

Salmonella enterica and *Escherichia coli* Cohabitation among Factors Increasing Antibiotic Resistance in Bukavu City, Democratic Republic of the Congo

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Abstract

Food- and water-borne diseases exacerbate cases of antimicrobial resistance (AMR), particularly in low- and middle-income countries. Since 2011, cases of enteric infections have been reported in Bukavu city, Democratic Republic of the Congo. The objectives of this study were to evaluate the rate of AMR and multidrug resistance (MDR) of *Salmonella enterica* and *Escherichia coli*, and to determine the effect of *S. enterica* and *E. coli* cohabitation on antibiotic resistance of *S. enterica*. Bacteria were isolated from 553 foods, milk, and water samples collected from restaurants, taps, tanks and wells in Bukavu. Microbial analyses involved bacterial culture, and morphological and biochemical characterization. Antibiotic susceptibility tests were performed before and after bacteria cohabitation of *S. enterica* and *E. coli* isolates in the same media. 152 (27.5%) and 27 (4.9%) of the samples tested positive for *S. enterica* and *E. coli*, respectively. *Salmonella* isolates were more susceptible to ciprofloxacin (75.7%) and co-trimoxazole (75.0%) and more resistant to ampicillin (82.2%). *E. coli* was more resistant to ciprofloxacin (59.3%). Overall, 90.5% of isolates (n = 179) were MDR. The origin (food, water) of *S. enterica* and *E. coli* isolates had no significant (p > 0.05) influence on their susceptibility to antibiotics. However, *S. enterica* isolates from milk were significantly (p = 0.00) antibiotic-resistant than those from food and water. The cohabitation between antibiotic-susceptible *S. enterica* and antibiotic-resistant *E. coli* significantly (p < 0.00) increased the rate of antibiotic resistance of *S. enterica* from 30% to 89.5%, implying that interactions of antibiotic-resistant and antibiotic-susceptible

bacteria in food and water could be among neglected factors promoting the spread of AMR, leading to increase AMR cases in Bukavu. Strong sanitation strategies and the operationalization of One Health approach could mitigate the spread of AMR in Bukavu city, DR Congo.

Keywords

Antimicrobial Resistance, Bacteria Cohabitation, *Salmonella*, *Escherichia*, One Health

1. Introduction

The triple planetary crisis consisting of climate change, loss of nature and biodiversity, pollution and waste increases the occurrence of antimicrobial resistance (AMR) cases. There is therefore a need for international commitment to curb the silent pandemic of AMR to protect the health of humans, animals and ecosystems. So far, environmental dimensions of AMR have been neglected, creating a gap in holistically understanding AMR issues [1]-[3]. From their niches, *Salmonella enterica* and *Escherichia coli* spread to other environments such as from food and water to humans and animals and vice-versa causing enteric infections which are difficult to treat, representing a challenge in low- and middle-income countries [4]-[6].

In the Democratic Republic of the Congo (DRC), *S. enterica* remains the most studied bacterium due to its multidrug resistance (MDR) caused by the presence of beta-lactamase enzyme or specific antibiotic resistance genes in *Salmonella* serotypes [7]-[9]. In Bukavu city, a study reported that none of the tested antibiotics had 100% inhibition activity face *Salmonella* strains isolated from blood [10], and 20.4% of *E. coli* strains causing bloodstream infection were MDR, and *S. enterica* produced the extended-spectrum β -lactamase and resisted to ampicillin, cefepime, erythromycin and co-trimoxazole [11]. From hemoculture of 150 newborns in Bukavu city, more than 85% of Enterobacteriaceae species were resistant to antibiotics [12]. Additionally, 68.7% of *E. coli* were MDR and positively correlated with high consumption of meat and milk [13].

