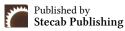


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Research Article

One Health Surveillance of ESBL-Producing Escherichia coli and Klebsiella pneumoniae in Pig Farms in Yaounde, Cameroon

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About Article

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ABSTRACT

A study was conducted to assess the prevalence and antimicrobial resistance pattern of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae in human, animal, and environmental sources of four pig farms in Yaoundé, Cameroon using a One Health approach. There were 338 samples collected from farmers (n = 42), pigs (n = 168), and farms (n = 42) 128) and ESBL Enterobacteriaceae were recovered in 55.3% of the samples with E. coli (49.1%) more prevalent than K. pneumoniae (29.6%). Among humans, the largest proportion of ESBL-producing isolates was encountered (90.5%), followed by pigs (58.3%) and the environment (39.8%). Farm 3 and the short dry season exhibited the highest frequencies of isolation, which suggests the occurrence of farm-level and climatic factors. The antibiotic susceptibility testing showed absolute resistance to third-generation cephalosporins (100%), monobactams (100%), and β-lactam/β-lactamase inhibitor combinations (100%) to confirm the ESBL phenotype. Co-resistance was high, particularly to tetracyclines, sulfonamides, and fluoroquinolones, and to carbapenems and aminoglycosides, the resistance was moderate to low. The prevalence of tetracycline and β-lactam resistance most likely reflects their frequent and poorly controlled use on the farms studied. Farmer self-treatment, poor biosecurity, and environmental contamination were recognized as potential explanations for multidrug resistance. This study provides useful evidence for multidrug-resistant ESBL-producing E. coli and K. pneumoniae that are circulating among humans, animals, and the environment in pig farms, with its major limitation being the absence of molecular characterization, which precludes verification of genetic relatedness and specific ESBL genes accountable. These findings highlight the urgent need for coordinated One Health interventions towards rational application of antibiotics, increased farm sanitation, and continued antimicrobial resistance surveillance towards counteracting public health threats resulting from the zoonotic transmission of resistant bacteria.

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1. INTRODUCTION

Antimicrobial resistance (AMR) has been ranked among the foremost 21st-century global public health challenges. The One Health approach has become crucial in predicting, preventing, and controlling the emergence and spread of resistant microorganisms, recognizing human, animal, and environmental health interrelatedness and demanding strong interdisciplinary collaborations (Atlas et al. 2010). AMR undermines the effectiveness of modern medicine, compromising the control of otherwise straightforward bacterial infections in animals and humans (AbuOun et al. 2020). Its effects are enormous, spanning from prolonged illness, increased mortality, and huge economic costs in animal husbandry (Garcias et al., 2024). 1.27 million deaths directly due to antibiotic-resistant infections were accounted for in 2019 (Murray et al., 2022). To guide international surveillance and control efforts, the World Health Organization (WHO) has developed a list of high-priority antibiotic-resistant bacteria, specifically ESBL-producing Enterobacterales (WHO, 2024). Of these, E coli and K pneumoniae are of particular concern because they frequently cause hospital-acquired infections, neonatal sepsis, urinary tract infections, and diarrheal disease (Ohene Larbi et al., 2022).

Their widespread occurrence is strongly associated with the overuse and abuse of antibiotics in agriculture, which accounts for nearly 73% of all antibiotics sold globally (Peng et al., 2022). A large proportion of these antibiotics get excreted without being metabolized and release active residues into the environment, while also imposing selective pressure on resistance (Barathe et al., 2024). Antimicrobial-resistant bacteria subsequently infect humans either directly through animal contact or indirectly through food chains and environmental pathways (Mouiche et al., 2019). Pig farming is indeed especially reliant on antimicrobials—with an estimated 64.04 mg of antibiotics used per kilogram of pig produced, higher than in cattle or sheep farming (Dohmen et al., 2023; Mouiche et al., 2020).

Most current studies have only considered clinical samples or one reservoir, without concurrently investigating bacterial transmission dynamics in compartments simultaneously. Moreover, research in Yaoundé ever investigating the ESBLproducing E. coli and K prevalence and resistance profiles systematically is scared. This study therefore fills an important knowledge gap by reporting the first integrated One Health assessment of ESBL-producing enteric bacteria from Yaoundé pig farms. It aims to (i) determine their prevalence among pigs, farm workers, and the environment; (ii) describe their antimicrobial resistance profiles; and (iii) determine putative transmission pathways between humans, animals, and environmental reservoirs. These findings are expected to inform the development of targeted surveillance and stewardship interventions against AMR emergence in Cameroon's rapidly expanding pig production sector.

