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Study of the correlation between antibiotic resistance genes, susceptibility profiles and biotopes of *enterobacteria* in Burkina Faso

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ABSTRACT

Background: The spread and emergence of antibiotic resistance in *enterobacteria* is a public health concern. This study aimed to evaluate the role of resistance genes in the development of antibiotic resistance and to assess the impact of biotope (living environment) on the prevalence of these genes. **Methods:** Animal products (eggs, milk, fish), human sources (urine, feces), and the environment (surface soil, lettuce) were employed using standard microbiology methods in order to evaluate antibiotic susceptibility and to detect resistance genes to beta-lactams (*blaTEM*, *blaSHV*, *blaCTX-M-G1*) and quinolones (*qnrA*, *qnrB*, *qnrS*) in strains. Conventional PCRs were used to detect resistance genes. **Results:** The occurrence of extended-spectrum beta-lactamase-producing Enterobacteria (ESBL-E) was determined to be 29.9%, 19.2%, and 12% in samples obtained from animal, environmental and human sources, respectively. Enterobacteria strains tested were found harboring at least one resistance gene consisting of 134(41.2%) *blaTEM*, 76(23.6%) *blaSHV*, 85(26.2%) *blaCTX-M-G1*, 54(16.6%) *qnrA*, 95(29.2%) *qnrB* and 104(32%) *qnrS*. The correlation study indicated that significant (p = 0.000) and strong (Cramer's V= 0.506) correlations were found between the presence of the *blaTEM* gene and resistance to sulfamethoxazole-trimethoprim. Additionally, the expression of the *CTX-M-G1* gene was strongly (Cramer's V $\geq$ 0.3) linked (p = 0.000) with resistance to the antibiotics ceftriaxone, cefepime, ceftazidime, sulfamethoxazole-trimethoprim, and ciprofloxacin. Significant (p = 0.000) strong (Cramer's V $\geq$ 0.3) associations were seen between the elevated expression of the *blaTEM* and *blaCTX-M-G1* genes and the impact of the biotope. **Conclusion:** The results of this study indicate that the bacterial environment can influence the expression of specific resistance genes in *enterobacteria*.

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Introduction

The rise of carbapenem-resistant enterobacteria has become a notable public health issue owing to its capacity to resist all current classes of antibiotics [1]. Carbapenem has been the drug of first choice for treating some Enterobacteriaceae-induced infections for a long time. This is changing, though, due to many factors including the recent emergence of carbapenemase-producing bacteria such as *Klebsiella* and *Enterobacter* species and *E. coli*. Thus, there is an urgent need to develop alternative approaches [2]. These enterobacterial strains constitute a substantial component of the pathogenic bacteria obtained from clinical samples [3].

The resistance of Enterobacteria to third-generation cephalosporins (C3G) is significantly enhanced by enzymes (TEM, SHV, CTX-M, and their derivatives), These enzymes give resistance to all beta-lactam antibiotics, except cephamycins, as well as other families of antibiotics [4]. These enzymes that are translocated into the periplasm in Gram-negative bacteria confers resistance by hydrolysing the central beta-lactam ring of beta-lactam molecules [5]. Although ESBL-E were initially found in hospital settings, the spread of these bacteria, which are resistant to several drugs, in the community is becoming worrisome. The fast spread of ESBL-producing bacteria and the rise in their prevalence can be attributed to the transfer of genes encoding for ESBLs through plasmids [6].

Plasmids containing the *qnr* genes have been associated with resistance to quinolone antibiotics. These genes encode for proteins that inhibit the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV [7], this leads to resistance against quinolone and an elevation in the minimum inhibitory concentration (MIC) of ciprofloxacin [8]. The *qnr* genes have been identified in multiple members of the Enterobacteria family, predominantly in *K. pneumoniae*, *E. coli* and *Enterobacter spp*. across various countries. Additionally, the plasmids carrying the *qnr* genes may also harbour ESBL [9,10]. The spatial distribution of the *qnrA* gene has been extensively described [7]. Other more recent *qnr* genes (*qnrB* and *qnrS*) are increasingly being isolated from biological products. For example, 26.2% of *qnrB* and 64.3% of *qnrS* were found in ESBL-producing Enterobacteriaceae among fecal commensal of children with severe malnutrition [11].

