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ORIGINAL RESEARCH

Prevalence and Antibiotic Susceptibility of Pathogenic Enterobacteria Strains from Three Biotopes in the City of Ouagadougou (Burkina Faso)

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Purpose: The emergence of antibiotic resistance in pathogenic *Enterobacteriaceae* is a public health problem in tropical countries such as Burkina Faso. Antibiotic resistance could be identified using a variety of approaches. This study aimed to estimate the prevalence of pathogenic enterobacteria strains from three sources, as well as their antibiotic resistance profile to biotope and climatic season.

Material and Methods: The methodological approach consisted of identifying *Enterobacteriaceae* from human (urine, stool), animal (eggs, milk, fish), and environmental (soil, lettuce) samples, followed by assessing their antibiotic susceptibility. Samples were collected from February to December 2023. Bacterial species were isolated and phenotypically identified (morphologically, culturally, biochemically, and antigenically) using standard methods. The prevalence of bacterial susceptibility to ten antibiotics was determined using the agar disk diffusion method. The collected data were analyzed with IBM SPSS Statistics 25 software.

Results: A total of 615 *Enterobacteriaceae* isolates were collected, including 300, 168, and 147 samples from human, animal, and environmental sources respectively. Phenotypic characteristics allowed to partially identify 43 species, among these 29.76% belonged to *Escherichia coli*, 24.72% *to Enterobacter cloacae*, 13.*82% to Klebsiella pneumoniae, 3.41% to Enterobacter sakazakii* and 2.6% to *Klebsiella oxytoca*. Bacterial resistance rates were: aminopenicillins (54.8%), first-generation cephalosporins (35.3%), sulfonamides (33.3%), third-generation cephalosporins (30.7%), fourth-generation cephalosporins (22.5%), fluoroquinolones (21.8%), phenicols (16.8%), and carbapenems (16.2%). The distribution of antibiotic resistance was 45.3% from human sources, 19.3% from animal sources, and 13.8% from environmental sources.

Conclusion: The results indicate that resistant bacteria can come from any of the three biotopes, with human origin being the most frequent. The high prevalence of resistance to the antibiotics tested in isolated bacteria raises interest in investigating the genetic factors responsible.

Keywords: pathogenic *Enterobacteriaceae*, biotopes, resistance, antibiotics, Burkina Faso

Introduction

The *Enterobacteriaceae* family consists of Gram-negative bacilli that generally reside in the intestinal tract of humans and animals. These bacteria are also found widely in the environment and can contaminate various food products such as milk,[1] eggs,[2,3] and fish[4,5] through excrement or polluted water. The presence of *Enterobacteriaceae* in food products poses a serious threat to public health. These microorganisms usually cause gastrointestinal diseases and urinary tract infections that need to be controlled.[6]

To prevent infections and economic losses, antibiotics are commonly used to eliminate pathogenic bacteria.[6] However, uncontrolled use of these drugs can lead to the emergence of antimicrobial resistance. This can occur in

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commensal bacteria found in the human and animal gut, as well as in the environmental bacteria. The spread of resistance genes can occur between bacterial populations. Studies have shown that bacteria found in aquatic environments, such as *Aeromonas salmonicida*, can transfer antimicrobial resistance genes to other bacteria like *Escherichia coli* through horizontal transfer.[7] Inappropriate use of antimicrobials has become a major concern for public health, leading to the development of antimicrobial resistance on a global scale.[7] In 2019, it was estimated that approximately 4.95 million deaths were associated with antimicrobial resistance, of which 1.27 million were directly caused by multidrug-resistant bacteria.[8] According to forecasts, antimicrobial resistance could lead to around 10 million deaths by 2050.[9] As a result, there has been a lot of research focused on isolating potentially pathogenic *Enterobacteriaceae* in several countries.

In Burkina Faso, many authors have taken an interest in the problem of food-borne bacterial diseases, which can affect all human organs, and particularly the gastrointestinal tract. These studies have focused on raw, ready-to-eat products[10–14] as well as products consumed after cooking.[11,12,15,16] Other studies have examined the knowledge, attitudes, and practices of poultry farmers regarding the use of antibiotics. This indicates that the majority of actors (85.65%) are not informed about the appropriate use of antibiotics. Furthermore, many actors utilize antibiotics without a medical prescription or seek veterinary advice during outbreaks.[17] These practices collectively contribute to the emergence of multidrug-resistant bacteria.

In a study conducted by Kagambèga et al in 2022, multidrug resistance was found in 36.2% of isolates for different classes of antibiotics, including fosfomycin and β-lactam antibiotics, in slaughtered and sold chickens.[18] The frequency of extended-spectrum betalactamase-producing *Enterobacteriaceae* was 58% (179/308) among enterobacterial isolates identified in human pathological products.[19] However, all these studies addressed the question of the prevalence of enterobacteria and their antibiotic resistance in a single biotope.

The One Health approach, which integrates human, animal, and environmental health issues, is recommended by global health authorities to address the complex issue of antibiotic resistance in enterobacteria. Despite these recommendations and the existence of a national program for combating antimicrobial resistance, Burkina Faso lacks updated data on the use of this approach to address the burden of antibiotic resistance. The objective of this study was to address the aforementioned gap by estimating the prevalence of enterobacteria in human, animal, and environmental habitats, as well as their antibiotic susceptibility.

Materials and Methods

Sampling

This study was conducted across three different biotopes: human, animal, and environment. It was a cross-sectional study, aimed at examining various aspects related to these biotopes. Samples were collected from February to October 2023 in the city of Ouagadougou and its surrounding areas.

Human Biotope

Samples (urine and stool) were collected in the microbiology laboratories of two medical centers that were observed to have a high patient throughput. These samples were pathological products that were obtained from hospitalized and non-hospitalized patients (men, women, and children).

Animal Biotope

Enterobacteriaceae were investigated in three animal products that are widely consumed by the population, but which are generally reservoirs of microorganisms. These products were eggs (white and red), unpasteurized milk from dairy cows and fish (carp). Animal samples were obtained from dams and breeding sites in the city of Ouagadougou and its outskirts (Nanoro, Saaba and Koubri), where these products were available all year round. Samples were taken at random.

Environment Biotope

Samples (soil and lettuce) were collected at gardening sites that differed in the source of water used for irrigation (canal/dam water and borehole/well water). Environmental samples were taken from market gardening sites in the city of

Ouagadougou (Juvenat Saint Camille, Bendogo and Tanghin) where market gardening is practiced throughout the year. Only edible-looking leaves were sampled. Damaged or old lettuce leaves were eliminated.