2. LITERATURE REVIEW

Pig production has increased dramatically in Africa, from below one million tons in the year 2000 to over two million tons in 2020 (Adesehinwa *et al.*, 2024). Pork continues to be the most consumed meat globally, contributing about 40% of the diet's

protein intake (Peng *et al.*, 2022). Apart from being a source of nutrients, pig production is a major livelihood enterprise; in Cameroon, it contributes nearly 15% of the GDP of Cameroon (De Angelis *et al.*, 2020). This two-way purpose places the pig industry both as an economic value and as a possible reservoir for the emergence and dissemination of ESBL-producing Enterobacterales.

One Health theory is the conceptual framework of this study. It hypothesizes that human, animal, and environmental health are interlinked in nature and that optimal management of antimicrobial resistance can be obtained through an approach based on systems covering all three spheres simultaneously. Ecoepidemiological resistance model is also shown to suggest that the agricultural environment provides an ecological interface for horizontal gene transfer and cross-species transfer of resistant bacteria and antibiotic resistance genes. Empirical evidence in Asia, Europe, and some parts of Africa has demonstrated high genetic relatedness between ESBL-producing E. coli strains and K. pneumoniae isolated from humans, animals, and the environment, demonstrating the zoonotic potential of these organisms (Robinson et al., 2016; Magiorakos et al., 2012; Matakone et al., 2024; Founou et al., 2019). Nonetheless, studies from sub-Saharan Africa and specifically Cameroon continue to be scarce, fragmented, and primarily set on hospital or clinical isolates, with hardly any investigations of community or farm-level transmission patterns. Placing empirical findings in such a theoretical framework enables the current study to examine how farm management routines, antibiotic use, and environmental contamination reciprocally influence each other to determine the ecology of resistance in an urban agricultural environment with low resources. Despite growing concern for AMR in sub-Saharan Africa, data on pig production systems in Cameroon are very limited, especially those that consider the human-animal-environment interactions within the One Health approach.

3. METHODOLOGY

3.1. Study design and sampling sites

Yaoundé was where the study took place, which is Cameroon's political capital (3°52′N, 11°31′E) (Figure 1). Sampling was conducted in four pig farms of Yaoundé I, Yaoundé III, Yaoundé IV, and Yaoundé VII districts, hereinafter referred to as Farm 1, Farm 2, Farm 3, and Farm 4, respectively.

3.2. Survey and data collection

A standardised questionnaire was used to collect farm- and household-level information. For farmers, the variables were sex, age, education level, source of income, history of use of antibiotics, awareness of antimicrobial resistance (AMR), use of personal protective equipment (PPE), and antibiotic prescription practice (antibiogram-based prescription versus self-medication). For pigs, the variables were use of veterinary services, reason for use of antibiotics (prophylaxis, treatment, or growth promotion), use of antibiograms, treatment approach (individual vs. group), and history of use of antibiotics. For environmental factors, data included farming system (pen or free-range), type of waste disposal, animal type (pure pigs or

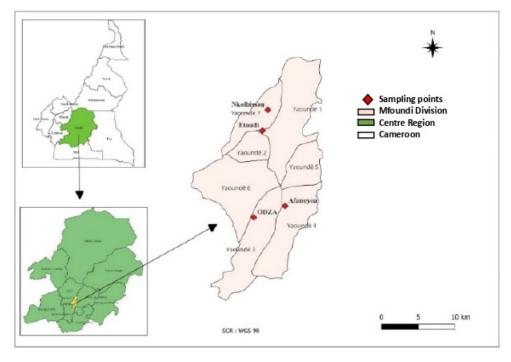


Figure 1: Sampling sites

mixed farming), cleaning frequency, and hygiene. The farm hygiene was categorized as high, medium, or low based on availability of running water, soap, and cleaning frequency.