Most of the antimicrobial resistance (AMR) studies conducted in DRC and Bukavu city have been focused on human samples and involved collecting data at hospital level. Studies focusing on AMR from environmental samples such as food, water, milk, air and soil remain rare. There is therefore a need to investigate environmental samples because the AMR bacteria and antibiotic resistance genes (ARGs) are mostly found in the environment, sewage and tap water [14]-[16]. Secondly, there have been limited studies focusing on the cause of AMR due to bacteria cohabitation and the possibility of horizontal or vertical transfer of ARGs between and among bacteria sharing the same environment [17] in DRC. The objectives of this study were therefore to determine the rate of AMR and MDR among *S. enterica* and *E. coli* isolates in Bukavu city; evaluate the MDR distribution

in food, water and milk samples; and determine the effect of *Salmonella* and *Escherichia* cohabitation on antibiotic resistance of *S. enterica*.

2. Materials and Methods

2.1. Description of the Study Area

Food, milk and water samples were collected in Bukavu city, Democratic Republic of the Congo located at 2°29' - 2°33' South and 28°48' - 28°52' East. The city is dominated by mountains with altitude range from 1463 to 2200 m and covers an area of approximately 60 km² with an estimated population of 1.5 million [18].

2.2. Sample Collection Approach

Prior sampling, the restaurants were grouped across the three municipality (Bagira, Ibanda, Kadutu) of Bukavu city, then 408 restaurants were identified among which 82 in Bagira, 187 in Ibanda and 139 in Kadutu. The water samples were collected from wells, taps and water-system network consisting of 8 water tanks of Bukavu city. The milk samples were taken from 3 dairy farms in the hinterland of Bukavu city. A systematic random sampling method was applied to identify restaurants and wells, and the first selected was determined by a lottery method to reach at least 30% [19] of the identified restaurants, wells and tanks proportionally across the 3 municipalities. A total of 415 foods, 34 milk and 104 water samples were collected. Approximately, 100 g of food samples were usually purchased from restaurants; 500 mL of milk from dairy farms and 500 mL of water sampled from taps, tanks, and wells, then deposited into sterile Erlen-Meyer flask and transported in a cooler box within eight hours post-sampling to the Microbiology and Biotechnology Laboratory of the “Université Officielle de Bukavu” for analysis.

2.3. Isolation and Identification of *S. enterica* and *E. coli* from Samples

25 g of food or 25 mL of milk/water sub-samples were aseptically weighed and deposited in 225 mL of peptone water (PW) (TM Media, India), vigorously agitated and incubated at 37°C for 24 hours. Subsequently, 1 mL of positive culture was aseptically streaked into Hektoen Enteric agar (HEA) (TM Media, India) and incubated at 37°C for 24 hours. Suspected colonies of *Salmonella* from HEA were streaked on Salmonella-Shigella agar (SS) (TM MEDIA, India) and incubated at 37°C for 24 hours. For *Escherichia*, 1 mL of positive culture from PW was streaked on Eosine Methyl Blue (EMB) agar and incubated at 37°C for 24 hours. Suspected colonies of *Escherichia* from EMB were then streaked on Mac-Conkey agar (MCA) and incubated at 44°C for 24 hours [20]. From SS-agar or MCA, 2 - 3 colonies were streaked on nutrient agar (Oxoid, England) for purification and incubated at 37°C for 24 hours. Finally, purified colonies of *Salmonella* or *Escherichia* from nutrient agar underwent morphological and biochemical characterization based on the result of gram staining, citrate, catalase, indole, urease, glucose,

lactose, H₂S production, and motility tests [21].

2.4. Antibiotic Susceptibility Testing

Susceptibility of *S. enterica* and *E. coli* isolates was tested against the antibiotics listed in **Table 1** by Kirby-Bauer's disk diffusion agar method on Mueller-Hinton agar [14] [22]. The antibiotic susceptibility was evaluated according to the guideline of European Committee on Antimicrobial Susceptibility Testing [23]. The multidrug resistance was determined at two levels. Firstly, against all tested antibiotics; and secondly, focusing on three antibiotics (co-trimoxazole, ampicillin and chloramphenicol) used as first-line treatment against *Salmonella* infections [24] [25].

Table 1. Theoretical inhibition diameter (TID) in millimeter (mm) corresponding to each tested antibiotic.