Isolation and Identification of Enterobacteria Strains

Pathogenic enterobacteria were investigated by conventional culture procedures. All samples were inoculated onto selective enterobacterial media, allowing for enumeration of these enterobacteria. The enterobacterial loads were calculated using the dilution factors and the volumes of the samples inoculated, in accordance with the formula "bacterial load (CFU/mL) = (number of CFU x dilution factor)/sample volume (mL)".

Preparation of Human Samples (Urine, Stool)

Clinical specimens were cultured on Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar for fecal samples, and Cystine Lactose Electrolyte Deficient (CLED) agar for urine samples, using standard microbiological procedures. For identification, Le Minor's minimal gallery was used and subcultured colonies from presumptive colonies were obtained on Mueller Hinton agar (MH).[20]

Preparation of Animal-Based Food Samples (Egg, Fish, Unpasteurized Milk)

Enterobacteriaceae from chicken eggs were isolated from eggshells using the method of Roberts and al. (1995). Briefly, 1 mL of diluted sample from nutrient broth enrichment was transferred to Violet Red Bile Glucose (VRBG) agar. The plates were then incubated for 24 hours at 37°C. After incubation, dark-red and purple colonies with violet-red halos were considered.[2]

To isolate potential bacteria in unpasteurized milk, samples were diluted by a factor of 10 and plated onto VRBG agar. The plates were then incubated at 37°C for 24 hours. Any colonies that met the following criteria were identified as typical: a red or purple color, diameter greater than 0.5 mm, and surrounded by a zone of precipitated bile.[1]

To isolate enterobacteria from fish, a swab was used to collect the bacterial population from the mucosa on both sides of the fish, while avoiding the opercular and anal regions. The skin swab was then dipped in 9 mL of sterile 0.1% peptone water and vortexed to loosen the bacteria. For microbiological analysis, the diluted enriched samples were plated on EMB and SS agar. These plates were incubated for 24 hours at 37°C.[21] *Enterobacteriaceae* of animal origin from the specific media were transferred to MH agar plates and identified using the Api 20E gallery from Biomerieux SA, France.

Preparation of Environmental Samples (Soil, Lettuce)

To isolate *Enterobacteriaceae* from lettuce, 25 grams of lettuce leaves (wet weight) were taken from each sample and placed in a suitable bag. Then, 225 mL of Butterfield phosphate buffer (42.5 g/l KH_2PO_4, pH 7.2, Merck) was added to the bag according to the method described by Keeratipibul et al (2011). After a series of 1:10 dilutions, the samples were cultured and processed using the same methods used for clinical samples.[22]

Soil samples were taken from the bottom of selected lettuce plants. For the isolation of enterobacteria, 0.5 g of sample was suspended in 50 mL of distilled water. After a series of 1:10 dilutions, the samples were plated on VRBG agar. Petri dishes were inverted and incubated at 37 °C for 24h. Plates with distinct colonies were selected for purification according to the colonies present.[23]

Enterobacteria of environmental origin were subcultured onto MH agar plates and identified using the Api 20E gallery from Biomerieux SA.

Phenotypic Characterization of Enterobacteria

The process of phenotypic characterization entails evaluating the biochemical parameters and antibiotic susceptibility profile of the isolated species. The investigation of biochemical characteristics started by verifying the oxidase-negative status of separated Gram-negative bacilli with oxidase discs from HiMedia, India. For all items except for clinical samples, bacterial species were identified by using the API 20E gallery from Biomerieux SA, France. For clinical samples, species were identified using Le Minor's minimal gallery. Antibiotic susceptibility was determined using the Kirby Bauer disc diffusion method on Mueller Hinton agar. Antibiotic discs from BioMérieux SA were used. The

antibiotics used were: amoxicillin + clavulanic acid (20 + 10 µg), cefalexin (30 µg), ceftriaxone (30 µg), cefixime (5 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25-–23.75 µg), and chloramphenicol (30 µg). *E. coli* ATCC 25922 was used as a control for sensitivity testing.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 25 software. The paired-sample *t*-test was used to compare prevalences and rates of resistance, while correlations between antibiotic resistance and biotope and season were determined by principal component analysis. Antibiotic resistance rates were used for this analysis. Results were considered statistically significant at $p < 0.05$.

Results

Distribution of Samples Collected

From February to December 2023, a total of 3786 samples were collected for laboratory analysis and presented in Table 1. Of these, 3529 were of human origin, while 153 and 104 were of animal and environmental origin, respectively.

Biochemical Characteristics of Isolated Enterobacteria

Biochemical tests were conducted to determine the species, and Table 2 presents the essential parameters for identifying enterobacteria using the tryptophan deaminase (TDA), acetoin production (VP), hydrogen sulfide (H_2S), and urease tests. The species reactions to the various tests were found to be variable. All *Enterobacter cloacae* and *Enterobacter sakazakii* strains showed negative reactions to the VP tests and positive reactions to the TDA tests. Only 25.9% and 15.8% of *Escherichia coli* and *Klebsiella* pneumoniae strains respectively were capable of producing hydrogen sulfide.

Prevalence of Enterobacteria in Biotopes

Biochemical characteristics were used to identify strains of the *Enterobacteriaceae* group. Table 3 shows the proportions of presumptive species identified. All potentially pathogenic enterobacteria were collected and identified from original samples. In total, 615 isolates were collected, of which 168 (27.3%) originated from animals, 147 (23.9%) from the environment, and 300 (48.8%) from human-made products. Forty-three (43) species were partially identified using

Table 1 Distribution, Origin and Number of Samples Collected and Coded

Biotope	Specimen Products	Sampling Site	Number of Samples	Total by Biotope
Human	Urine	CERBA	669	3529
		CHUP-CDG	1522	
	Stool	CERBA	579	
		CHUP-CDG	759	
Animal	Milk	Saaba	65	153
	Egg	Koubri	44	
	Fish	Tanghin	28	
		Nanoro	16	
Environment and vegetable	Soil	Bendogo	22	104
		Juvenat S.C.	8	
		Tanghin	22	
	Lettuce	Bendogo	22	
		Juvenat S.C.	8	
		Tanghin	22	
Total				3786