3.3. Sampling strategy

Sampling was done during the four climatic seasons in Yaoundé (long dry, short rainy, short dry, and long rainy seasons). Two visits were made each season, providing a total of 338 samples from January to December 2024: 168 rectal swabs of pigs, 128 environmental samples (swabbing of feeders, drinkers, and footbaths; wastewater; soil) and 42 stool samples from six farmers. Samples were sent in ice to the National Veterinary Laboratory (LANAVET), Yaoundé, and were processed within 6 hours.

Inclusion and exclusion criteria: Only apparently healthy pigs from home farms where the farmer resided on the same premises were included. Farmers who were under antibiotic treatment at the time of sampling were excluded.

3.4. Bacterial culture and isolation

- Pig samples: Rectal swabs placed in peptone water, vortexed, and 0.1 mL of suspension plated on MacConkey agar supplemented with cefotaxime (MacConkey + CTX) and without supplementation plain MacConkey agar (control). Incubated at 37 $^{\circ}$ C for 24 h.
- Human samples: Suspended 10 mg stool in 90 mL sterile distilled water, mixed and 0.1 mL suspension plated on MacConkey + CTX and control MacConkey agar and incubated at 37 $^{\circ}$ C for 24 h.
- Environmental samples: Swabs were handled as with rectal swabs. Soil samples (10 g) were suspended in 90 mL sterile distilled water, vortex mixed and 0.1 mL inoculated to selective and control media. Water samples were cultured directly. All plates were incubated at 37 $^{\circ}$ C for 24 h.

3.5. Identification of isolates

Colony of normal morphology of *K. pneumoniae* and *E. coli* were grown on nutrient agar and incubated at 37 °C for 24 h. Biochemical characterization was performed on Kliger Iron Agar, Simmons Citrate, Urea-Indole medium, and Eosin Methylene Blue (EMB) agar for *E. coli*, and Hektoen agar for *K. pneumoniae*. The isolates were confirmed and stored in Brain Heart Infusion (BHI) broth containing 20% glycerol at -4 °C.

3.6. Phenotypic ESBL production detection

ESBL production was identified using the double-disc synergy test (DDST) as proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Robinson et al., 2016). Glycerol stock colonies were transferred to nutrient agar and incubated at 37 °C for 24 h. Fresh colonies were resuspended in sterile distilled water to a turbidity of 0.5 McFarland standard. Inoculum was spread on Mueller-Hinton agar and at the center, a disc of amoxicillin-clavulanic acid (AMC, 20/10 µg) was placed with discs of cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), cefixime (CFM, 5 μg), and aztreonam (ATM, 30 μg) around it (20-30 mm between centers). Plates were incubated for 18-24 h at 37 °C. Colonies with larger inhibition zone (champagne cork-shaped or keyhole effect) toward AMC were taken as ESBL producers. The presumptive E. coli and K. pneumoniae isolates showing an ESBL production phenotype were identified using API 20E.

3.7. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion technique. Sixteen antibiotics were screened against $E.\ coli$ and fifteen against $K.\ pneumoniae$, a naturally ampicillin-resistant organism. Colonies were reconstituted on nutrient agar at -4 °C to maintain fresh isolates. After 24 h of incubation, an inoculum was prepared of each

isolate to obtain a turbidity of 0.5 McFarland. The antibiotics employed were: amoxicillin–clavulanic acid (20/10 μg), ampicillin (10 μg), cefixime (5 μg), cefotaxime (5 μg), cefepime (30 μg), ceftazidime (10 μg), aztreonam (30 μg), piperacillin–tazobactam (30/6 μg), cefoxitin (30 μg), imipenem (10 μg), amikacin (30 μg), chloramphenicol (30 μg), sulfamethoxazole–trimethoprim (1.25/23.75 μg), gentamicin (10 μg), ciprofloxacin (5 μg), and tetracycline (30 μg). Plates were incubated at 37 °C for 24 h, and diameters of the inhibition zones were measured. Results were read according to the EUCAST guidelines.

3.8. Statistical analysis

Descriptive statistical analyses were conducted using Microsoft Excel. Descriptive statistics were created with Microsoft Excel, and quantitative analyses were done in R (v4.5.1) through RStudio (2024.09). The changes in isolate abundance across farms and seasons, and the relationships between farm management or environmental factors and ESBL carriage were evaluated by the chi-square test.