A significant challenge for Africa is a scarcity of data regarding antimicrobial resistance (AMR). AMR surveillance in West Africa is hindered by the insufficient capacity of laboratories, infrastructure, and data administration. As a result, data are scarce regarding the magnitude of antibiotic resistance in humans, animals, and the environment [12] in a context where the forecasts are hardly rosy: beyond the financial costs, deaths attributable to antimicrobial resistance will be estimated at 10 million by 2050 [13].

In Burkina Faso, the proper assessment of the degree of antibiotic resistance gene transmission across humans, animals, and the environment is hindered by the absence of updated data, despite it being a priority to control this issue. The high consumption of antibiotics outside of medical prescriptions renders the Burkinabé context particularly noteworthy. According to a hospital-based study, 99% of patients had taken at least one antibiotic in the ten days prior to inclusion in the study for acute febrile illness of unknown cause or gastrointestinal infections [14]. These practices, combined with the uncontrolled marketing of antibiotics, contribute significantly to the spread of antibiotic resistance in the country. Enterobacteriaceae circulate between the three bacterial biotopes of humans, animals and the environment through direct contamination by drinking water or consumption of contaminated food, it would be beneficial to investigate the prevalence of enterobacterial pathogens from three sources, as well as their antibiotic resistance profiles in relation to the habitat.

The objective of this study was to ascertain the prevalence of resistance genes and to investigate the correlations between these genes and both phenotypic resistance and the environment of pathogenic Enterobacteriaceae.

Methods

Study's typology, duration, and conceptual framework.

This study is a cross-sectional experimental investigation that analyzed bacterial samples (specifically pathogenic enterobacteria) obtained from February to October 2023 in the city of Ouagadougou and its environs. The clinical strains were carefully chosen and identified at the Pietro Annigoni Biomolecular Research Centre (CERBA) and the bacteriology-serology section of the Charles de Gaulle University Hospital (CHUP-

CDG). The selection of these two structures was based on their high patient traffic, which indicates the diversity of the locations they serve. The selected products are pathological products that are frequently requested for use in a wide range of pathologies. The strains coming from animal source were derived from commonly consumed food products, which were sourced from poultry farmers in Koubri, livestock producers in Saaba, and fishermen in Nanoro. The selection of sites was based on their species variety, the temporal availability of products, and antibiotic usage patterns. The environmental strains obtained from goods sourced from vegetable growers located adjacent to the Kossodo canal and the Tanghin dam. It is hypothesized that these places are contaminated with a diverse range of chemical and microbiological contaminants. The Saint Camille juvenile garden was chosen as the third location. The three sites varied in terms of the water supply utilized for irrigation and the techniques adopted for soil enrichment. These products were lettuce, a vegetable that is widely consumed and which harbours bacteria, and soil taken from the surface just above the lettuce plants that were retained. The determination of bacterial strain sensitivity profiles and the molecular characterisation of bacterial strains were conducted at the Biomedical Research Laboratory (LaReBio) of the IRSS/CNRST in Ouagadougou.

Research and identification of enterobacteria species

The research and identification of enterobacterial species was conducted at LaReBio. The bacterial strains were obtained from biological samples derived from human (n=3529), animal (n=153), and environmental sources (n=104). The human samples consisted of pee and feces, whereas the animal samples consisted of milk, eggshells, and fish. The environmental samples were soil and lettuce. The collection was conducted between February and December 2023. The human samples were obtained from patients in accordance with standard procedures, with the samples being transferred to the laboratory within an hour of the initial sampling. The remaining samples were collected in appropriate containers and transported to the laboratory in a refrigerated cooler set at 4°C. All samples were analyzed within an hour of collection. Following isolation on a specific medium for enterobacteria and purification on a Mueller-Hinton agar plate, the colonies were identified using the API 20E system (bioMérieux, France) and a combination of cultural and morphological characteristics. Following the sensitivity tests, the identified samples were stored at a temperature below -80°C.

Antibiotic susceptibility and detection of ESBL phenotypes

Susceptibility to antibiotics was tested using the Kirby-Bauer disc diffusion method. The diameters of the zones of antibiotic inhibition were interpreted in accordance with of the European Committee on Antimicrobial Susceptibility Testing (EUCAST)/The Clinical and Laboratory Standards Institute (CLSI) 2021 recommendations. Ten (10) discs from various antibiotic families manufactured by BioMérieux SA, a company based in France, were used. The antibiotics employed were amoxicillin + clavulanic acid (20 + 10 µg), cefalexin (30 µg), ceftriaxone (30 µg), cefixime (5 µg), ceftazidime (30 µg), The antibiotics tested were cefepime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25-23.75 µg), and chloramphenicol (30 µg). The *E. coli* ATCC® 25922 strain was employed for the quality control of the sensitivity tests.