Table 2 Biochemical Parameters for Identification of Enterobacteriaceae

Species	Biochemical Parameters			
	TDA +(%)	H_2S +(%)	VP +(%)	UREASE +(%)
Citrobacter arizonae (N=*3*)	0.0	100.0	100.0	0.0
Citrobacter braakii (N=*5*)	0.0	100.0	100.0	0.0
Citrobacter freundii (N=*5*)	0.0	80.0	80.0	0.0
Citrobacter youngae (N=*1*)	0.0	100.0	100.0	0.0
Enterobacter aerogenes (N=*9*)	0.0	0.0	100.0	11.1
Enterobacter cloacae (N=*151*)	0.0	4.0	100.0	0.0
Enterobacter sakazakii (N=*21*)	0.0	0.0	100.0	0.0
Escherichia coli (N=*27*)	7.4	25.9	66.7	11.1
Escherichia coli 1 (N=*2*)	0.0	0.0	0.0	0.0
Klebsiella ornithinolytica (N=*3*)	0.0	0.0	66.7	66.7
Klebsiella oxytoca (N=*2*)	0.0	0.0	50.0	100.0
Klebsiella planticola (N=*1*)	0.0	0.0	100.0	100.0
Klebsiella pneumoniae (N=*19*)	0.0	15.8	89.5	100.0
Klebsiella pneumoniae pneumoniae (N=*10*)	0.0	0.0	100.0	90.0
Klebsiella terrigena (N=*3*)	0.0	0.0	100.0	0.0
Pantoea spp (N=*1*)	0.0	0.0	100.0	0.0
Pantoea spp 1 (N=*1*)	0.0	0.0	0.0	0.0
Pantoea spp 2 (N=*6*)	0.0	0.0	100.0	0.0
Pantoea spp 3 (N=*7*)	0.0	0.0	100.0	0.0
Proteus mirabilis (N=*10*)	100.0	100.0	100.0	90.0
Proteus vulgaris (N=*14*)	100.0	100.0	100.0	92.9
*Providencia alcali*faciens (N=*1*)	100.0	0.0	100.0	0.0
Providencia spp (N=*1*)	100.0	0.0	100.0	100.0
Rahnella aquatilis (N=*1*)	0.0	0.0	100.0	0.0
Salmonella arizonae (N=*4*)	25.0	50.0	50.0	0.0
Salmonella odorifera(N=*1*)	0.0	0.0	100.0	0.0
Serratia ficaria (N=*2*)	0.0	0.0	50.0	0.0
Serratia liquefaciens (N=*10*)	0.0	20.0	100.0	0.0
Serratia marcescens (N=*4*)	0.0	50.0	100.0	50.0
Serratia odorifera 1 (N=*1*)	0.0	0.0	0.0	0.0
Serratia plymuthica (N=*2*)	0.0	0.0	100.0	0.0
Shigella spp (N=*4*)	0.0	0.0	75.0	0.0

Note: +, capacity of strains to metabolize.

Table 3 Species Identified by Biotope

Species	Biotope			Total
	Animal[a,b] N(%)	Environment[a,c] N(%)	Human[b,c] N(%)	
Escherichia coli	15 (8.2)	4 (2.2)	164 (89.6)	183(100)
Enterobacter cloacae	80 (52.6)	70 (46.1)	2 (1.3)	152(100)
Klebsiella pneumoniae	4 (4.7)	11 (12.9)	70 (0.0)	85(100)
Enterobacter sakazakii	9 (42.9)	12 (57.1)	0 (0.0)	21(100)
Klebsiella oxytoca	0 (0.0)	1 (6.3)	15 (93.8)	16(100)
Enterobacter sp	0 (0.0)	0 (0.0)	14 (100)	14(100)
Proteus mirabilis	9 (64.3)	1 (7.1)	4 (28.6)	14(100)
Proteus vulgaris	10 (71.4)	4 (28.6)	0 (0.0)	14(100)
Serratia liquefaciens	10 (90.9)	0 (0.0)	1 (9.1)	11(100)
Klebsiella pneumoniae pneumoniae	1 (10.0)	9 (90.0)	0 (0.0)	10(100)

(*Continued*)

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Table 3 (Continued).

Species	Biotope			Total
	Animal[a,b] N(%)	Environment[a,c] N(%)	Human[b,c] N(%)	
Enterobacter aerogenes	5 (55.6)	4 (44.4)	0 (0.0)	9(100)
Citrobacter freundii	2 (28.6)	3 (42.9)	2 (28.6)	7(100)
Pantoea spp 3	0 (0.0)	7 (100)	0 (0.0)	7(100)
Pantoea spp 2	2 (33.3)	4 (66.7)	0 (0.0)	6(100)
Citrobacter braakii	2 (40.0)	3 (60.0)	0 (0.0)	5(100)
Klebsiella ornithinolytica	1 (20.0)	1 (20.0)	3 (60.0)	5(100)
Salmonella arizonae	2 (40.0)	1 (20.0)	2 (40.0)	5(100)
Escherichia coli 1	2 (50.0)	0 (0.0)	2 (50.0)	4(100)
Serratia marcescens	2 (50.0)	2 (50.0)	0 (0.0)	4(100)
Shigella spp	3 (75.0)	1 (25.0)	0 (0.0)	4(100)
Citrobacter arizonae	2 (66.7)	1 (33.3)	0 (0.0)	3(100)
Klebsiella terrigena	0 (0.0)	3 (100)	0 (0.0)	3(100)
Salmonella groupe B	0 (0.0)	0 (0.0)	3 (100)	3(100)
Salmonella sp	0 (0.0)	0 (0.0)	3 (100)	3(100)
Salmonella spp	0 (0.0)	0 (0.0)	3 (100)	3(100)
Citrobacter koseri	0 (0.0)	0 (0.0)	2 (100)	2(100)
Klebsiella spp	0 (0.0)	0 (0.0)	2 (100)	2(100)
Salmonella paratyphi	0 (0.0)	0 (0.0)	2 (100)	2(100)
Salmonella typhimurium	0 (0.0)	0 (0.0)	2 (100)	2(100)
Serratia ficaria	2 (100)	0 (0.0)	0 (0.0)	2(100)
Serratia plymuthica	1 (50.0)	1 (50.0)	0 (0.0)	2(100)
Citrobacter sp	0 (0.0)	0 (0.0)	1 (100)	1(100)
Citrobacter youngae	1 (100)	0 (0.0)	0 (0.0)	1(100)
Klebsiella planticola	0 (0.0)	0 (0.0)	1 (100)	1(100)
Pantoea spp	0 (0.0)	1 (100)	0 (0.0)	1(100)
Pantoea spp 1	1 (100)	0 (0.0)	0 (0.0)	1(100)
Providencia alcalifaciens	0 (0.0)	1 (100)	0 (0.0)	1(100)
Providencia spp	0 (0.0)	1 (100)	0 (0.0)	1(100)
Rahnella aquatilis	0 (0.0)	1 (100)	0 (0.0)	1(100)
Salmonella groupe A	0 (0.0)	0 (0.0)	1 (100)	1(100)
Salmonella groupe C	0 (0.0)	0 (0.0)	1 (100)	1(100)
Salmonella odorifera	1 (100)	0 (0.0)	0 (0.0)	1(100)
Serratia odorifera 1	1 (100)	0 (0.0)	0 (0.0)	1(100)
TOTAL	168 (27.3)	147 (23.9)	300 (48.8)	615(100)