3.9. Ethical considerations

Ethical clearance was obtained from the Centre Region Ethics Committee (0125-/CRERSHC/2024) and authorization by the Ministry of Livestock, Fisheries and Animal Industries (101/L/DREPIA-CE/DD-MFDI). Written consent was obtained from all participants after explaining the aim and research procedure clearly.

4. RESULTS AND DISCUSSION

During the study, 338 samples were examined, with 42 human, 168 pig, and 128 environmental samples (Table 1). *Enterobacteriaceae* were detected in 55.3% of total samples, of which most were *E. coli* (49.1%) as opposed to *K. pneumoniae* (29.6%). The highest prevalence of *Enterobacteriaceae* was in the human samples (90.5%), followed by pigs (58.3%) and the environment (39.8%). The same trend was noted for *E. coli* and *K. pneumoniae*, with the former being highest among humans (76.1%) and lowest in environmental (36.7%), and the latter highest among humans (69%) and lowest in the environment (23.4%).

Table 1. Prevalence of Enterobacteriaceae, ESBL-producing E. coli, and K. pneumoniae from human, animal, and environmental samples

	N	Enterobacteriaceae	E. coli	K. pneumoniae
Men	42	38 (90.5%)	32 (76.1%)	29 (69%)
Pig	168	98 (58.3%)	87 (51.8%)	41 (24.4%)
Environment	128	51 (39.8%)	47 (36.7%)	30 (23.4%)
Total	338	187 (55.3%)	166 (49.1%)	100 (29.6%)

N = Number of samples

The incidence of ESBL-producing isolates varied from farm to farm and season to season. The most frequently isolated species was *E. coli*, with the greatest incidence on Farm 3 (59 isolates), followed by Farm 2 (46 isolates) (Table 2). *K. pneumoniae* was also most frequently found on Farm 3 (39 isolates). There were differences seasonally, with both *E. coli*

and *K. pneumoniae* with increased isolation rates during the short dry season (SDS; 60 and 30 isolates, respectively). Farm 3 and short dry season recorded the highest total number of isolates (98 and 90, respectively), and farm-specific and seasonal variables may have an impact on the detection of ESBL-producing bacteria.

Table 2. Distribution of ESBL-producing *E. coli* and *K. pneumoniae* isolates by farm and season

ESBL strains	Farm				Season			
	Farm 1	Farm 2	Farm 3	Farm 4	LRD	SRS	SDS	LDS
E. coli	35	46	59	26	40	39	60	27
K. pneumoniae	28	19	39	14	27	29	30	14
Total	63	65	98	40	67	68	90	41

LDS: Long dry season; SRS: Short rainy season; SDR: Short dry season; LDS: Long rainy season

4.1. Socio-demographic characteristics of farmers and prevalence of ESBL- producing *Escherichia coli* and *Klebsiella pneumoniae* carriage

Of the survey participants, the majority were men (83.3%), aged mainly between 20 and 50 years, and with the secondary education level being the most prevalent (50%) (Table 3). Men had higher carriage rates of ESBL-producing *E. coli* and *K. pneumoniae* (57.1% and 54.8%, respectively) than women (1% and 14.3%). The age group 20–30 years had the greatest carriage of *E. coli* ESBL

(35.7%), while comparatively high carriage was observed for both $E.\ coli\ (19\%)$ and $K.\ pneumoniae\ (23.8\%)$ in the 40–50 years age group. Secondary education and primarily occupation in farming showed the highest prevalence of ESBL carriage.

Low awareness of AMR (only 16.7% reported awareness) and increased carriage levels for *E. coli* (59.5%) and *K. pneumoniae* (66.7%) for those not aware of AMR were noted. Notably, participants' self-medication was high (100%), and all self-medicated cases harboured ESBL-producers, suggesting that



misuse of antibiotics could be the primary factor driving ESBL dissemination among this group.

