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Posted Date: 8 October 2024

doi: 10.20944/preprints202410.0580.v1

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Article

Extended Spectrum Beta-Lactamase-Producing and Multidrug Resistant *Escherichia coli* and *Klebsiella* spp. from the Human-Animal-Environment Interface on Cattle Farms in Burkina Faso

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Abstract: Extended-spectrum beta-lactamase (ESBL) -producing and multidrug resistant *Enterobacteriales* pose a major threat to both human and animal health. This study assessed the prevalence of ESBL-producing *Escherichia coli* (ESBL-*Ec*) and *Klebsiella* spp. (ESBL-*K*) in cattle farms in Ouagadougou, Burkina Faso, using a One Health approach. From May 2021 to September 2022, cattle faeces, farmers' stools, their drinking water and farm soil samples were collected from semi-intensive and traditional farms. ESBL-selective medium was used to obtain resistant isolates, which were further characterized using biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. ESBL-*Ec* and/or ESBL-*K* were detected in 188 of 322 samples (58.0%). The prevalence of ESBL-*Ec* isolates was 42.2% (136/322) and ESBL-*K* isolates 24.5% (79/322). Notably, 156 of the 188 ESBL isolates (83.0%) exhibited multidrug resistance. The highest resistance rates were observed against tetracycline and cotrimoxazole. Importantly, no isolates showed resistance to meropenem, which was used to test for carbapenem resistance. This study highlights the presence of ESBL-*Ec* and ESBL-*K* among humans, animals and the environment of the cattle farms. Good hygiene and biosafety practices are essential to limit the potential spread of multidrug resistant bacteria between different interfaces on farms.

Keywords: *Escherichia coli*; *Klebsiella* spp.; ESBL; cattle farms; one health; Burkina Faso

1. Introduction

Antimicrobial resistance (AMR) is a serious public health problem, that threatens human health and can cause large economic losses. Infections caused by multidrug resistant bacteria challenge the

hospital practices, since they may be associated with increased severity of infections and with a high mortality rate. Recently, in 2019, approximately 4.95 million deaths were associated to AMR, with 1.27 million directly attributable to multidrug resistant bacteria with the highest number recorded in western Sub-Saharan Africa, with 27.3 deaths per 100,000 population [1]. Moreover, the economic cost of AMR will continually increase over the next 10 years [2]. However, there are data gaps in many low-income settings, including Burkina Faso. ESBL and carbapenemase-producing *Enterobacterales*, particularly *Escherichia coli* (ESBL-*Ec*) and *Klebsiella pneumoniae* (ESBL-*Kp*), contribute to exacerbate the AMR crisis in healthcare and community in low- and middle income countries (LMICs), due to their resistance to beta-lactams, commonly used antibiotics in treatment of bacterial infections [4–6].

Estimated 131,109 tons of antimicrobials were used for food animals in 2013 [5]. Since 2000 to 2017, the demand for animal protein in Africa has increased by 64%, driven by demand for protein-rich diets, consumer preferences and population growth, accelerating transfer to both more intensive and extensive animal production [6]. With regard to livestock production in Africa, cattle accounted for 53% of the total population produced in 2017. The quantity of antimicrobials used for animal production was estimated at 4,279 tonnes [7]. In Burkina Faso, cattle farming significantly contributes to rural households' income, estimated at 71 to 115 million dollars [8]. The intensification of animal production has led to an increased use of veterinary medicines including antimicrobials, accelerating the rise of AMR [4,8]. Antimicrobials are used to treat sick animals, but also for prophylaxis, as growth promoters and to compensate inadequate hygiene on the farm [9]. The misuse or excessive use of antimicrobials contribute to the emergence and dissemination of resistant bacteria including resistant *Enterobacterales*, which can be transferred to people through the food chain [10]. ESBL production is one of the most common mechanisms of multidrug resistance in *Enterobacterales* and occurrence of ESBL-producing bacteria on farms has been documented in many parts of the world [11]. From African countries, the occurrence of ESBL-producing *Enterobacterales* (ESBL-*E*) in cattle have been reported, for example, from Egypt, Madagascar, Nigeria, South Africa and Tunisia [12–16]. In Nigeria, the horizontal transfer of ESBL genes from cattle to slaughterhouse workers was reported [17]. In Burkina Faso, ESBL-*E* were previously isolated from farms, ESBL-*Ec* and ESBL-*K* being the most common isolates [18]. Studies carried out in 2014 in three regional hospitals in Burkina Faso showed that 58% of enterobacterial isolates were ESBL producers and *E. coli* and *Klebsiella pneumoniae* were the most frequently isolated ones [19]. However, data on the occurrence of ESBL-producing bacteria at the human-animal-environment interface on Burkinabe farms remains limited. Therefore, this study assessed the occurrence of ESBL-*Ec* and ESBL-*K* on farms near the capital city Ouagadougou, among cattle, farmers, their drinking water and soil using a One Health approach.