Characteristic	*CFT	CIP	AZM	DO	CTX	AK	COT	AX	NAC	C
*TID (mm)	25	25	21	17	20	18	14	14	14	17
Concentration of antibiotic (µg)	30	30	15	30	30	30	25	10	30	50

*Theoretical inhibition diameter (TID) means the inhibition diameter from which an isolate is considered as susceptible to the tested antibiotic. Susceptible isolate means inhibition diameter (ID) measured \geq TID; Resistant isolate means ID measured $<$ TID. CFT: ceftriaxone, CIP: ciprofloxacin, AZM: azithromycin, DO: doxycycline hydrochloride, CTX: cefotaxime, AK: amikacin, COT: co-trimoxazole, AX: ampicillin, NAC: nalidixic acid, C: chloramphenicol.

2.5. Evaluation of the Effect of Bacteria Cohabitation on the Antibiotic Resistance of *S. enterica*

The effect of bacteria cohabitation on antibiotic resistance profile of *S. enterica* was performed according to Faure *et al.* [17] with modification. *S. enterica* and *E. coli* having undergone the first antibiotic susceptibility test before cohabitation (AST1) were separately cultured in peptone water at 37°C for 24 hours. Subsequently, 3 mL of antibiotic-susceptible *S. enterica* and 3 mL of antibiotic-resistant *E. coli* were mixed aseptically in the same tube and incubated at 37°C for 10 days. Next, 1 mL of the growth mixture was then streaked on SS-agar and EMB-agar and incubated at 37°C for 18 hours. Finally, antibiotic susceptibility test was reformed on the isolated bacteria after cohabitation (AST2). The inhibition diameters of antibiotics of AST2 were compared to those of AST1 to determine antibiotic resistance of *S. enterica* before and after cohabitation.

2.6. Data Analysis

The data were analyzed on RStudio 4.4.0 [26]. Analysis of variance was applied to compare the inhibitory activity between the 10 tested antibiotics. Student t test was used to compare the rate of antibiotic resistance before and after cohabitation of the isolates, and the inhibition diameter of each antibiotic to its theoretical inhibition

diameter. The chi-square test was used to assess the independence of the distribution of antibiotic resistance associated with the origin (food, milk, water) of isolates. The least significant difference test was performed to rank the level of the inhibition activity for each antibiotic in the context of the cohabitation effect on isolates sharing the same media. Mean differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Occurrence of *Salmonella enterica* and *Escherichia coli* in Food, Milk and Water

A total of 152 *S. enterica* and 27 *E. coli* were isolated from 553 foods, milk and water samples. Out of 415 foods samples, 29.4% were positive for *S. enterica* and 3.4% for *E. coli*. Among 104 water samples, 23.1% were positive for *S. enterica* and 12.5% for *E. coli*. 17.6% of the 34 milk samples were positive for *S. enterica* while none was positive for *E. coli* (Table 2).

Table 2. Occurrence of *S. enterica* and *E. coli* in food, milk and water samples.

Sample	<i>S. enterica</i>		<i>E. coli</i>	
	n	%	n	%
Foods (n = 415)	122	29.4	14	3.4
Water (n = 104)	24	23.1	13	12.5
Milk (n = 34)	6	17.6	0	0
Total (n = 553)	152	27.5	27	4.9

3.2. In Vitro Antimicrobial Resistance Rate in Food, Milk and Water

The rate of antibiotic resistance of *S. enterica* isolates was more than 25% (n = 152) against ceftriaxone, azithromycin, doxycycline, cefotaxime, amikacin, nalidixic acid, ampicillin and chloramphenicol. *E. coli* isolates resisted to more than 29% (n = 27) of all tested antibiotics. The highest antibiotic resistance of *S. enterica* was to ampicillin (82.2%), while *E. coli* was most resistant to ciprofloxacin (59.3%). Among the *S. enterica* isolates, 75.7% were susceptible to ciprofloxacin and 75% to co-trimoxazole. 70.4% of *E. coli* isolates were susceptible to ampicillin. The inhibition activity of antibiotics was significantly different ($p < 0.00$), suggesting that each tested antibiotic has presented a different level of inhibitory activity on the isolates. The rate of antibiotic resistance of *S. enterica* was not significantly different from *E. coli* against the tested antibiotics ($p = 0.23$) (Table 3; Figure 1). On average, 54.9% (n = 122), 57.5% (n = 24) and 75% (n = 6) of *S. enterica* from food, water and milk, respectively were antibiotic-resistant. On the other hand, 47.9% (n = 14) and 45.4% (n = 13) of *E. coli* from food and water, respectively were antibiotic-resistant. There was no significant difference ($p = 0.79$) in the rate of antibiotic resistance of *S. enterica* isolates based on their origin (food and water); as well as *E. coli* in food and water ($p = 0.68$) (Table 4). However, the average inhibition diameter of antibiotics on *S. enterica* isolates from