Table 3. Association between socio-demographic characteristics and ESBL-producing *E. coli* and *K. pneumoniae* carriage among human participants

Variables	Sub-variables	Frequency (n)	Carriage of ESBL E. coli	Carriage of ESBL K. pneumonia
Sex	Men	83.3% (5)	57.1% (24)	54.8% (23)
	Women	16.7% (1)	1% (8)	14.3% (6)
p-value			0.03	0.12
	[20-30]	33.3% (2)	35.7% (15)	28.6% (12)
A	[30-40]	16.7% (1)	16.7% (7)	2.4% (1)
Age group	[40-50]	33.3% (2)	19% (8)	23.8% (10)
	[50-60]	16.7% (1)	19% (8)	14.3% (6)
p-value			0.12	0.20
	Primary	33.3% (2)	28.6% (12)	23.8% (10)
Level of study	Secondary	3 (50%)	30.9% (13)	42.9% (18)
	University	16.7% (1)	16.7% (7)	2.4% (1)
p-value			0.05	0.07
C C:	Farm	66.7% (4)	47.6% (20)	45.2% (19)
Source of incomes	Other	33.3% (2)	28.6% (12)	23.8% (10)
p-value			0.15	0.10
IZ 1.1. AMD	Yes	16.7% (1)	16.7% (7)	2.4% (1)
Knowledge on AMR	No	83.3% (5)	59.5% (25)	66.7% (28)
p-value			0.02	0.01
Self-medication	Yes	100% (6)	76.2% (32)	100% (29)
	No	0	0	0

Bold text indicates $p \le 0.05$

4.2. Evaluation of some Farming Practices and Prevalence of ESBL-Producing *Escherichia coli* and *Klebsiella pneumoniae* in the Environment

All the pig farms surveyed in this study practiced enclosed animal rearing and reported the use of veterinary services (Table 4). The farming systems were either exclusive (pigs alone) or mixed (other animals raised together with pigs), 50% of the mixed farms reported a higher carriage rate of ESBL-producing *E. coli* (65.9%) and *K. pneumoniae* (36.2%) compared to pure pig farms (34% and 27.7%, respectively). The above result suggests that mixed farming systems may enhance transmission of bacteria and dissemination of antimicrobial resistance between species.

All of the farms had indicated the use of antibiotics, with oxytetracycline the only antimicrobial that was being given (100%), used almost exclusively for treatment, although prophylactic use was indicated on some (34% *E. coli*; 17% *K. pneumoniae*). No farms indicated use of antibiotics for growth promotion. Despite regular use of veterinary services, use of antibiotics appeared to be largely unregulated.

Biosecurity standards were poor across all farms. No farmer

used personal protective equipment (PPE) and there were low levels of hygiene across all, none approaching medium or high hygiene standards. Liquid waste control was poor—75% of farms discharged wastewater straight into the environment, with only one farm (25%) having a basic system for drainage and treatment. Solid waste was often used as fertilizer.

Environmental contamination was rampant: all the environmental locations sampled within the farms harbored ESBL-producing $E.\ coli$ and $K.\ pneumoniae$. Most heavily contaminated were feeders (34% $E.\ coli$; 21.3% $K.\ pneumoniae$) and drinkers (21.3%; 8.5%), followed by soil, wastewater, and footbaths, indicating that these niches may be reservoirs of resistant bacteria within the environment. A total of 128 environmental samples were collected with an ESBL prevalence of 39.8% (n = 51).

4.3. Antibiotic Susceptibility Testing of Multidrug-Resistant ESBL-Producing *E. coli* and *K. pneumoniae*

producing *E. coli* and *K. pneumoniae* in pig farms A few isolates were ESBL production phenotype with β -lactams antibiotics resistance only and some isolates were infrequently

Table 4. Farm management and environmental determinants with carriage of ESBL-producing *E. coli* and *K. pneumoniae* in pig farms

Variables	Sub-variables	Frequency (n)	Carriage of ESBL E. coli	Carriage of ESBL K. pneumoniae
D 11	Exclusive	50% (2)	34% (16)	27.7% (13)
Breeding type	Mixed	50% (2)	65.9% (31)	36.2% (17)
p-value			0.04	0.20
	Wastewater	75% (3)	14.9% (7)	12.8% (6)
	Soil	100% (4)	19.1% (9)	17% (8)
Environmental reservoirs	Feeder	100% (4)	34% (16)	21.3% (10)
reservoirs	Water	100% (4)	21.3% (10)	4(8.5%)
	Footbath	100% (4)	10.6% (5)	4.3% (2)
p-value		_	0.15	0.25
PPE	Yes	0%	0%	0%
	No	100% (4)	100 % (47)	100% (30)
Antibiotic used	Oxytetracycline	100% (4)	100 % (47)	100% (30)
	Other	0%	0%	0%
	Therapeutic	100% (4)	100 % (47)	100% (30)
Reason for antibiotic administration	Prophylactic	50% (2)	34.04% (16)	17.02% (8)
	Growth promotion	0%	0%	0%
p-value			0.06	0.09
	Low	100% (4)	100 % (47)	100% (30)
Hygiene level	Middle	0%	0%	0%
	High	0%	0%	0%

Bold text indicates $p \le 0.05$

resistant to two groups of antibiotics. Their antibiotic susceptibility was not recorded. We recorded the antibiogram of multidrug-resistant isolates only.