2. Materials and Methods

2.1. Ethics Committee Approval

The ethical committee approval was obtained from the Health Research Ethics Committee (CERS) of Burkina Faso (N°2018-15-1153). The purpose of the study and the sampling procedure were explained to the farmers orally, after which written consent to participate in the study was requested.

2.2. Study Design

Samples were collected from May 2021 to September 2022 from 39 semi-intensive and 28 traditional farms located in the peri-urban area of Ouagadougou (Figure 1). In Burkina Faso, animal production is commonly located in the peri-urban areas surrounding cities. On semi-intensive farms, cattle graze during the day and receive feed supplements in the evening. They are dewormed, treated, and their health is continuously monitored. On traditional farms, cattle are constantly on the move, guided by a shepherd in search for the best pastures. Treatment is only administered in cases of illness or during vaccination campaigns.

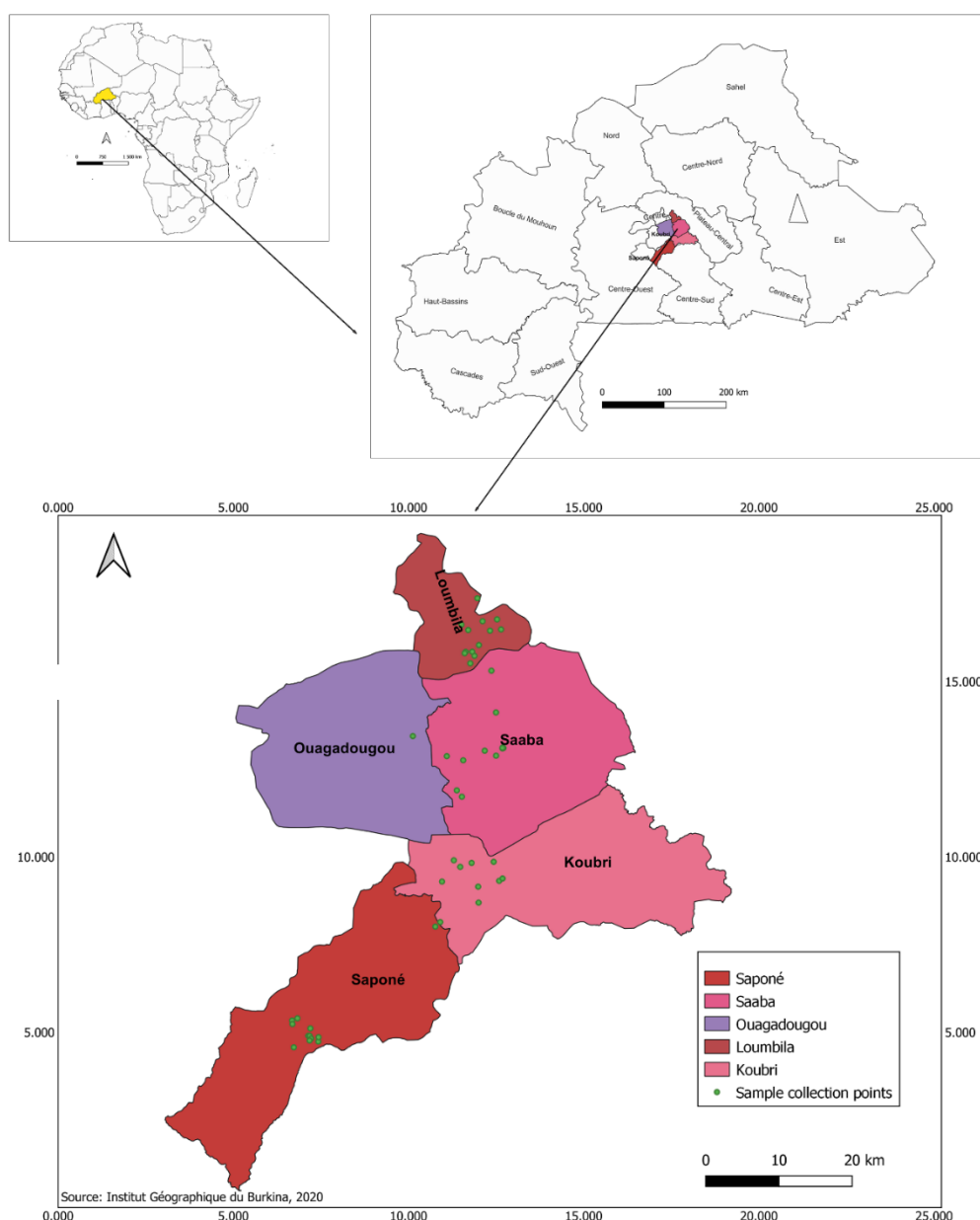


Figure 1. Map of Burkina Faso with locations of the cattle farms, where the samples were collected from.

2.3. Sampling

Approximately 100g of faeces ($n=68$) and soil samples ($n=68$) were collected into sterile bags from cattle enclosures from both semi-intensive and traditional farms. At least five points were sampled for each sample type and pooled. Farmers' drinking water, sourced from taps on the farm, was collected into sterile 500 mL bottles. Additionally, farmers provided stool samples in sterile 120 mL containers. All the samples were placed in a cooler box containing ice blocks and transported to the laboratory for analysis.

2.4. Bacterial Isolation and Identification

Water, soil and cattle faeces samples were enriched using buffered peptone water (BPW). An amount of 10 g of soil or faeces were mixed into 90 mL of BPW. The drinking water samples were first concentrated by filtering 250mL water through a 0.45 μ M membrane filter, after which it was

transferred into BPW. The inoculated BPW tubes were incubated at 37°C for 18 to 24 h. After incubation, 10 µL of each enriched sample was plated onto selective ESBL CHROMagar plates (CHROMagar™ ESBL, Paris, France). The human stool samples were directly plated without enrichment onto the ESBL CHROMagar plates, which were incubated at 37°C for 18 to 24 h. For each sample, a positive control was inoculated on non-selective cystine lactose electrolyte deficient (CLED) agar plate to ensure that the sample contained bacteria. After incubation, the ESBL CHROMagar plates were inspected and following the manufacturer’s