A modified double synergy test was employed to identify BLSE-producing strains. This involved the placement of discs (2-3 cm in diameter) of ceftriaxone, ceftazidime, and cefixime around a disc of amoxicillin-clavulanic acid on an MH agar plate, followed by observation of the resulting synergies[15].

Detecting selected resistance genes

The bacterial DNA extraction was conducted using thermal shock, following the methodology described by Moyo et al. (2007) [16]. A single colony of pure culture was aseptically transferred from an MH agar plate to 200 µL of distilled water in an Eppendorf tube. The suspension was heated to 99°C for 17 minutes using a rotary thermal block. Subsequently, the supernatant, which contained the released DNA, was subjected to centrifugation at 12,000 rpm for 10 minutes. Subsequently, the obtained pellet was transferred to a fresh Eppendorf tube and kept at a temperature of -20°C. Before performing the traditional PCR, the Nanodrop and Biodrop (Montréal Biotech Inc., Canada) were used to confirm the quantity and purity of the extracts. The polymerase chain reaction (PCR) was conducted using the QuantStudio 5 (ThermoFisher Scientific Inc., USA), in a 20 µL reaction mixture as recommended by the manufacturer of the master mix (SOLIS BIODYNE). The master mix consisted of FirePol DNA polymerase (4 µL), sense primer (0.5 µL, 10 µM), antisense primer (0.5 µL, 10 µM), and PCR water (10 µL). This mixture, which had been previously distributed into the wells of a PCR plate, was then supplemented with an additional 5 µL of DNA extracts. The characteristics of the primers are summarized in Tables 1. Negative controls (PCR water) and positive controls for each gene were included with all PCRs. Genes involved in antibiotic resistance in both human and veterinary medicine were investigated.

The *qnr* genes (*qnrA*, *qnrB* and *qnrS*) were amplified using the same following programme: 32 cycles consisting of 45 seconds at 95°C for denaturation, 45 seconds at 53°C for hybridisation and 60 seconds at 72°C for extension. The *blaTEM* and *blaSHV* ESBL genes used the same programme comprising the following steps: initial denaturation step at 94°C for 10 minutes followed by 30 cycles of denaturation at 94°C for 40 seconds, hybridisation at 60°C for 40 seconds, extension at 72°C for 1 minute and a final extension step at 72°C for 7 minutes. As for the *BlaCTX-M-G1* gene, its amplification programme comprised: initial denaturation at 96°C for 10 minutes, 35 cycles of denaturation at 94°C for 1 minute, hybridisation at 50°C for 1 minute and extension at 72°C for 1 minute followed by a final extension at 72°C for 10 minutes.

The PCR products were subjected to gel electrophoresis on 2% agarose gel at 100 V for one hour in Tris Borate EDTA (TBE) buffer 1X, containing Sybr Green (Thermo Fisher Scientific, USA). A 100 bp marker (Promega, USA) was used for band identification.

Statistical analyses

The data was analyzed using the IBM SPSS Statistics 25 software (IBM Corp., Armonk, NY, USA). Quantitative data were represented as percentages. The Pearson chi-squared test was used to compare categorical variables, and a p-value below 0.05 was considered statistically significant. Cramer's V was employed to evaluate the strength of various associations.

Results

ESBL phenotype prevalence

The prevalence of ESBL phenotypes is presented in Table 2. A total of 613 strains of enterobacteria were identified, with 3 strains found in all three biotopes examined. The strains that occurred most commonly were *E. coli*, *E. cloacae*, and *K. pneumoniae*. The sensitivity testing indicated that 325 commonly found species were resistant to at least one of the ten investigated antibiotics. Several strains had an Extended-Spectrum Beta-Lactamase (ESBL) trait. The prevalence of enterobacterial ESBL in samples derived from animal, environmental and human sources were 29.9%, 19.2%, and 12%, respectively. A total of 19 (15.8%) *E. coli*, 38 (25%) *E. cloacae*, and 36 (8.3%) *K. pneumoniae* were identified as ESBL-E (Table 3).

Prevalence of betalactam (*bla*) and quinolone (*qnr*) resistance genes

Resistance genes detected in enterobacteria strains were mentioned in table 3.