Notes: [a]Animal vs environment frequency with p-value = 0.203; [b]Animal vs human frequency with p-value = 0.246; [c]Environment vs human frequency with p-value = 0.207.

phenotypic characteristics, with 29.76% belonging to *Escherichia coli*, 24.72% to *Enterobacter cloacae*, 13.82% to *Klebsiella pneumoniae*, and 3.41% to *Enterobacter sakazakii*.

Prevalence of Enterobacteriaceae by Season

The prevalence of strains of enterobacteria isolated was presented in Table 4. The dry season is defined as the period from November to May, while the rainy season is the period from June to October in Burkina Faso. These two periods are distinguished by the presence or absence of precipitation, respectively. Based on the two kinds of seasons, 251 (40.8%) isolates were collected during the rainy season. The isolates were dominated by the species *Enterobacter sakazakii* and *Klebsiella pneumoniae. Serratia liquefaciens and Pantoea spp 3* were more frequently related to the dry season. Other species were evenly distributed including *Proteus mirabilis, Proteus vulgaris and Klebsiella pneumoniae*.

Table 4 Distribution of Species According to Season

Species	Saison		Total
	Rainy[d]	Dry[a]	
Escherichia coli	79 (43.2)	104 (56.8)	183(100)
Enterobacter cloacae	51 (33.6)	101 (66.4)	152(100)
Klebsiella pneumoniae	39 (45.9)	46 (54.1)	85(100)
Enterobacter sakazakii	14 (66.7)	7 (33.3)	21(100)
Klebsiella oxytoca	6 (37.5)	10 (62.5)	16(100)
Enterobacter sp	5 (35.7)	9 (64.3)	14(100)
Proteus mirabilis	7 (50.0)	7 (50.0)	14(100)
Proteus vulgaris	8 (57.1)	6 (42.9)	14(100)
Serratia liquefaciens	3 (27.3)	8 (72.7)	11(100)
Klebsiella pneumoniae pneumoniae	7 (70.0)	3 (30.0)	10(100)
Enterobacter aerogenes	2 (22.2)	7 (77.8)	9(100)
Citrobacter freundii	1 (14.3)	6 (85.7)	7(100)
Pantoea spp 3	0 (0.0)	7 (100)	7(100)
Pantoea spp 2	1 (16.7)	5 (83.3)	6(100)
Citrobacter braakii	3 (60.0)	2 (40.0)	5(100)
Klebsiella ornithinolytica	1 (20.0)	4 (80.0)	5(100)
Salmonella arizonae	1 (20.0)	4 (80.0)	5(100)
Escherichia coli 1	1 (25.0)	3 (75.0)	4(100)
Serratia marcescens	4 (100)	0 (0.0)	4(100)
Shigella spp	1 (25.0)	3 (75.0)	4(100)
Citrobacter arizonae	2 (66.7)	1 (33.3)	3(100)
Klebsiella terrigena	1 (33.3)	2 (66.7)	3(100)
Salmonella groupe B	1 (33.3)	2 (66.7)	3(100)
Salmonella sp	3 (100)	0 (0.0)	3(100)
Salmonella spp	0 (0.0)	3 (100)	3(100)
Citrobacter koseri	2 (100)	0 (0.0)	2(100)
Klebsiella spp	2 (100)	0 (0.0)	2(100)
Salmonella paratyphi	1 (50.0)	1 (50.0)	2(100)
Salmonella typhimurium	1 (50.0)	1 (50.0)	2(100)
Serratia ficaria	0 (0.0)	2 (100)	2(100)
Serratia plymuthica	1 (50.0)	1 (50.0)	2(100)
Citrobacter sp	0 (0.0)	1 (100)	1(100)
Citrobacter youngae	0 (0.0)	1 (100)	1(100)
Klebsiella planticola	0 (0.0)	1 (100)	1(100)
Pantoea spp	1 (100)	0 (0.0)	1(100)
Pantoea spp 1	0 (0.0)	1 (100)	1(100)
Providencia alcalifaciens.	0 (0.0)	1 (100)	1(100)
Providencia spp	1 (100)	0 (0.0)	1(100)
Rahnella aquatilis	0 (0.0)	1 (100)	1(100)
Salmonella groupe A	0 (0.0)	1 (100)	1(100)
Salmonella groupe C	0 (0.0)	1 (100)	1(100)
Salmonella odorifera	0 (0.0)	1 (100)	1(100)
Serratia odorifera 1	1 (100)	0 (0.0)	1(100)
Total	251 (40.8)	364 (59.2)	615(100)

Note: [a]Rainy vs dry season resistance with p-value = 0.054.

Enterobacteria Count from Samples

The count of strains of enterobacteria is presented in Table 5. The higher load of bacteria in CFU/mL was $3.25x10^6$, 2.15×10^5 and 1.42×10^5 in urine, soil and egg respectively (Table 5).

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Table 5 Bacterial Load According to the Collected Products

Products	Bacterial Load (CFU/mL)			
	Minimum	Maximum	Average	Standard Deviation
Urine	1.00 x 10^{3}	1.00x10^{8}	3.25x10^{6}	7.54x10^{6}
Egg	0.00x10^{0}	5.90x10^{6}	2.15x10^{5}	5.90x10^{6}
Soil	1.89x10^{1}	2.89x10^{6}	1.42x10^{5}	4.08x10^{5}
Fish	2.00x10^{3}	1.78x10^{6}	7.81x10^{4}	1.10x10^{4}
Lettuce	1.50x10^{2}	1.20x10^{5}	7.06x10^{4}	4.50x10^{4}
Milk	0.00x10^{0}	4.81x10^{5}	8.60x10^{3}	5.83x10^{4}

Antibiotic Resistance Profile of Isolated Enterobacteriaceae

The results of enterobacteria strain's reaction against antibiotics are presented in Table 6. A percentage of 16.2% of species were found to be resistant to imipenem and 54.8% for amoxicillin + clavulanic acid).