instructions, the red or pink colonies were identified as *E. coli* and the green, blue-green or blue as KESC group (*Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*). Five colonies of the each morphotype of *E. coli* or KESC group were picked and purified using eosin methylene blue agar (EMB). The purified colonies were transferred to Muller-Hinton agar and subsequently identified using six biochemical tests: indole test, citrate utilization, lactose utilization, glucose fermentation, motility and gas production. The verified isolates were stored in 30% glycerol at -40°C for further analysis.

2.5. Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was performed using the disk diffusion method on Muller Hinton agar (HiMedia, India). The isolates were tested against 13 antibiotics: amoxicillin+ clavulanic acid (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefepime (30µg), meropenem (10 µg), gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nalidixic acid (30 µg), tetracycline (30µg), sulfametoxazole+ trimethoprim (25 µg) and chloramphenicol (30 µg) (Liofilchem, Italy). *Escherichia coli* ATCC 25922 was used for quality control of the antibiotic discs. The results were interpreted according to the American Clinical and Laboratory Standards Institute guidelines [20].

2.6. Phenotypic Detection of ESBL Production

ESBL production was detected using double disk synergy test (DDST) between cefotaxime, cefepime and amoxicillin + clavulanic acid. An isolate was considered ESBL-producing when there was a visible synergy inhibition zone between the tree antibiotic disks.

2.7. Data Analysis

Data were analysed using R software version 4.2.2. Data were subjected to the Chi-square test, and a probability value of $p \leq 0.05$ was considered statistically significant. GraphPad Prism Version 10.0.3 (275) was used to produce a heat map visualizing the antibiotic resistance profiles.

3. Results

3.1. Prevalence of ESBL-Producing *E. coli* and *Klebsiella* spp. Isolates by Sample and Farm Type

A total of 322 samples including cattle faeces (n=68), soil (n=68), farmers’ stools (n=120) and their drinking water (n=66) were collected. Of these, 188 contained at least one ESBL-producing *E. coli* (ESBL-*Ec*) and/or ESBL-producing *Klebsiella* spp. (ESBL-K), the overall prevalence being 58.4%. The highest prevalence was observed in cattle faeces (58/68, 85.3%), and the lowest in farmers’ drinking water (25/66, 37.9 %) (Table 1). Of the two enterobacteria, ESBL-*Ec* was more prevalent than ESBL-K spp. in all sample types. The highest prevalence of ESBL-*Ec* (76.5%) was detected in cattle faeces.