The results demonstrated that the strains exhibited the presence of 16.6% *qnrA*, 29.2% *qnrB*, 32% *qnrS*, 41.2% *blaTEM*, 23.4% *blaSHV* and 26.2% *blaCTX-M-G1*. The *blaTEM* gene had the highest prevalence (65.2%) in *E. coli*, whereas the CTX-M-G1 gene had the lowest prevalence (2.5%) in *E. cloacae.* The findings indicated that none of the bacterial strains derived from animals carried the *blaCTX-M-G1* gene. The *blaTEM* gene was most commonly found in human habitat, with a prevalence rate of 65% (Table 3).

Table 4 provides an overview of the potential resistance levels of isolated strains. A total of 25.2% of *E. cloacae* strains were found to have a minimum of two resistance genes. The percentage of occurrence was 68.8% for *E. coli* and 60.3% for *K. pneumoniae.*

Relationship between the presence of resistance genes and phenotypic resistance

The data of association between resistance genes and antibiotics according Chi2 test. The results showed some statistically significant correlations between the identification of resistant genes and the expression of antibiotic resistance at the phenotypic level. ($p < 0.05$). (Table 5).

The expression of the *qnrA* gene was not correlated ($p > 0.05$) with antibiotic resistance, except amoxicillin-clavulanic acid and sulfamethoxazole-trimethoprim, for which a weak association was observed ($0.1 \leq$ Cramer's $V < 0.2$). The *qnrB* gene has shown a limited association with resistance to cefixime, cefepime, ceftazidime, and sulfamethoxazole-trimethoprim. Furthermore, the presence of *qnrS* did not correlate with resistance to the antibiotics that were evaluated. Nevertheless, a weak association was observed between this gene and resistance to imipenem.

Additionally, the beta-lactam resistance genes demonstrated significant associations ($p < 0.05$) with the antibiotics. Consequently, the *blaTEM* gene was found to be associated with resistance to most of antibiotics except chloramphenicol, cefalexin and amoxicillin-clavulanic acid. A strong association (Cramer's V ≥ 0.3) was observed between the *blaTEM* gene and sulfamethoxazole-trimethoprim. The *blaSHV* gene was found to have a weak correlation with resistance to cefalexin. No association was observed with the other antibiotics tested. The *blaCTX-M-G1* gene was strongly associated with resistance to ceftriaxone, cefepime, ceftazidime, sulfamethoxazole-trimethoprim, and ciprofloxacin.

Relationship between resistance genes and bacterial species

The correlation between resistance genes and bacterial species is mentioned in Table 6.

The analysis of the Pearson's chi-squared value revealed a highly significant correlation (Cramer's V ≥ 0.3) between the presence of the *blaTEM*, *blaSHV* and *blaCTX-M-G1* genes and the following species *E. coli*, *E. cloacae* and *K. pneumoniae.* In addition, the data indicate that the three species show a minimal predominance of *qnrA*, *qnrB*, and *qnrS*.

Relationship between resistance genes and biotopes of bacterial

The relationship between genes that provide resistance and the specific environments where bacteria are found is shown in Table 7.

The results revealed a strong and statistically significant link (Cramer's V > 0.3) between the presence of the *blaTEM* and *blaCTX-M-G1* genes in samples obtained from human, animal, and environmental sources. Nevertheless, a rather low correlation (0.1 ≤ Cramer's V < 0.2) was detected between the genes *qnrA* and *qnrB* and these specific environments. The relationship between the *blaSHV* gene and the biotopes exhibited moderate strength, with 0.2 ≤ Cramer's V < 3.

Table 1. Primer sequences used.

Genetic resistance factors	Genes	Primer sequences (5'-3')	Size (pb)	References
Betalactam resistance genes (*bla*)	*bla*$_{CTX-M}$	For: GTTACAATGTGTGAGAAGCAG Rev.: CCGTTTCCGCTATTACAAAC	1000	[17]
	bla$_{TEM}$	For: CATTTCCGTGTCGCCCTTATTC Rev.: CGTTCATCCATAGTTGCCTGAC	800	[17]
Quinolone resistance genes (*qnr*)	*bla*$_{SHV}$	For: AGCCGCTTGAGCAAATTAAAC Rev.: ATCCCGCAGATAAATCACCAC	713	[17]
	qnrA	For: ATTTCTCACGCCAGGATTTG Rev.: GATCGGCAAAGGTTAGGTCA	516	[18]
	qnrB	For: GATCGTGAAAGCCAGAAAGG Rev.: ACGATGCCTGGTAGTTGTCC	469	[18]
	qnrS	For: ACGACATTCGTCAACTGCAA Rev.: TAAATTGGCACCCTGTAGGC	417	[19]