food and water was significantly different to those from milk ($p = 0.00$), suggesting that *S. enterica* from milk displayed higher resistance than those from food and water.

Table 3. Antimicrobial resistance rate of *S. enterica* and *E. coli* isolates against 10 commonly used antibiotics in Bukavu city, DR Congo.

Antibiotics	<i>S. enterica</i>		<i>E. coli</i>		Sum of AMR of all isolates		p-value (Anova)
	n = 152	%	n = 27	%	n = 179	%	
Ceftriaxone	74	48.7	14	51.9	88	49.2	<0.00
Ciprofloxacin	37	24.3	16	59.3	53	29.6	
Azithromycin	105	69.1	15	55.6	120	67.0	
Doxycycline	118	77.6	15	55.6	133	74.3	
Cefotaxime	108	71.1	13	48.1	121	67.6	
Amikacin	62	40.8	13	48.1	75	41.9	
Co-Trimoxazole	38	25.0	9	33.3	47	26.3	
Ampicillin	125	82.2	8	29.6	133	74.3	
Nalidixic Acid	104	68.4	9	33.3	113	63.1	
Chloramphenicol	78	51.3	14	51.9	92	51.4	
Mean	84.9	55.9	12.6	46.7	97.5	54.5	

p-value (T test *S. enterica* and *E. coli*) = 0.23

Table 4. Antimicrobial resistance rate of *S. enterica* and *E. coli* based on the pathogen origin (food, water and milk).

Antibiotics	Food		Water		Milk
	<i>S. enterica</i> (n = 122)	<i>E. coli</i> (n = 14)	<i>S. enterica</i> (n = 24)	<i>E. coli</i> (n = 13)	<i>S. enterica</i> (n = 6)
Ceftriaxone	46.7	64.3	54.2	38.5	66.7
Ciprofloxacin	13.9	42.9	75.0	76.9	33.3
Azithromycin	75.4	57.1	41.7	53.8	66.7
Doxycycline	80.3	57.1	70.8	53.8	66.7
Cefotaxime	77.9	50.0	37.5	46.2	66.7
Amikacin	31.1	42.9	75.0	53.8	100.0
Co-Trimoxazole	19.7	35.7	41.7	30.8	66.7
Ampicillin	85.2	35.7	66.7	23.1	100.0
Nalidixic Acid	73.8	35.7	37.5	30.8	100.0
Chloramphenicol	45.1	57.1	75.0	46.2	83.3
Mean (%)	54.9	47.9	57.5	45.4	75.0

p-value (T test) *S. enterica* in food and water = 0.79
p-value (T test) *E. coli* in food and water = 0.68