Antibiotic Susceptibility of Enterobacteria from Animal Samples

Enterobacteria from animal samples were tested and their antibiotic susceptibility is shown in Figure 1. *Enterobacteriaceae* strains exhibited high resistance to amoxicillin + clavulanic acid (48.2%), cefalexin (43.5%), and imipenem (26.2%). However, the lowest resistance was revealed for ciprofloxacin (2%). Additionally, 17.9% of species in this particular habitat displayed intermediate susceptibility to imipenem.

Antibiotic Susceptibility of Enterobacteria from the Environment Samples

Enterobacteria from environment samples were tested and their antibiotic susceptibility is shown in Figure 2. Enterobacteria strains from the environment were resistant to amoxicillin + clavulanic acid (41.5%), cefixime (32.7%) and cefalexin (28.6%). The rate of resistance to imipenem was 6.1%.

Antibiotic Susceptibility of Enterobacteria from Human Pathological Products

Enterobacteria from human pathological products were tested and their antibiotic susceptibility is shown in Figure 3. It was detected that strains were resistant to amoxicillin + clavulanic acid (67.5%), trimethoprim + sulfamethoxazole

Table 6 Antibiotic Resistance Profile of Isolated Enterobacteriaceae

Groups	ATB	Susceptibility Rate (%)		
		I	R	S
Aminopenicillin	AUG	1.1	54.8	44.1
C1G	CN	1.1	35.3	63.6
C3G	CRO	1.8	29.4	68.8
	CAZ	6.3	23.2	70.5
	CFM	0.2	39.5	60.4
C4G	FEP	5	22.5	72.4
Carbapenem	IMI	11	16.2	72.8
Fluoroquinolone	CIP	1.8	21.8	76.4
Sulphamide	SXT	0.2	33.3	66.5
Phenicol	C	0.2	16.8	83.1

Abbreviations: CRO, Ceftriaxone; CN, Cefalexin; AUG, Amoxicillin-Clavulanic Acid; CAZ, Ceftazidime; IMI, Imipenem; FEP, Cefepime; CIP, Ciprofloxacin; SXT, Sulfamethoxazole-trimethoprim; C: Chloramphenicol; I, Intermediate; R, Resistant, S, Sensitive.

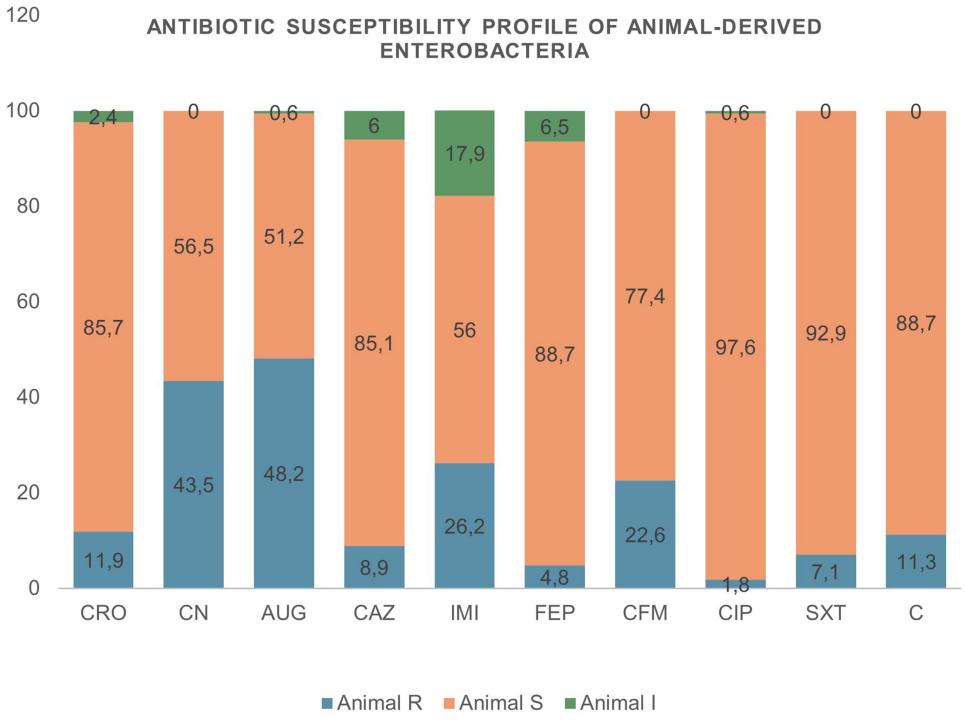

Figure 1 Antibiotic susceptibility profile of animal-derived Enterobacteria. Animal (**R**) resistance rates in animal, Animal (**S**) sensitivity rates in animal, Animal (**I**) intermediate susceptibility rate in animal.