For : Forward, Rev : Reverse

Table 2. Distribution of ESBL phenotypes according to species and biotopes

Parameters	ESBL **n (%)**	**P-value**
Species		
E. coli (n=183)	29(15.8)	0.014
E. cloacae (n=152)	38(25)	
K. pneumoniae (n=84)	7(8.3)	
Biotopes		
Animal (n=167)	50(29.9)	0.002
Environment (n=146)	28(19.2)	
Human (n=300)	36(12)	

n: number of ESBL-producing isolates, %: approximate percentage

Table 3. Resistance genes detected in enterobacteria strains.

	Resistance genes					
Parameter	***qnrA* n°(%)**	***qnrB* n°(%)**	***qnrS* n°(%)**	***blaTEM* n°(%)**	***blaSHV* n°(%)**	***BlaCTX-M-G1* n°(%)**
Species						
E. cloacae (n=119)	19(16)	23(19.3)	48(40.3)	12(10.1)	11(9.2)	3(2.5)
E. coli (n=138)	33(23.9)	48(34.8)	36(26.1)	90(65.2)	24(17.4)	60(43.5)
K. pneumoniae (n=68)	2(2.9)	24(35.3)	20(29.4)	32(47.1)	41(60.3)	22(32.4)
Total Species (n=325)	54(16.6)	95(29.2)	104(32)	134(41.2)	76(23.4)	85(26.2)
P value	0.001	0.012	0.044	0.000	0.000	0.000
Biotopes						
Animal (n=80)	16(20)	16(20)	29(36.3)	5(6.3)	9(11.3)	0(0)
Environment (n=62)	3(4.8)	14(22.6)	29(46.8)	10(16.1)	10(16.1)	1(1.6)
Human (n=183)	35(19.1)	65(35.5)	46(25.1)	119(65)	57(31.1)	84(45.9)
P value	0.021	0.017	0.004	0.000	0.001	0.000

Table 4. Number of resistance genes expressed by the species isolated

Resistance genes per species	***E. cloacae,* N = 119[1] n(%)**	***E. coli,* N = 138[1] n(%)**	***K. pneumoniae,* N = 68[1] n(%)**	**Total Espèces N=325[1] n(%)**
0	43 (36.1)	15 (10.9)	14 (20.6)	72 (22.1)
1	46 (38.7)	28 (20.3)	13 (19.1)	87 (26.8)
2	21 (17.7)	48 (34.8)	14 (20.6)	83 (25.5)
3	8 (6.7)	29 (21.0)	13 (19.1)	50 (15.4)
4	1 (0.8)	12 (8.7)	9 (13.2)	22 (6.8)
5	0 (0.0)	4 (2.9)	5 (7.4)	9 (2.8)
6	0 (0.0)	2 (1.4)	0 (0.0)	2 (0.6)

[1]n : total isolates

Table 5. Correlation between resistance genes and antibiotics according Chi2 test

Relationship between resistance genes and antibiotics	**Chi2 test**	**Cramer's V**
qnrA / Chloramphenicol, Cefalexin, Ceftriaxone, Cefixime, Cefepime, Ceftazidime, Ciprofloxacin, Imipenem	>0.05	NR
qnrA / Amoxicillin-Clavulanic acid	0.045	0.138
qnrA / Sulfamethoxazole-trimethoprim	0.003	0.190
qnrB / Chloramphenicol, Cefalexin, Ceftriaxone, Ciprofloxacin, Imipenem	>0.05	NR
qnrB / Cefixime	0.013	0.164
qnrB / Cefepime	0.013	0.163
qnrB / Ceftazidime	0.014	0.162