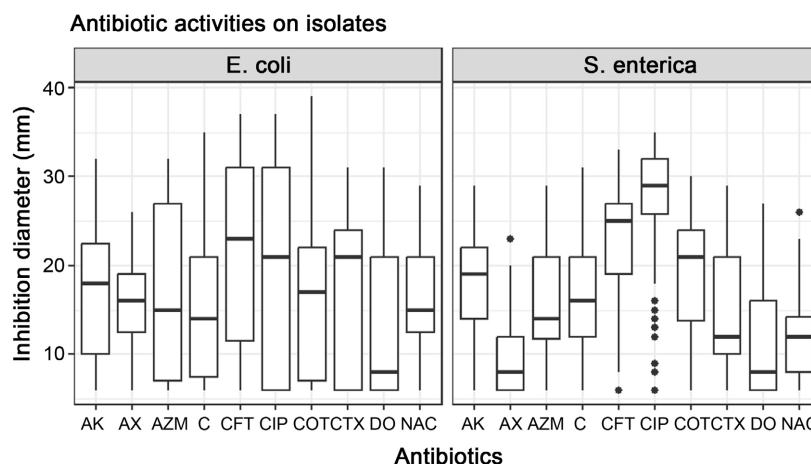


Figure 1. Boxplot summarizing the inhibition diameter (ID) for 10 tested antibiotics on *S. enterica* and *E. coli* before cohabitation. Three antibiotics (CIP, CFT, and AK) displayed average ID reaching the TID for *S. enterica* but none of the antibiotics had an average ID reaching the TID for *E. coli*.

3.3. Multidrug Resistance Profile of *S. enterica* and *E. coli* Isolated from Food, Water and Milk

In total, 90.5% (n = 179) of isolates were multidrug-resistant. The rate of multidrug resistance of *S. enterica* and *E. coli* against 10 tested antibiotics was 94.1% (n = 152) and 70.4% (n = 27), respectively. *S. enterica* and *E. coli* were multidrug-resistant at 11.2% (n = 152) and 14.8% (n = 27), respectively against the three first-line antibiotics (co-trimoxazole, ampicillin, chloramphenicol) used in treatment of enteric infections. The distribution of multidrug resistance was significantly associated with *S. enterica* isolates from food, and *E. coli* from water samples (p = 0.00) (Table 5).

Table 5. Multi drug resistance (MDR) profile of *Salmonella enterica* and *Escherichia coli* in food, water, and milk.

Antibiotics (ATB)	<i>S. enterica</i>		<i>E. coli</i>		Sum of MDR of all isolates		p-value* (chi-square test)
	n = 152	%	n = 27	%	n = 179	%	
MDR to 3 first-line ATB [AX, C, COT]							
a. Food	9	5.9	4	14.8	13	7.3	=0.00
b. Milk	3	2.0	0	0	3	1.7	
c. Water	5	3.3	0	0	5	2.8	
MDR against all 10 tested ATB							
a. Food	114	75.0	10	37.0	124	69.3	=0.01
b. Milk	6	3.9	0	0	6	3.4	
c. Water	23	15.1	9	33.3	32	17.9	
Total	143	94.1	19	70.4	162	90.5	

*p-value: probability based on 0.05 significance threshold to test the distribution of MDR associated with *S. enterica* in food, milk, and water and *E. coli* in food and water.

3.4. Influence of *S. enterica* and *E. coli* Cohabitation on Antibiotic Resistance of *S. enterica*

This study revealed that *S. enterica* lost antibiotic susceptibility after cohabitation with antibiotic-resistant *E. coli* in the same culture media (Figure 2). The average inhibition diameter (ID) for each antibiotic tested on 21 *S. enterica* highly antibiotic-susceptible decreased significantly from 20.5 mm before cohabitation to 10.2 mm after cohabitation (Figure 3). The average rate of antibiotic resistance of *S. enterica* significantly increased from 30% before cohabitation to 89.5% after cohabitation ($p < 0.00$) (Figure 4). The least significant difference (LSD) test showed that before cohabitation (AST1), the activity of antibiotics (ciprofloxacin, ceftriaxone and co-trimoxazole) was statistically different from one another and the rest of antibiotics, while after cohabitation (AST2) only ciprofloxacin presented an inhibition activity significantly different to other antibiotics. The average ID of each antibiotic measured on 152 *S. enterica*, and 27 *E. coli* isolates was significantly different against their theoretical inhibition diameter (TID) of susceptibility, except three antibiotics (ciprofloxacin, amikacin and chloramphenicol), suggesting the decrease in antibiotic activity after bacteria cohabitation (Table 6).