4.3.1. Case of Multidrug-Resistant ESBL-producing *E. coli* ESBL-producing *E. coli* isolate resistance patterns revealed high-level multidrug resistance in all three reservoirs humans, pigs, and the environment showing high-level dissemination of β -lactam resistance genes in the ecosystem (Table 5). All (100%) isolates were resistant to third-generation cephalosporins (ceftazidime, cefixime, cefotaxime), monobactams (aztreonam), and amoxicillin–clavulanic acid, confirming their ESBL phenotype. Ampicillin resistance was very high overall (81.5%), with frequencies being comparable in human and pig isolates (85.7% each), and slightly lower in environmental isolates (66.7%). Resistance to fourth-generation

cephalosporin (cefepime) was moderate (49.4%), but lower in the environmental reservoir (22.2%) than in pigs and humans (57.1% each). Carbapenem resistance (imipenem) was moderate (22.2%), but its presence in all the reservoirs is concerning since it suggests potential emergence of carbapenemaseproducing E. coli piperacillin-tazobactam resistance was low (overall 33.3%), indicating partial regain of β-lactam efficacy in some isolates. Among non- β -lactams, high resistance rates were recorded for sulfamethoxazole-trimethoprim (65.4%), tetracycline (46.9%), and ciprofloxacin (46.9%), displaying coresistance patterns that further limit therapy. Aminoglycoside resistance remained low (gentamicin 14.8%, amikacin 18.5%), which means that such drugs still retain some therapeutic utility. In general, pig isolates had the most frequent resistance to all but two antibiotic classes, then came environmental and human isolates.

Table 5. Antibiotic resistance patterns among ESBL-producing *E. coli* strains from human, pig, and environmental reservoirs

Classe	Antibiotic	% Resistance (n)				
Classe	Antibiotic	Men	Pig	Environment	Total	
Pénicilline	Ampicilline	85.7% (18)	85.7% (36)	66.7% (12)	81.2% (64)	
C2G	Cefoxitine	38.1% (8)	40.5% (17)	27.8% (5)	37% (30)	
C3G	Ceftazidime	100% (21)	100% (42)	100% (18)	_	
	Cefixime	100% (21)	100% (42)	100% (18)	100% (81)	
	Cefotaxime	100% (21)	100% (42)	100% (18)		
C4G	Cefepime	57.1% (12)	57.1% (24)	22.2% (4)	49.4% (40)	
Monobactam	Aztreonam	100% (21)	100% (42)	100% (18)	100% (81)	
Penicilline + β -lactamases inhibitor	Amoxicilline + clavulanic acid	100% (21)	100% (42)	100% (18)	100% (81)	
	Piperacilline + tazobactam	38.09% (8)	38.9% (16)	16.66% (3)	33.3% (27)	
Carbapénème	Imipenème	19.04% (4)	28.57% (12)	11.11% (2)	22.22% (18)	
Quinolones	Ciprofloxacine	38.09% (8)	57.14% (24)	33.33% (6)	46.91% (38)	
Sulfamides	Sulfametoxazole + trimethoprime	61.90% (13)	80.95% (34)	33.33% (6)	65.43% (53)	
Aminosides	Gentamicine	19.04% (4)	9.52 % (4)	22.22% (4)	14.81% (12)	
	Amikacine	23.80% (5)	14.28% (6)	22.22% (4)	18.51% (15)	
phenicoles	Chloramphénicol	16.66% (6)	33.33% (14)	16.66% (3)	37.17% (29)	
Cyclines	Tétracycline	28.57% (5)	61.90% (26)	38.88% (7)	46.91% (38)	