qnrB / Sulfamethoxazole-trimethoprim	0.002	0.198
qnrS / Chloramphenicol, Cefalexin, Ceftriaxone, Cefixime, Amoxicillin-Clavulanic acid, Cefepime, Ceftazidime, Sulfamethoxazole-trimethoprim, Ciprofloxacin	>0.05	NR
qnrS / Imipenem	0.044	0.138
blaTEM / Chloramphenicol, Cefalexin, Amoxicillin-Clavulanic acid	>0.05	NR
blaTEM / Ceftriaxone	0.006	0.179
blaTEM / Cefixime	0.022	0.153
blaTEM / Cefepime	0.009	0.170
blaTEM / Ceftazidime	0.000	0.250
blaTEM / Sulfamethoxazole-trimethoprim	0.000	0.509
blaTEM / Ciprofloxacin	0.000	0.281
blaTEM / Imipenem	0.002	0.195
blaSHV / Chloramphenicol, Ceftriaxone, Cefixime, Amoxicillin-Clavulanic acid, Cefepime, Ceftazidime, Sulfamethoxazole-trimethoprim, Ciprofloxacin, Imipenem	>0.05	NR
blaSHV / Cefalexin	0.045	0.138
blaCTX-M-G1 / Chloramphenicol, Cefalexin, Amoxicillin-Clavulanic acid, Imipenem	>0.05	NR
blaCTX-M-G1 / Ceftriaxone	0.000	0.484
blaCTX-M-G1 / Cefixime	0.000	0.268
blaCTX-M-G1 / Cefepime	0.000	0.538
blaCTX-M-G1 / Ceftazidime	0.000	0.496
blaCTX-M-G1 / Sulfamethoxazole-trimethoprim	0.000	0.324
blaCTX-M-G1 / Ciprofloxacin	0.000	0.547

NR : Not relevant

Table 6. Correlation between resistance genes and bacterial species.

Relationship between resistance genes and species	**Chi2 test**	**Cramer's V**
qnrA / E. cloacae, E. coli, K. pneumoniae	0.001	0.211
qnrB / E. cloacae, E. coli, K. pneumoniae	0.012	0.166
qnrS / E. cloacae, E. coli, K. pneumoniae	0.044	0.138
blaTEM / E. cloacae, E. coli, K. pneumoniae	0.000	0.500
blaSHV / E. cloacae, E. coli, K. pneumoniae	0.000	0.457
blaCTX-M-G1 / E. cloacae, E. coli, K. pneumoniae	0.000	0.420

Table 7. Statistical associations between resistance genes and environment of bacteria

Relationship between resistance genes and biotopes	**Chi2 test.**	**Cramer's V**
***qnrA* / Animal, Environment, Human**	0.012	0.154
***qnrB* / Animal, Environment, Human**	0.017	0.158
***qnrS* / Animal, Environment, Human**	0.004	0.183
***blaTEM* / Animal, Environment, Human**	0.000	0.553
***blaSHV* / Animal, Environment, Human**	0.001	0.212
***blaCTX-M-G1* / Animal, Environment, Human**	0.000	0.510

Discussion

The *E. cloacae* bacteria had the greatest prevalence rate of ESBL at 25% followed by *E. coli* at 15.8%, and *K. pneumoniae* at 8.3%. These high rates are to be expected, as Bezabih et al. (2021) [20] have demonstrated that during the last two decades, the prevalence of *E. coli* producing ESBL in the community has increased eightfold globally. The variations in the prevalence of ESBL among species could be attributed to the varying frequency of isolation. *E. coli* and *K. pneumoniae* are the two most common causes of bacterial pathogens [3,21]. However, E. cloacae is an environmental bacterium, and its relatively high BLSE rate compared to that of *E. coli* and *K. pneumoniae* may be a physiological response to environmental stress. Further investigation could elucidate this specific reaction.

Depending on the bacterial biotopes, animals (29.9%), the environment (19.2%) and humans (12%) showed significant ESBL prevalences with significant differences suggesting that animals harbour more E-BLSEs than the other two biotopes (p = 0.002). However, when it comes to the prevalence of Enterobacteriaceae at the human-animal-environment interface, the results are mixed. This is illustrated by the differences in prevalence between the studies by Bézabih et al. 2021 and Gonçalves et al. 2016 [20,22]. According to the work of Mahamat et al., 2021, the prevalence of E-BLSE varied from 11% to 72% in humans and from 7% to 79% in aquatic environments (wastewater) (Mahamat et al., 2021). In animals, the prevalence of E-BLSE varied enormously: 0% in cattle, 11-36% in chickens, 20% in rats, 21-71% in pigs and 32-75% in dogs. E-BLSEs are not limited to the hospital environment, they are also present as human intestinal commensals [22,24]. The presence of E-BLSE in several ecological niches, as commensals in humans and animals and as environmental contaminants, is reported worldwide. However, in recent decades, one niche that has been of great concern, as it can serve as a reservoir and vehicle for transmission and dissemination of E-BLSE, is that of production animals, due to their direct link to the food chain [25].