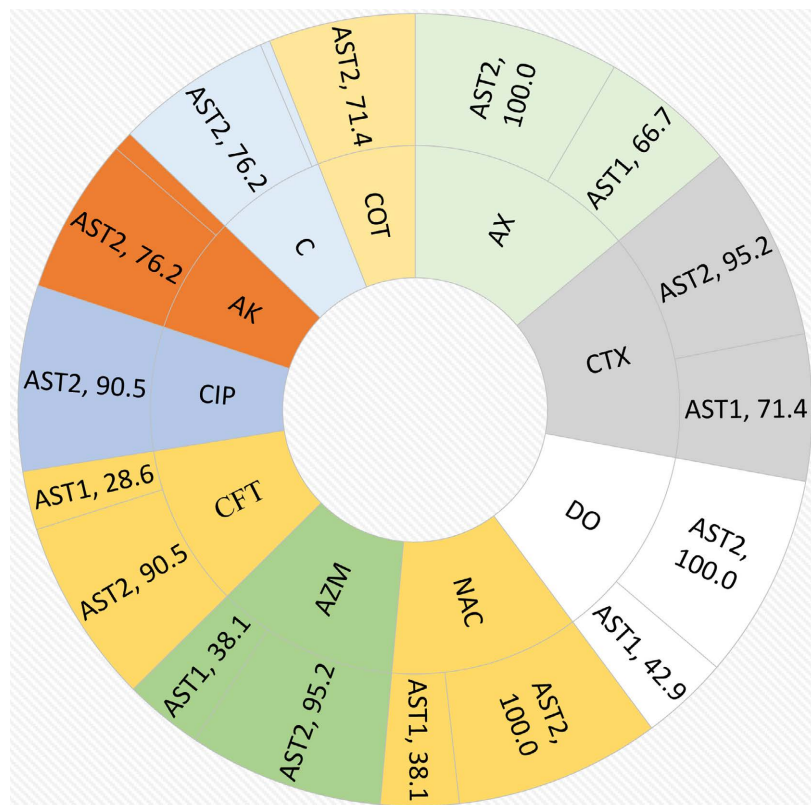


Figure 2. Rate (%) of antibiotic resistance of *Salmonella* before and after cohabitation with *E. coli*. CFT: ceftriaxone, CIP: ciprofloxacin, AZM: azithromycin, DO: doxycycline hydrochloride, CTX: cefotaxime, AK: amikacin, COT: co-trimoxazole, AX: ampicillin, NAC: nalidixic acid, C: chloramphenicol. AST1 = Test of antibiotic susceptibility before cohabitation. AST2 = Test of antibiotic susceptibility after cohabitation. Numbers in the chart indicate the antibiotic resistance rate (%) of *S. enterica*.

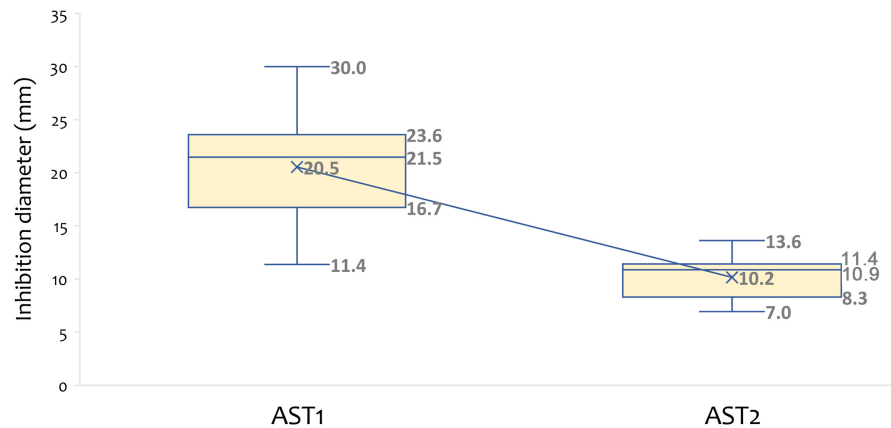


Figure 3. Evolution of inhibition diameter (ID) of antibiotics on antibiotic-susceptible *S. enterica* isolates from food, water and milk. The average ID decreased from 20.5 mm before cohabitation (AST1) to 10.2 mm after cohabitation (AST2) with antibiotic-resistant *E. coli*.