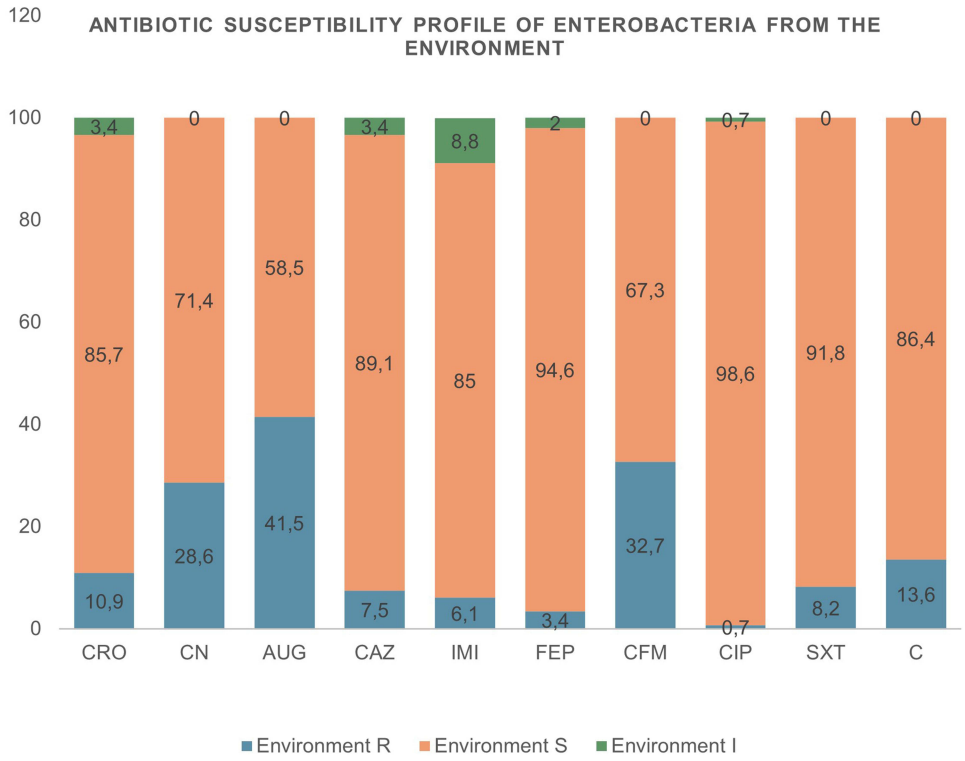

Figure 2 Antibiotic susceptibility profile of environmental Enterobacteria strains. Environment (**R**) resistance rates in environment, Environment (**S**) sensitivity rates in environment, Environment (**I**) intermediate susceptibility rate in environment.

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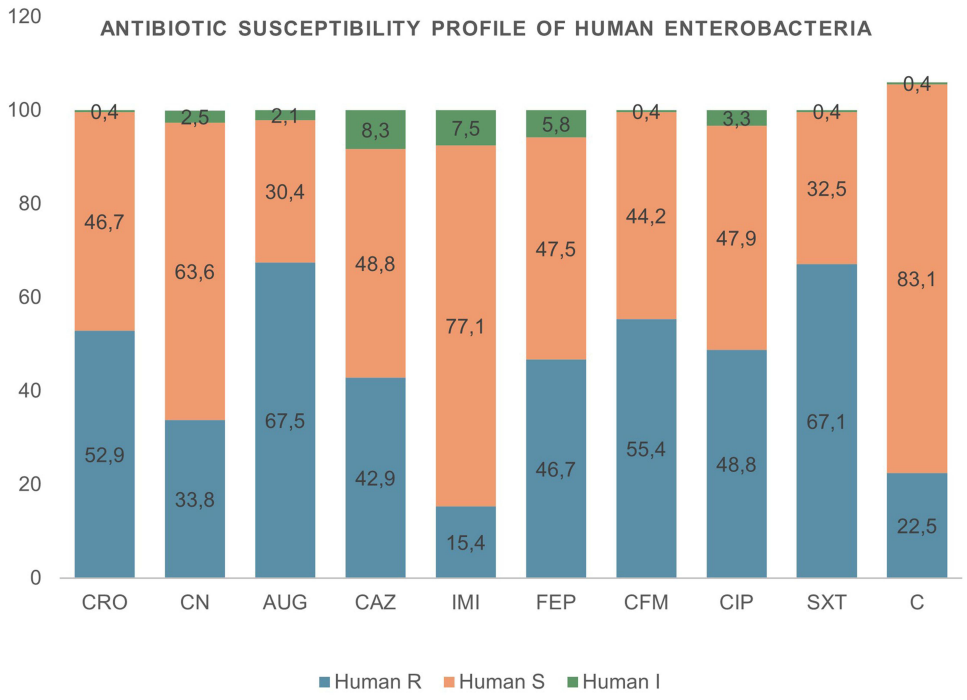

Figure 3 Antibiotic susceptibility profile of human Enterobacteriaceae. Human (**R**) resistance rates in human, Human (**S**) sensitivity rates in human, Human (**I**) intermediate susceptibility rate in human.

(67.1%), cefixime (55.4%) and ceftriaxone (52.9%). Resistance rates of 33.8% and 15.4% were observed for cefalexin and imipenem respectively.

Prevalence of Antibiotic Resistance of Enterobacteria Strains According to Climatic Season

Antibiotic susceptibility prevalence of Enterobacteria are shown in Table 7. Enterobacteria strains exhibited resistance to amoxicillin + clavulanic acid and cefalexin in both wet and dry seasons. However, difference has been noted in behavior towards cefepime, a C4G, as recorded 17% resistance in the wet season versus 26.2% in the dry season.

Table 7 Enterobacteria Strains Susceptibility Profile According to the Season

Familles	ATB	Rainy Season			Dry Season		
		I	R[a]	S	I	R[a]	S
Aminopenicillin	AUG	0.9	61.9	37.2	1.2	50	48.8
C1G	CN	0.4	39	60.5	1.5	32.8	65.7
C3G	CRO	1.8	28.3	70	1.8	30.1	68.1
	CAZ	4.9	22.4	72.6	7.2	23.8	69
	CFM	0.4	38.6	61	0	40.1	59.9
C4G	FEP	8.5	17	74.4	2.7	26.2	71.1
Carbapenem	IMI	12.6	16.6	70.9	9.9	16	74.1
Fluoroquinolone	CIP	1.3	20.6	78	2.1	22.6	75.3
Sulphamide	SXT	0	30.5	69.5	0.3	35.2	64.5
Phenicol	C	0.4	18.8	80.7	0	15.4	84.6

Note: [a]Wet vs dry season resistance with p-value = 0.937.

Principal Component Analysis of Antibiotic Resistance Rates

The correlation between antibiotic resistance rates and biotope and seasonal variables was analyzed using Principal Component Analysis (PCA) and presented in Figure 4. The first two dimensions (Dim1 and Dim2) showed a variability of 96.86%, indicating good representation. Dim2 was influenced by cefalexin and imipenem, while Dim1 was induced by the other eight antibiotics in the study. The human biotope was closer to Dim 1, while the animal biotope was closer to Dim 2. Results showed that resistance of strains to antibiotics (CAZ, CRO, CFP, CIP, SXT) could be correlated to human biotope source but no relationship has been found with the season.

Correlation Analysis of Tested Antibiotics

Results of the correlation analysis of tested antibiotics are presented in Table 8. The correlation between antibiotic resistance rates and Dim 1 antibiotics ranged from 0.761 to 1. For antibiotics in Dim 2, the correlation was r = 0.928.