The results indicated the occurrence of *qnrA* (16.6%), *qnrB* (29.2%), *qnrS* (32%), *blaTEM* (41.2%), *blaSHV* (23.4%), and *blaCTX-M-G1* (26.2%) in the three species being studied. Statistically significant changes ($p < 0.05$) were detected in the genes across the different species. Overall, however, these results indicate a high prevalence of genes encoding for *qnr* and ESBL. Additionally, the results demonstrated that the majority of the isolated strains exhibited multidrug resistance. Indeed, 68.8%, 60.3% and 21.08% of the *E. coli, K. pneumoniae,* and *E. cloacae* isolates had a minimum of two resistance genes. Furthermore, two *E. coli* strains exhibited all six of the targeted genes (Table 4). Several other studies have reported a high prevalence of genes encoding ESBL [21,24] and *qnr* [19,26].The prevalence of ESBL class A colonization was found to be 14%, with an observed increase of 5.38% per annum (P = 0.003). The prevalence of the condition was found to be higher in Asia and Africa, with rates ranging from 46% to 95%. In Central Europe, the incidence was lower but still significant at 3%, while in the Americas it was even lower at 2% [24]. Since 2000, there has been a significant rise in the prevalence of β-lactamases CTX-M in both human and animal populations. These enzymes have now become the most common variety of extended-spectrum β-lactamases (ESBL), surpassing the previously dominating kinds of TEM and SHV in most regions worldwide [27].The considerable genetic diversity among the isolates is a cause for concern, suggesting the existence of established reservoirs that warrant further investigation. However, it is crucial to ascertain the correlation between resistance genes and antibiotic classes.

Concerning bacterial biotopes, the findings revealed notable discrepancies in the prevalence of resistance genes across all samples. Overall, it can be observed that humans appear to harbour a greater number of resistance genes than animals and the environment (Table 3). To illustrate, the prevalence of *blaTEM* was 65% in humans, compared to 16.1% and 6.3% in the environment and animals, respectively. It is also noteworthy that the *blaCTX-M-G1* prevalence was 45.9% in humans, 1.6% in the environment, and 0% in animals. This trend in results towards humans as the primary reservoir for resistance genes may be justified by the fact that humans use more antibiotics than other biotopes, leading to the selection of resistant strains. Furthermore, humans consume foods of animal or vegetable origin that often contain resistant strains [25,28].

The aim of this study was to detect the presence of the genes *qnrA*, *qnrB*, and *qnrS* in enterobacteria that were exposed to 10 different categories of antibiotics, including fluoroquinolones. The results demonstrated a weak correlation between the detection of the *qnr* genes and the tested antibiotics, including fluoroquinolones. These findings are consistent with those of Salah et al. (2019) [26], who did not detect any *qnr* in strains that were resistant to ciprofloxacin. These results indicate that these genes may be associated with other antibiotic families or genes. Hooper et al. (2015) [29] have observed that bacterial resistance to quinolones is extensive. Other studies [30,31] have proposed an alternative mechanism of quinolone resistance, namely mutations in the DNA gyrase and topoisomerase IV genes. These two enzymes function in conjunction during the replication, transcription, recombination, and repair of DNA [32]. In highly resistant strains, simultaneous mutations in both enzymes would reduce quinolone sensitivity due to a reduction in drug affinity [33].

A significant association ($p < 0.05$) was observed between the *blaTEM* gene and sulfamethoxazole-trimethoprim and between the *blaCTX-M-G1* gene and the antibiotics ceftriaxone, cefepime, ceftazidime, sulfamethoxazole-trimethoprim and ciprofloxacin. The robust associations observed suggest that these two genes have a substantial impact on the resistance of enterobacteria to beta-lactams and other types of antibiotics. These findings are consistent with the results obtained by Sarshar et al. (2021) [34] who reported that these genes encode for enzymes capable of causing resistance to penicillin and to a broad spectrum of third-generation cephalosporins such as ceftazidime, ceftriaxone, cefotaxime and monobactams such as aztreonam. It was unexpected that the detection of the *blaTEM* and CTX-M-G1 genes was not associated with resistance to cefalexine, a first-generation cephalosporin. In contrast, the *blaSHV* gene demonstrated a weakly significant association ($p = 0.045$). This observation indicates that each protein encoded by these genes may have a distinct mechanism of action. Furthermore, no association was identified between the quinolone resistance genes and chloramphenicol, or between the beta-lactamase genes and chloramphenicol. This finding is consistent with other researches. It should be noted that other resistance mechanisms exist. The most common mechanism of resistance to chloramphenicol in bacteria is enzymatic inactivation by acetylation, primarily by acetyltransferases or, in some cases, by chloramphenicol phosphotransferases [35]. Additionally, resistance to chloramphenicol may result from a mutation or modification of the target site [36], a reduction in the permeability of the external membrane[37], or the presence of efflux pumps that frequently act as drug exporters, thereby reducing the intracellular concentration of the drug [38].

