of resistance genes. These observations are consistent with those reported in [20], which demonstrated that aquatic ecosystems play a key role in the interspecies dissemination of resistance genes. The absence of resistance to colistin is particularly significant, given its role as a last-resort antibiotic against multidrug-resistant Enterobacteriaceae [19]. However, monitoring of this gene must be maintained due to its potential for horizontal transfer via plasmids. Although this result is reassuring, it should be interpreted with caution, as several recent studies have reported the presence of mcr-1 in agricultural and urban environments, particularly in poultry droppings [29,30] and surface waters [31,32]. The absence of this gene in our study may reflect a still low prevalence in the investigated area, but its surveillance remains crucial given its epidemic potential.

Conclusion

In summary, this study highlights the role of animal droppings and wastewater as reservoirs and vectors of major resistance genes in the city of Daloa. The predominance of *E. coli* and the high frequency of *bla* and *qnr* genes confirm that domestic and agricultural environments are key links in the transmission chain of multidrug-resistant Enterobacteriaceae. These findings fall within the framework of the One Health approach, which advocates integrated surveillance of human, animal, and environmental settings to curb the spread of antimicrobial resistance. They also emphasize the need to strengthen waste management practices and promote the prudent use of antibiotics in livestock production in order to reduce selection pressure.

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