Regarding farm types, the prevalence of samples containing ESBL-*Ec* and/or ESBL-K isolates was quite similar in both semi-intensive and traditional farms (no significant difference). The overall prevalence of ESBL-*Ec* (55.2%) was higher than that of ESBL-K spp. (34.3%) on the two types of farms.

Table 1. Prevalence of ESBL-*Ec* and ESBL-K isolates in four different sample types and on two different farm types.

	Number of samples/ farms analysed	Samples containing ESBL-	Samples containing	Samples containing ESBL- K
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	N	<i>Ec</i> and/or ESBL- <i>K</i> spp. n (%)	ESBL- <i>Ec</i> n (%)	n (%)
Sample type				
Cattle faeces	68	58 (85.3) ¹	52 (76.5) ²	17 (25.0)
Soil	68	41 (60.3) ¹	32 (47.0) ²	13 (19.1)
Human stools	120	64 (53.3) ¹	47 (39.2) ²	27 (22.5)
Drinking water	66	25 (37.9) ¹	5 (7.6) ²	22 (33.3)
Total	322	188 (58.4)	136 (42.2)	79 (24.5)
Farm type				
Semi-intensive	39	36 (92.3)	21(53.8)	15 (38.5)
Traditional	28	24 (85.7)	16 (57.1)	8 (28.6)
Total	67	60 (89.5)	37 (55.2)	23 (34.3)

N= number of samples tested; n= number of positive samples. ¹ Mean statistically significant difference (p<0.05) between the prevalence of ESBL-*Ec* and/or ESBL-*K* spp in the different samples ² Mean statistically significant difference (p<0.05) between the prevalence of ESBL-*Ec* in the different samples.

3.2. Antibiotic Resistance of ESBL-Producing *E. coli* and *Klebsiella* spp. Isolates

A total of 136 ESBL-*Ec* and 79 ESBL-*K* isolates were tested for their susceptibility to 13 antibiotics belonging to seven different antibiotic groups. Apart from beta-lactams, the resistance levels were highest against tetracycline and cotrimoxazole (Figure 2, Tables 2 and 3). The lowest resistance rates among both ESBL-*Ec* and ESBL-*K* were observed against amikacin, gentamicin and ciprofloxacin (Tables 2 and 3). All the isolates were susceptible to meropenem.

The multidrug resistance rates of ESBL-*Ec* and ESBL-*K* were high, between 59.4-84.6% (Tables 2 and 3). As many as 3.1% the ESBL-*Ec* and 1.4% of the ESBL-*K* isolates were resistant to all the antibiotic groups tested, excepted carbapenems (Figure 3).