The highest prevalence of beta-lactamase genes was 65.2% for *blaTEM* in *E. coli* and the lowest 2.5% for *blaCTX-M-G1* in *E. cloacae*. This study demonstrated a strong correlation ($p = 0.000$) between the beta-lactamase genes and the species *E. coli, K. pneumoniae* and *E. cloacae.* The reason for this is that the *blaCTX-M-G1* and *blaTEM* genes are the most common beta-lactamase genes found in *E. coli* and *K. pneumoniae*, respectively. These genes produce enzymes that make the bacteria resistant to treatment. Currently, the enzyme TEM is considered to be one of the main mechanisms responsible for resistance to beta-lactam antibiotics in Gram-negative bacteria. The cefotaximases (CTX-M-ases) are a new group of plasmid-encoded beta-lactamases that have a wide range of activity against different types of antibiotics. They are produced by several species within the Enterobacteriaceae family, including *K. pneumoniae* [34]. As demonstrated by Sarshar et al. (2021) [34], of the 50 isolates examined, 34% exhibited the *blaCTX-M* gene, 28% displayed the *blaTEM* gene, and 11 (22%) demonstrated the presence of both genes simultaneously. Furthermore, over 77% of the positive ESBL isolates also exhibited the *blaCTX-M* gene, while approximately 63.64% of the positive ESBL isolates also exhibited the *blaTEM* gene.

The present study has identified the correlations between resistance genes and bacterial biotopes. The results demonstrated a weak association (Cramer's V <0.2) between the *qnr* genes and the biotopes. Additionally, it was observed that *qnrB* and *qnrS* appear to be more closely associated with humans and with environment respectively. Additionally, we observed strong associations (Cramer's V ≥0.3) between the *blaTEM* and *blaCTX-M-G1* genes and the biotopes. The *blaSHV* genes, on the other hand, demonstrated a medium association (0.2 ≤ Cramer's V < 3) with the biotopes. It is noteworthy that this study demonstrated that the *blaTEM, blaSHV* and *blaCTX-M-G1* genes are exclusive to the human habitat. Some studies [24,34] have reported high levels of these genes in humans. Further investigation should explain and yield insights into the medical implications.

This study demonstrated the presence of antibiotic resistance genes in various products, which are involved in resistance to a range of clinically significant antibiotic families in human and veterinary medicine. These findings highlight the necessity for improved regulation of antibiotic access by the general public. Furthermore, the routine identification of resistance genes with each antibiotic susceptibility test could help to reduce the emergence of antibiotic-resistant bacteria and enhance the precision of therapeutic strategies.

Conclusion

This study revealed correlations between the *blaTEM* gene and phenotypic resistance to sulfamethoxazole-trimethoprim, as well as between the *CTX-M-G1* gene and the antibiotics ceftriaxone, cefepime, ceftazidime, sulfamethoxazole-trimethoprim, and Ciprofloxacin. Additionally, the study revealed correlations between all the detected resistance genes and the isolated species, as well as between these genes and the bacterial biotopes. The results of this study will help create a monitoring system for antibiotic resistance and aid in the creation of new treatment approaches for multidrug-resistant bacteria in Burkina Faso. These results also highlight the importance of raising awareness about the potential risks associated with the misuse of antibiotics outside of medical guidance.

Conflict of interest

No conflict of interest.

Financial disclosure

No financial disclosure.

Data availability

Data available on request.

Authors' contribution

Conceptualization, MKS, LWN, and MHD; methodology, SCD, MKS, LWN, DK, AAZ, TRC, STS, HGO; software, SCD; validation, MKS, LWN, and MHD; formal analysis, SCD; investigation, SCD, KS, TRC, STS; resources, HGO; writing - preparation of the original version, SCD, TS, OO, RAN, AK; writing - revision and editing, MKS, LWN and MHD. All authors read and approved the final manuscript.

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