4.3.2. Case of multidrug-resistant ESBL-producing K. pneumoniae

There was a generalized trend towards multidrug resistance in ESBL-producing K. pneumoniae isolates from all three reservoirs. There was a uniform 100% third-generation cephalosporin (ceftazidime, cefixime, cefotaxime) resistance, aztreonam resistance, and amoxicillin–clavulanic acid resistance, as evidenced by the ESBL phenotype and pervasive β -lactam resistance. Fourth-generation cephalosporin (cefepime) resistance was moderate, ranging from 36.4% in humans to 57.1% in pigs. Carbapenem resistance (imipenem) was relatively low in general (11.5%), present only in human

and pig isolates, and not at all in the environment, suggesting little spreading of carbapenemase-producing strains. Resistance non- β -lactam was also high: sulfamethoxazole–trimethoprim (67.3%) and tetracycline (63.5%) showed high frequencies and possible co-resistance to commonly used drugs in both hospital and farm settings. Aminoglycoside resistance was variable, being highest among pig isolates (61.9% gentamicin, 28.6% amikacin) compared to human and environmental isolates, consistent with selective pressure due to veterinary antibiotic usage. Resistance to quinolones was moderate (26.9%), whereas chloramphenicol resistance was approximately 30.7% in total.

Table 6. Antibiotic resistance patterns of ESBL-producing *K. pneumoniae* isolates between the three reservoirs.

Classe	Antibiotic	% Resistance	% Resistance					
		Men	Pig	Environment	Total			
C2G	Cefoxitine	27.3% (6)	33.3% (7)	33.3% (3)	30.8% (16)			
C3G	Ceftazidime	100% (22)	100% (21)	100% (9)	100% (52)			
	Cefixime	100% (22)	100% (21)	100% (9)	100% (52)			
	Cefotaxime	100% (22)	100% (21)	100% (9)	100% (52)			
C4G	Cefepime	36.4% (8)	57.1% (12)	44.4% (4)	48.7% (38)			
Monobactam	Aztreonam	100% (22)	100% (21)	100% (9)	100% (52)			



Pénicilline + β-lactamases inhibitor	Amoxicilline + clavulanic acid	100% (22)	100% (21)	100% (9)	100% (52)
	Piperacilline + tazobactam	22.7% (5)	52.4% (11)	22.2% (3)	36.5% (6)
Carbapénème	Imipenème	9.1% (2)	19% (4)	0% (0)	11.5% (18)
Quinolones	Ciprofloxacine	22.7% (5)	28.6% (6)	33.3% (3)	26.9% (14)
Sulfamides	Sulfametoxazole + trimethoprime	72.7% (16)	76.6% (16)	33.3% (3)	67.3% (35)
Aminosides	Gentamicine	27.3% (6)	61.9% (13)	22.2% (2)	40.4% (21)
	Amikacine	13.6% (3)	28.6% (6)	0% (0)	17.3% (9)
phenicoles	Chloramphénicol	36.36% (8)	28.6% (6)	22.2% (2)	30.7% (16)
Cyclines	Tétracycline	59.09% (13)	71.4% (15)	55.5% (5)	63.5% (33)

4.4. Discussion

This study conducted a One Health-derived surveillance of extended-spectrum β-lactamase (ESBL)-producing E. coli and K. pneumoniae in pig farms, farmers, and their surroundings in Yaoundé. AMR is the essence of the One Health concept because AMR links human, animal, and environmental health into one continuum (Robinson et al., 2016). In Cameroon, increased demand for pork particularly in the Centre region, where Yaoundé is located has initiated a rapid expansion in pig farming, which has become an important livelihood activity. Indeed, 66.7% of the interviewed farmers relied primarily on pig farming as their main source of income, confirming earlier studies that pig production is an important economic activity for the majority of sub-Saharan African households (Adesehinwa et al., 2024). However, pig production systems have proven to be most dependent on antibiotics, much more so than goat or cattle farming (Mouiche et al., 2020). Misuse and unwarranted use of antibiotics in animal husbandry have been reported to be a major reason for the emergence and transmission of resistant bacteria, especially ESBL-producing Enterobacterales (Magiorakos et al., 2012). Our findings showed a global prevalence of 90.5% in humans, 54.2% in pigs, and 39.8% in the environment, that suggest possibly vast bacterial transmission among compartments. These estimates were largely different and indicate heterogeneity in ESBL distribution among reservoirs. High prevalence among pigs and humans may be indicative of direct and frequent exposure to antibiotic therapy, where there is strong selective pressure towards acquisition and maintenance of resistance genes. Alternatively, the environment is only indirectly exposed, chiefly through effluent and animal faeces, and thus has relatively lower selective pressure. These findings are in line with earlier work suggesting evidence for a causal relationship between the use of antibiotics and the emergence of antibiotic-resistant bacteria (Klein et al., 2024). Our results are in accordance with previous studies in Yaounde which reported prevalences around 70% in humans and pigs (Matakone et al., 2024; Founou et al., 2019). The higher prevalence in our human population is potentially due to occupational exposure and the absence of proper biosecurity, facilitating zoonotic transmission. Environmental prevalence in the current study (39.8%) was higher than some reported in