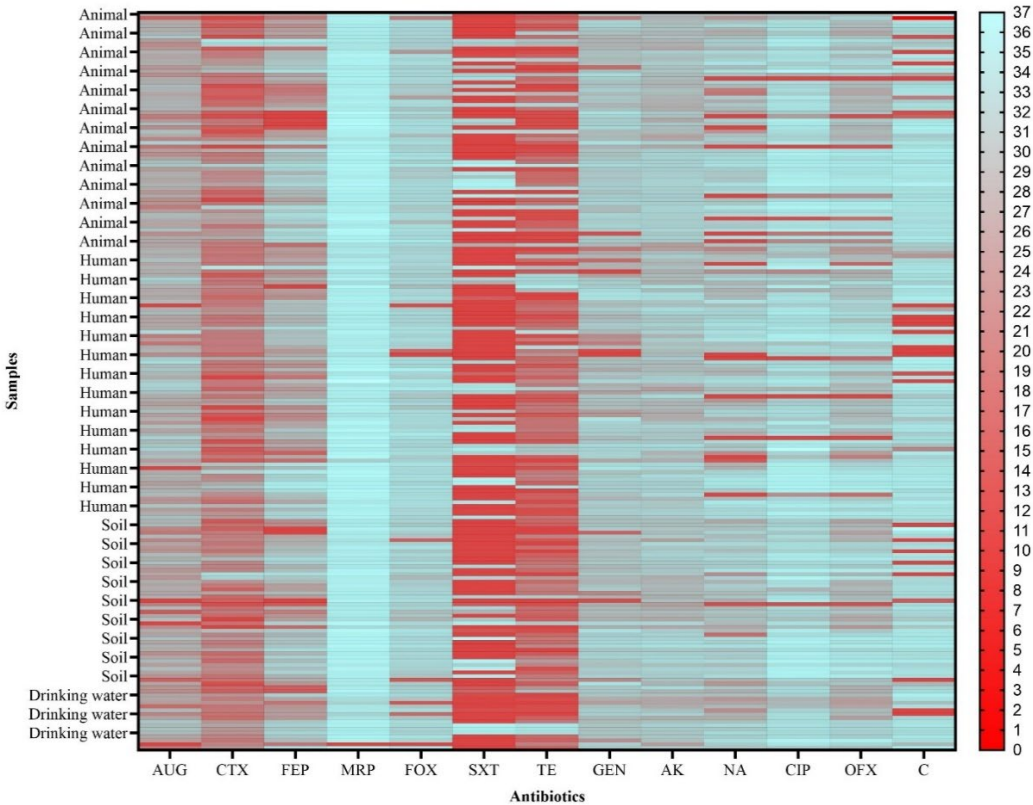


Figure 2. A heatmap showing the distribution of antibiotic resistance among the ESBL-producing *E. coli* and *Klebsiella* spp. isolates from cattle, humans, soil and drinking water. amoxicillin+ clavulanic acid (AUG), cefotaxime (CTX), cefepime (FEP), meropenem (MRP), ceftazidime (FOX), cotrimoxazole (STX), tetracycline (TE), gentamicin (GEN), amikacin (AK), nalidixic acid (NA), ciprofloxacin (CIP), ofloxacin (OFX), chloramphenicol (C). Red colour indicates resistance, blue colour indicates no resistance.

Table 2. Resistance to various antibiotics among 136 ESBL-producing *E. coli* isolates from cattle faeces, soil, human stools and drinking water.

Antibiotic group	Antibiotics (µg)	Cattle	Soil	Human	Water
		N=52 (%)	N= 32 (%)	N= 47 (%)	N= 5 (%)
Beta-lactamins	Cefoxitin (30)	1 (1.9)	2 (6.3)	1 (2.1)	0 (0.0)
	Cefotaxime (30)	52 (100)	30 (93.8)	46 (97.9)	5 (100)
	Cefepime (30)	39 (75.0)	27 (84.4)	40 (85.1)	3 (60.0)
	Meropenem (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Penicillin and inhibitors	Amoxicillin+ clavulanic acid (30)	24 (46.2)	20 (62.5)	12 (25.5)	4 (80.0)
Sulfonamides	Cotrimoxazole (25)	30 (57.7)	21 (65.6)	32 (68.1)	4 (80)
Quinolones, fluoroquinolones	Ciprofloxacin (5)	5 (9.6)	2 (6.3)	5 (10.6)	1 (20.0)
	Ofloxacin (5)	4 (7.7)	5 (15.6)	5 (10.6)	0 (0.0)
	Nalidixic acid (30)	13 (25.0)	11 (34.4)	12 (25.5)	1 (20.0)
Aminoglycosides	Amikacin (30)	1 (1.9)	3 (9.4)	2 (4.3)	0 (0.0)
	Gentamicin (10)	3 (5.8)	3 (9.4)	6 (12.8)	0 (0.0)
Phenicol	Chloramphenicol (30)	6 (11.5)	3 (9.4)	6 (12.8)	1 (20.0)
Cyclins	Tetracycline (30)	42 (80.8)	26 (81.3)	41 (87.2)	4 (80.0)
Multidrug resistance		36 (69.2)	19 (59.4)	33 (70.2)	4 (80.0)

Table 3. Resistance to various antibiotics among 79 ESBL-producing *Klebsiella* spp. isolated from cattle faeces, soil, human stools and drinking water.

Antibiotic group	Antibiotics (µg)	Cattle	Soil	Human	Water
		N=17 (%)	N= 13 (%)	N= 27(%)	N= 22 (%)
Beta-lactamins	Cefoxitin (30)	4 (23.5)	0 (0.00)	2 (7.4)	2 (9.1)
	Cefotaxime (30)	14 (82.4)	13 (100)	24 (88.9)	19 (86.4)
	Cefepime (30)	14 (80.4)	13 (100)	23 (85.2)	21 (95.5)
	Meropenem (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Penicillin and inhibitors	Amoxicillin+ clavulanic acid (30)	9 (52.9)	9 (69.2)	16 (59.3)	14 (63.6)
Sulphonamides	Cotrimoxazole (25)	12 (70.6)	11 (84.6)	22 (81.5)	17 (77.3)
Quinolones, fluoroquinolones	Ciprofloxacin (5)	0 (0.0)	2 (15.4)	1 (3.7)	0 (0.0)
	Ofloxacin (5)	0 (0.0)	0 (0.0)	1 (3.7)	3 (13.6)
	Nalidixic acid (30)	1 (5.9)	2 (15.4)	4 (14.8)	5 (22.7)
Aminoglycosides	Amikacin (30)	0 (0.0)	0 (0.0)	1 (3.7)	0 (0.0)
	Gentamicin (10)	2 (11.8)	2 (15.4)	4 (14.8)	1 (4.6)