Discussion

Prevalence of Enterobacteria According to Biotopes

In this study, a total of 3,786 samples have been analyzed. Out of these, 3,529 were human samples, 153 were animal samples, and 104 were environmental samples. Upon bacteriological analysis, a high diversity of enterobacteria was found and comprised of 11 genera and 43 species. Among these, *Escherichia coli* accounted for 29.76%, *Enterobacter cloacae* for 24.72%, *Klebsiella pneumoniae* for 13.82%, *Enterobacter sakazakii* for 3.41%, and *Klebsiella oxytoca* for 2.6%. These findings indicate that enterobacteria are highly prevalent among humans, animals, and the environment. They are consistent with numerous studies that have reported high prevalence rates of *Escherichia coli* between 48.5% and 63%, and *Klebsiella pneumoniae* at 33% in clinical samples.[24,25] This study conducted on three biotopes isolated approximately the same number of species in each. Specifically, we identified 168 species in animals, 147 in the environment, and 300 in humans. These results did not differ significantly, with p-values of 0.203, 0.246, and 0.207 for animal vs environment, animal vs human, and environment vs human comparisons, respectively. These results would indicate a homogeneous distribution of enterobacteria in the three biotopes. Studies have shown that multi-resistant bacteria like extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* species, are often transmitted between

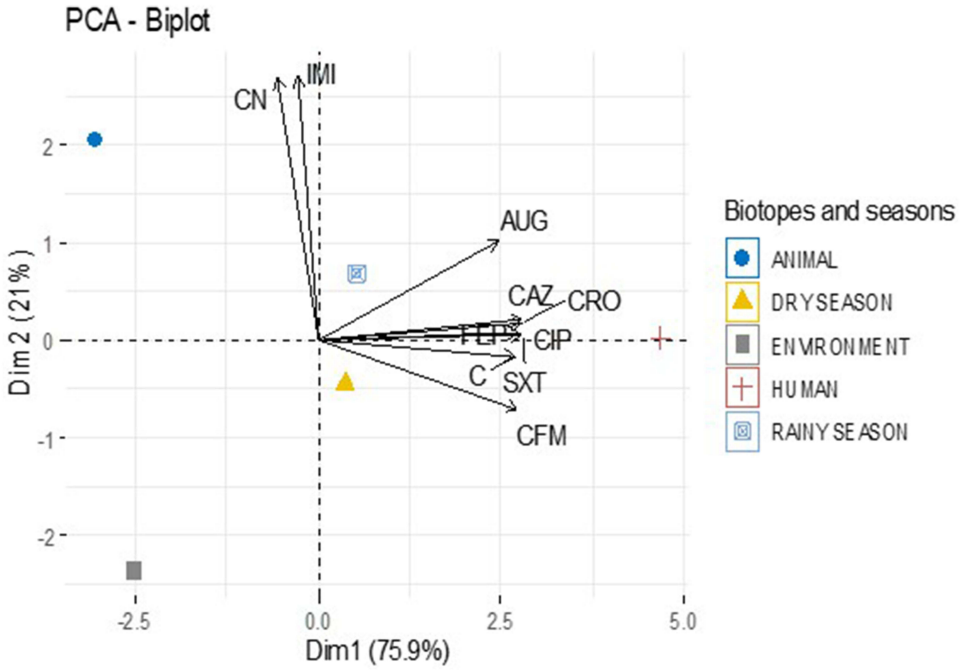

Figure 4 PCA of resistance rates by biotope and season.

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Table 8 Correlations Between Rates of Resistance to the Antibiotics Studied

		CRO	CN	AUG	CAZ	IMI	FEP	CFM	CIP	SXT	C
Corrélations	CRO	1.000	−0.145	0.877	1.000	−0.019	0.989	0.948	1.000	0.999	0.929
	CN	−0.145	1.000	0.225	−0.132	0.928	−0.193	−0.432	−0.146	−0.190	−0.203
	AUG	0.877	0.225	1.000	0.881	0.203	0.804	0.761	0.877	0.855	0.907
	CAZ	1.000	−0.132	0.881	1.000	−0.005	0.989	0.943	1.000	0.998	0.926
	IMI	−0.019	0.928	0.203	−0.005	1.000	−0.017	−0.336	−0.020	−0.057	−0.211
	FEP	0.989	−0.193	0.804	0.989	−0.017	1.000	0.937	0.989	0.992	0.871
	CFM	0.948	−0.432	0.761	0.943	−0.336	0.937	1.000	0.948	0.959	0.942
	CIP	1.000	−0.146	0.877	1.000	−0.020	0.989	0.948	1.000	0.999	0.929
	SXT	0.999	−0.190	0.855	0.998	−0.057	0.992	0.959	0.999	1.000	0.925
	C	0.929	−0.203	0.907	0.926	−0.211	0.871	0.942	0.929	0.925	1.000

pets or horses and their owners, as well as between livestock and humans who are exposed to them through occupation.[26] Transmission of these bacteria from animals to humans can occur through different ways such as direct contact with animals and their feces, as well as ingestion of food and water containing the bacteria.[7]

Prevalence of Enterobacteria Strains According to Season

This research collected 364 enterobacteria of different species during the dry season, versus 251 enterobacteria during the wet season, with a p-value of 0.054. Based on this data, we concluded that the frequency and profile of enterobacteria are not affected by seasonal changes. These findings contradict many other studies. Apart from various host-related risk factors such as the destruction of commensal flora and immunosuppression, non-intrinsic factors like weather conditions and latitude also contribute to the seasonality of several bacterial infections.[27–29] For instance, research has revealed that enterotoxigenic *Escherichia coli* and enteroaggregative *Escherichia coli* infections are more common during the rainy season.[30] A certain seasonality has been documented for some bacterial infections; for instance, higher incidence of Gram-negative infections occurs during the warmer months, reflecting the optimal growth conditions for many Gram-negative bacteria at 32–36°C.[31]

Enterobacteria Counts from Samples

CFU/mL counts ranged from 8.6×10^3 to 3.25 x 10^6, with the highest in urine and lowest in milk samples. The low bacterial load our unpasteurized milks may be associated with the high level of hygiene observed among farm personnel in caring for the cows and the milking process. Our bacterial loads were significantly lower than those reported by Sobeih et al[1], which ranged from 1.02×10^6 to 1.98×10^5 CFU/mL. We also found a bacterial load of 2.15×10^5 CFU/mL on the surface of eggshells. The reason for the contamination of eggs may be due to the presence of fecal matter during oviposition or shortly after in the surrounding environment. Other factors can also contribute to bacterial contamination of eggs, such as dust in the area where the eggs are stored, the hygiene and quality of the eggshell (including the presence of cracks, cuticle, and membrane quality), the season, and the conditions in which the eggs are stored.[3,32]