sub-Saharan Africa (24.8%) (Zaatout et al., 2021; Berendonk et al., 2015). This difference could be explained by local factors such as insanitary conditions, regular discharge of raw sewage, and use of animal dung as manure. It is also to be noted that the environment is primarily a transit and mixing reservoir and not a permanent niche for ESBL-producing bacteria. Natural dilution processes in water and soil, combined with a changing state of temperature, nutrients, and pH, can limit bacterial survival and detection by routine culture methods. Moreover, bacteria under such low-selective-pressure conditions can lose plasmids carrying resistance determinants to avoid a fitness cost, explaining the relatively lower occurrence rates in environmental samples (Carroll et al., 2018).

In comparison with ESBL-producing bacterial species, we observed higher counts of ESBL-producing E. coli (n=81) than K. pneumoniae (n=52). This in in contrast to some hospital surveys, where K. pneumoniae predominates among the β -lactamase producers (Kazemian et al., 2019), the predominance of E. coli in the current study may be due to it being a ubiquitous gut commensal of humans and animals, in contrast to K. pneumoniae, which is opportunity-oriented and tends to be more prevalent in a clinical setting.

The high levels of contamination that were witnessed are consistent with the general unsatisfactory sanitation practices of the farms, the non-existence of personal protective equipment use, and the unsatisfactory waste management systems. The nonexistence of systematic biosecurity and antibiotic stewardship is expected to facilitate bacterial transmission within and between farms. Similar results of the correlation between poor sanitation, uncontrolled use of antibiotics, and the transmission of resistant bacteria have been reported by many authors (Traoré *et al.*, 2024; Alarcón *et al.*, 2021; Scollo *et al.*, 2023).

Antibiotic susceptibility tests disclosed ESBL-producing isolates as having strong resistance to third-generation cephalosporins, β -lactams, and tetracyclines, moderate resistance to carbapenems and non- β -lactam classes such as quinolones and sulfonamides. These patterns are suggestive of the most frequently applied antibiotics in surveyed farms particularly β -lactams and tetracycline analogs therefore substantiating the notion that bacterial resistance is likely to mimic antibiotic

exposure (Bedekelabou *et al.*, 2022; Leangapichart *et al.*, 2021; Tebug *et al.*, 2021). Excessive use and absence of regulation in the acquisition of antibiotics by farmers are likely to exacerbate such resistance patterns (Bennadi, 2013).

Overall, the study emphasizes interconnectedness of humans, animals, and the environment in the dissemination of ESBL-producing Enterobacterales in Yaoundé. The ubiquitous occurrence of multidrug resistance in compartments is suggestive of inadequate antibiotic stewardship, inadequate biosecurity, and environmental routes of contamination. Addressing these challenges requires concerted One Health interventions that are directed towards promoting rational use of antibiotics, farm hygiene, intensified surveillance systems, and public education on the dangers of AMR.

5. CONCLUSION

Throughout this study. we conducted an integrated surveillance of extended-spectrum β -lactamase-producing $\it E.~coli$ and $\it K.~pneumoniae$ in certain pig farms in the city of Yaounde. following the "One Health" concept. We found that these β -lactamase-producing $\it Enterobacteriaceae$ pose a significant risk to the development of pig farming and public health. The similarity in resistance profiles suggests a circulation of resistant bacteria between farmers. pigs. and the farm environment. This is exacerbated by shortcomings such as inadequate facilities and practices like self-medication and group therapy for animals. A genomic study including an epidemiological map of resistance genes and a phylodynamic analysis of the bacteria would be highly beneficial to better understand the spread of AMR in pig farms.

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