Phenicol	Chloramphenicol (30)	6 (35.3)	3 (28.1)	10 (37.0)	1 (4.5)
Cyclins	Tetracycline (30)	12 (70.6)	13 (100)	16 (59.3)	16 (72.7)
	Multidrug resistance	14 (82.4)	11 (84.6)	21 (77.8)	18 (81.8)

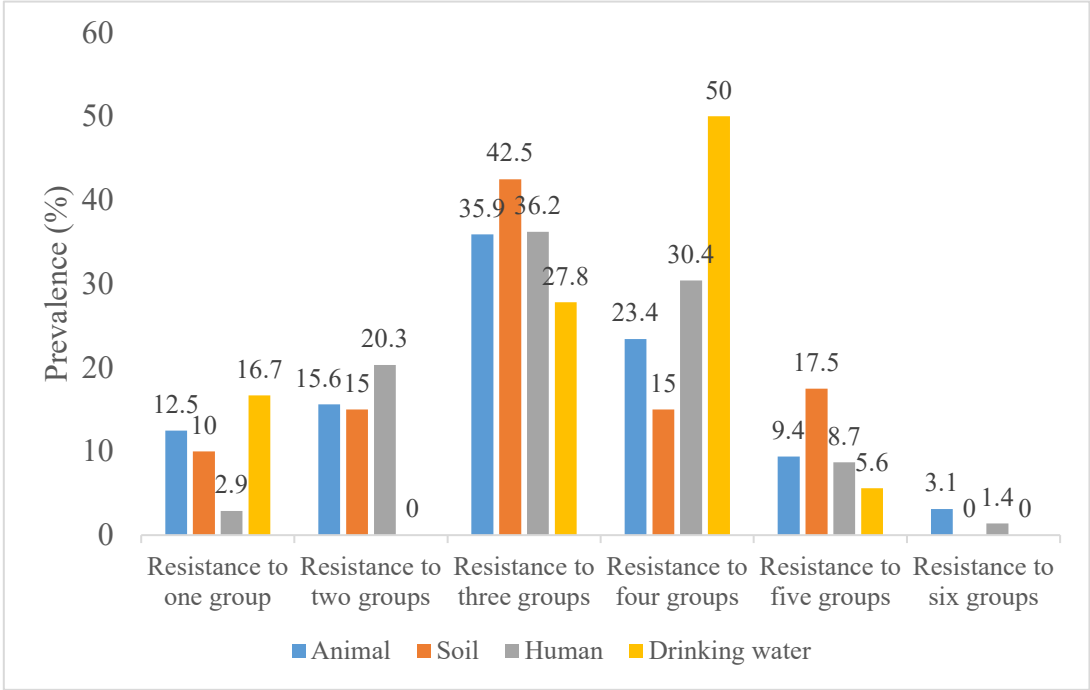


Figure 3. Multidrug resistance of ESBL-producing *E. coli* and *Klebsiella* spp. to one or more antibiotic groups in cattle faeces, soil, farmers’ stools and their drinking water.

4. Discussion

Antimicrobial resistance poses a serious threat to health globally, and the World Health Organisation (WHO) classifies ESBL-*Ec* and ESBL-*K* among the highest priority pathogens [21]. Multidrug-resistant *E. coli* can be considered as indicator of antibiotic resistant bacteria in general, as *E. coli* is a ubiquitous and commensal species in animals and can provide relevant indication of the spread of antibiotic resistance [22]. Our study assessed the presence of ESBL-*Ec* and ESBL-*K* in animals, soil, humans and drinking water on two types of farms located in the semi-urban area in Burkina Faso. The study revealed that 58% of all the samples from farms yielded at least one ESBL-*Ec* or ESBL-*K* isolate. Researchers from other parts of Africa have reported lower rates, on the average 25% occurrence of ESBL-*Ec* and ESBL-*K* in animals, environment and humans in Egypt [23], Nigeria [17] , Ghana [24] and in Uganda [25]. Likewise, a study conducted in Rwanda among livestock, environment, community members and farm products showed a relatively low prevalence of 14.8% [26]. In Burkina Faso and other LMICs, the occurrence of resistant bacteria correlates with poor sanitation, close interactions with livestock, easy access and irrational use of antibiotics [27]. Misuse of antibiotics in humans and animals has been documented in the literature to promote a selective increase in some bacterial populations as well as dissemination of resistant strains [28].