Antibiotic Resistance Profile of Enterobacteria Strains

Antibiotic resistance rates were 54.8%, 35.3%, 33.3%, 30.7%, 22.5%, 21.8%, 16.8% and 16.2% for aminopenicillins, C1Gs, sulfonamides, C3Gs, C4Gs, fluoroquinolones, phenicols and carbapenems. When tested individually, species from all three biotopes exhibited a similar resistance profile for most of the ten antibiotics that were tested. Nevertheless, it is possible that these results have been influenced by natural resistance observed in certain species. However, it is worth noting that species originating from humans seem to be considerably more resistant to C3Gs, fluoroquinolones, and sulfonamides when compared to animal and environmental species. An analysis of the correlations between ten different antibiotics was conducted, and it revealed some strong positive correlations. Most of the antibiotics showed a high correlation, with an r-value greater than 0.76 (Table 8). The high correlation,

with an r-value of 0.928, between cefalexin and imipenem suggests that these two antibiotics share similar mechanisms of resistance. Additionally, negative correlations were found between imipenem and all eight other antibiotics. This finding contradicts the results of previous studies,[33,34] which have established a connection between exposure to carbapenems and resistance to other antimicrobials. These analyses have shown that the progression towards carbapenem-resistant or intermediate enterobacteria is likely due to a combination of factors related to antibiotic exposure. This may indicate that replacing multiple antibiotics with carbapenems may have potential drawbacks. Carbapenems are a type of β-lactam antibiotics that are often used as a last resort in treating bacterial infections caused by multi-resistant Gram-negative bacilli. The results of this study show a relatively high and concerning level of resistance to these drugs. In fact, we observed imipenem resistance rates of 26.2%, 6.1%, and 15.4% in animals, the environment, and humans, respectively. According to a study by Kock et al in 2018, the rate of carbapenem resistance among livestock and pets in Europe was less than 1%, while in Africa it ranged from 2–26% and in Asia it ranged from 1–15%. The rate in wild animals in Australia and Europe varied between 16–19%. The study also found that 33–67% of humans who were exposed to poultry farms carried carbapenem-resistant *Enterobacteriaceae*.[26] In some enterobacteria, resistance to carbapenem antibiotics can result from a combination of mechanisms, which involve β-lactamases that have very low carbapenemase activity and reduced outer membrane permeability. Alternatively, it can result from the presence of true carbapenemases.[35] Carbapenemases that are acquired are one of the most serious threats to public health in terms of antibiotic resistance. These carbapenemases provide resistance not only to carbapenems but also to almost all β-lactams. Moreover, they are encoded by genes that can be transferred from one bacterium to another.[36]

Prevalence of Antibiotic Resistance of Enterobacteria Strains from Biotopes

Overall resistance of isolated species was 18.63% for animals, 15.32% for the environment, and 45.3% for humans. Surprisingly, it has been found that resistance to selected antibiotics was similar in animal and environmental species (p=0.258), indicating a certain relationship between the animal and the environment in their behavior towards antibiotics. This finding is supported by the PCA representation as shown in Figure 4. Animals would be more likely to come into contact with the soil, which can lead to them being more susceptible to bacterial infections. Indeed, most bacteria attach to biotic or abiotic surfaces and integrate into a complex matrix known as a biofilm. The environment is now considered to be a determining factor in biofilm formation, and in the development and dissemination of antibiotic resistance through biofilms. Several studies have demonstrated that environmental biofilms can be hotspots for the dissemination of antibiotic resistance genes. These genes can be rapidly transferred via horizontal gene transfer, which is more frequent in biofilms.[37]

This research showed that humans have higher rates of antibiotic resistance compared to animals (p=0.006), and the environment (p=0.001). The results of the PCA analyses (Figure 4) reinforce this observation that humans have a higher prevalence of antibiotic resistance compared to animals and the environment. This can be attributed to the fact that humans tend to use more antibiotics than other living beings. Moreover, humans also consume food items from animal and plant sources that often contain antibiotic-resistant strains.[38] Although there is evidence to suggest that livestock farmers who come into direct contact with animals acquire antibiotic resistance genes linked to the use of antibiotics in animals, it is unclear whether there is a wider spread of antibiotic resistance in the general human population.[39] The evidence for the transmission of resistance genes between humans, livestock, and the environment is inconclusive. Hence, the spread of antibiotic-resistant infections is not the sole way in which the use of antibiotics in one population can impact others.[40,41]

Prevalence of Antibiotic Resistance of Enterobacteria Strains According to Season

In both the dry and wet seasons, the average resistance rates were 29.2% and 29.4%, respectively (p = 0.937). These results show that there is no significant difference in the prevalence of resistance between dry and wet seasons. The enterobacterial resistance mechanisms remained stable across climatic seasons, and high resistance potential enterobacteria were constantly distributed throughout the study period, as confirmed by the PCA analyses (Figure 4). Contrary to these results, numerous studies have established relationships between climate change and antibiotic resistance.[42–44] Increasingly higher temperatures in fact are intimately linked to antimicrobial resistance, because they are associated

with increased bacterial growth rates.[45] A cross-sectional study conducted by Kaba and et al shows that carbapenem-resistant *Klebsiella pneumoniae* and multi-resistant *Escherichia coli* are significantly associated with the warm-season change in temperature.[46] Understanding how antimicrobial resistance evolved alongside climatic change can provide insights to better design future efforts and interventions.[47]

Conclusion

This study reported a high prevalence of potentially pathogenic *Enterobacteriaceae* in the three biotopes of humans, animals, and the environment. These biotopes contain enormous quantities of enterobacteria. We have also demonstrated that humans harbor more resistant enterobacteria than animals and the environment. This work underlines the importance of continuous monitoring of antibiotic administration. It also highlights the need for multidisciplinary collaboration and the establishment of an effective antimicrobial use monitoring program, to improve the quality of care in all structures concerned with public health, animal health and environmental health.

Ethics Statement

This research was authorized by the National Ethics Committee for Health Research of Burkina Faso after verification of the study protocol. Approval No. 2023-06-198 was issued for the study following the guidelines set out in the Declaration of Helsinki. The study did not directly recruit patients as the samples were not taken directly from the human body. So bacterial strains were collected from clinical sources on faeces and pathological fluids, but not directly from human products or patient's bodies. We also received authorisation No. 2023-193/MSHP/SG/CHUP-CDG/DRH/SRF for collection from the Safety and Public Healthy Ministry, respecting patients' rights and confidentiality to collect bacteria from pathological fluids.

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Disclosure

The authors report no conflicts of interest in this work.

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