In the cattle faeces, the detected prevalence of 85.3% was detected for ESBL-*Ec* and/or ESBL-*K*, which is higher than previously reported from Burkina Faso [18] and Cote d’Ivoire [29]. A study on cattle faeces reported 45.4% prevalence of ESBL-*Ec* among animals in Nigerian slaughterhouses[17]. In Ghana, the prevalence of ESBL-*Ec* was 31% in cattle faeces [30] and in Egypt 42.8%[12]., while ESBL-*Ec* and ESBL-*K* prevalence on cattle farms were reported to be 76.4% in South Africa [15]. In Burkina Faso, antibiotics are used by farmers to treat animals, for prophylactic purposes, and more critically, as growth promoters [8] and, consequently, the selection pressure on commensal and

pathogenic microorganisms has led to the proliferation of antibiotic resistant bacteria. These bacteria can be transferred to humans through direct contact with animals or indirectly via the food chain or environmental pollution from agricultural effluents [31].

In the farmers' stools, the prevalence of ESBL-*Ec* and ESBL-*K* was 76.5% and 25.0 %, respectively. Two previous studies carried out in Burkina Faso reported a prevalence of 22% and 53% of ESBL-*E* in healthy volunteers, and 42% and 56% among inpatients [32,33]. Among slaughterhouse workers in Nigeria, 50% prevalence of ESBL-*Ec* was detected [34]. A study conducted among poultry workers in Nigeria reported as low prevalence as 2.7 % of ESBL-*Ec* [35]. Over the last 20 years, the prevalence of community-acquired ESBL carriage has increased tenfold worldwide, reaching 26% in 2016-2020 [36]. Cumulative prevalence was highest in South-East Asia (35.1%, 95% CI, 10.3%–60.0%) and lowest in Europe (6.0%, 95% CI, 4.6%–7.5%) , whereas it was 21.4% (95% CI, 12.7%–30.1%) in Africa [36]. Compared to these figures, the prevalence we detected in farmer's stools is high. This could be due to common use of antibiotics on the studied farms and farmers being in contact with farm animals and sharing the same environment with them. Resistant bacteria can be transmitted between humans, cattle and their environment through contact with faeces or via the food chain, including raw milk and contaminated meat [37]. Several studies have highlighted the potential contribution of poor hygienic practices, lack of personal protective equipment and an abundance of bacterial pathogens in the environment to contamination especially with ESBL-*Ec* [19,36–38].

In drinking water collected from farmers' taps our study found prevalence of 37.9 % of ESBL-producing bacteria. The prevalence of ESBL-*Ec* was 7.6% and of ESBL-*K* 33.3%. A study conducted in Kenya found an association between domestic animal presence and ownership and household drinking water contamination, reporting approximately 70% of water samples to be contaminated by enterococci in different peri-urban areas [39]. The prevalence of ESBL-*K* and ESBL-*Ec* in the current study aligns with results from Ethiopia [40], whereas a study in Nigeria reported a prevalence of 7.14% of ESBL-*E* in drinking water sources [41]. The general poor quality of drinking water in Ouagadougou was reported by a study on borehole water in the city, with 59% of water samples being contaminated by coliforms, including *E. coli*, indicating faecal contamination [42]. ESBL-producing *Proteus*, *Klebsiella* spp and *Bacillus* were isolated from the treated water, municipal water and raw water in a study in Nigeria [43,44]. Indeed, the spread of ESBL-*E* via drinking water poses a serious health risk to consumers and can compromise empirical treatment of invasive infections such as urinary tract and bloodstream infections [43].

ESBL-*Ec* and ESBL-*K* were also found in farm soil, with a prevalence of 47% and 19%, respectively. The recent study in Burkina Faso reported a prevalence of 28.0% and 36.0% of ESBL-*Ec* and ESBL-*K* in manure from cattle markets from two livestock markets [45]. A study from Nigeria on ESBL-*Ec* in drainage and washing water in slaughterhouses showed prevalence of 40% and 22%, respectively [46]. The presence of ESBL-*Ec* and/or ESBL-*K* in slaughterhouses can lead to contamination of meat, which is a risk for consumers if good hygiene and proper cooking conditions are not respected. Livestock production wastewater, soil and manure from dairy and beef production can also add AMR genes and bacteria into the environment [47]. Wastewater, humans, pets, domestic animals, and industry have all been identified as potential sources of resistant bacteria in the African environment [48].

The present study also compared the prevalence of ESBL-*Ec* and ESBL-*K* on semi-intensive and traditional farms. The prevalence of ESBL-*Ec* and/or ESBL-*K* was 92% on semi-intensive farm and 85% on traditional farms, not a significant difference between these two types. This is possibly because cattle on semi-intensive farms also graze in the surroundings of the farm during the day. Our figures from the traditional farms are much higher than that reported from Tanzanian traditional cattle farm, where the prevalence was 10% [49]. At the global level, in 2022, the prevalence of AMR varied between 18% and 28%, depending on the farming system [50]. In comparison to these figures, the AMR on cattle farms in Burkina Faso seems very high. We can speculate that here animals that roam free in the environment are exposed to various AMR sources resulting from deficient sanitation and waste management, exposing free-roaming animals to eg. human and abattoir solid waste and wastewaters that contain resistant bacteria and antibiotic residues.

The multidrug resistance rates we detected were high, close to 70% of ESBL-*Ec* and ESBL-*K* isolates were multidrug resistant, with highest resistance rates against tetracycline and cotrimoxazole. Tetracycline and cotrimoxazole antibiotics are relatively cheap and readily available over the counter in LMICs. Tetracycline belongs to one of the most commonly used classes of antimicrobial agents in veterinary medicine due to their broad spectrum of activity [29] and it contributed 63% of the quantity of antibiotics used in 2016, 11.6% in 2017, 31.7% in 2018 and 28.7% in 2020 [7]. Our results showed that 3.1% of the animal faeces and 1.4% of the human stools contained ESBL-*Ec* and ESBL-*K* resistant to all the groups of antibiotics tested, except carbapenems. Carbapenems are used to treat serious human infections and their use is not licensed in livestock or veterinary fields [51,52]. However, One Health approach is needed to prevent dissemination of carbapenem resistance from humans to animals and the environment.

5. Conclusion

This study demonstrated high prevalence of ESBL-*Ec* and ESBL-*K* in cattle faeces, farm soil environment, farmers' stools and their drinking water in the surroundings of Ouagadougou in Burkina Faso. The isolates were resistant to many commonly used antibiotics and a high rate of multidrug resistance was observed. The faecal and environment carriage of ESBL-producing *Enterobacterales* among humans, animals and the environment underscores the need for increased AMR surveillance in veterinary and human medicine to discover the potential sources and the dynamics of transmission. It is also necessary to raise farmers' awareness on the appropriate use of antibiotics, hygienic measures (e.g., disinfection, water quality control, detergent use) and biosafety on farms. A One Health approach as a comprehensive strategy for addressing antibiotic resistance and limiting the spread of multidrug-resistant bacteria across different ecosystems, by sharing data between the human, animal, and environmental health sectors.

Author Contributions: Conceptualization, D.S., I.J.O.B., F.B.J.D K.H.; methodology, I.J.O.B., D.S., F.B.J.D and Z.G.; formal analysis, S.D., writing—original draft preparation, S.D.; writing—review and editing, D.S., I.J.O.B., K.H., F.B.J.D., Z.G., N.S., and N.S.S., E.B., M.E.M.N, supervision, Z.G. and I.J.O.B.; project administration, I.J.O.B.; funding acquisition, I.J.O.B and K.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EU/Erasmus+ funded SEBA-project (Strengthening expertise and bioinformatics to control antimicrobial resistance in West Africa) 619000-EPP-1-2020-1-FI-EPPKA2-CBHE-JP.

Informed Consent Statement: Prior to data collection, each participant was fully informed about the study, and consent was obtained. The purpose of the study was explained to the farmers to secure their consent. Each participant provided written and signed consent. For participants who spoke a local language (mooré), the consent form was translated. Participants were informed of their right to withdraw from the study at any time.

Acknowledgments: We thank the staff of the clinical laboratory of the clinical research unit of Nanoro, for their support in the preparation of culture media. The managers of the Livestock Technical Support Zones and the farmers involved in the study are thanked for their participation and accompaniment in the sites and for facilitating data collection.

Conflicts of Interest: The authors declare no conflicts of interest

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