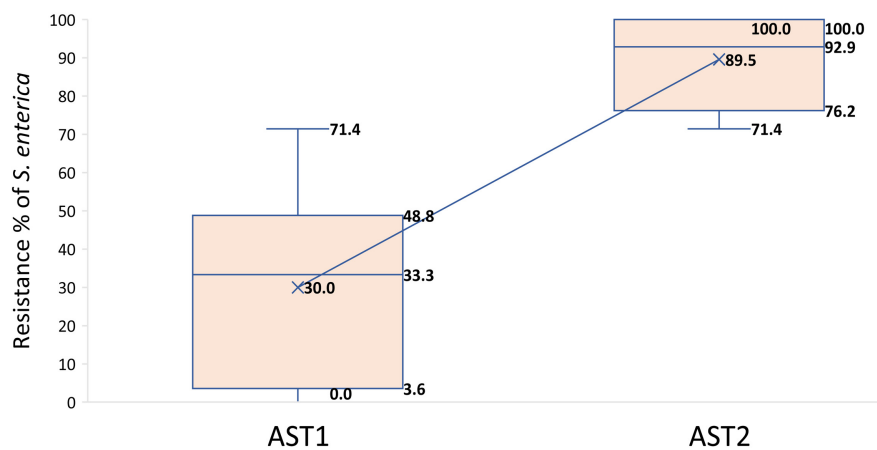


Figure 4. Summary of rate of antibiotic-resistant *S. enterica* before (AST1) and after cohabitation (AST2). The rate of antibiotic-resistant *S. enterica* increased from 30% during AST1 to 89.5% at AST2 translating to significant loss of antibiotic-activity in AST2 due to cohabitation between antibiotic-susceptible *S. enterica* and antibiotic-resistant *E. coli* in the same environment.

4. Discussion

4.1. Occurrence and Antimicrobial Resistance of *S. enterica* and *E. coli* Isolates from Food, Milk and Water

Enterobacteriaceae can spread from an unsafe environment to humans, animals or plants. In the current study, food, milk and water samples tested positive for *S. enterica* (27.5%) and *E. coli* (4.9%). A similar study conducted in Bukavu city reported that foods sold and consumed in unsafe conditions presented a high risk of contracting enteric bacteria and could lead to infectious diseases [27]. Furthermore, the current study confirmed a high rate of antimicrobial resistance (AMR), an average of 55.9% for *S. enterica* and 46.7% for *E. coli*, implying a high risk to consume an antimicrobial-resistant bacteria through food, milk and water. These findings are consistent with the results of previous studies conducted in

Table 6. Ranking of antibiotic activity through ID according to LSD test on 179 isolates.

Antibiotics	TID* (mm)	ID mean (mm)		t. test	t. test	LSD test	LSD test
		AST1	AST2	p-value AST1	p-value AST2	AST1	AST2
Ciprofloxacin	25	25.7	13.6	0.24	0.00	a	a
Ceftriaxone	25	22.3	12.5	0.00	0.00	b	ab
Co-Trimoxazole	14	18.5	11.0	0.00	0.00	c	abc
Amikacin	18	17.3	10.9	0.15	0.00	cd	abc
Chloramphenicol	17	16.1	10.9	0.08	0.00	d	abc
Azithromycin	21	15.7	8.6	0.00	0.00	d	abc
Cefotaxime	20	15.1	11.0	0.00	0.00	d	abc
Nalidixic Acid	14	12.6	7.5	0.00	0.00	e	bc
Doxycycline	17	11.9	8.7	0.00	0.00	e	abc
Ampicillin	14	10.5	7.0	0.00	0.00	e	c

Letters (a-e) are the levels of difference among inhibition activity of each antibiotic based on the average ID measured for each antibiotic, e.g. during AST1, the average of inhibition activity of ciprofloxacin (a) was significantly different comparatively to other antibiotics. Co-trimoxazole (c) and amikacin (cd) means that the inhibition activity of co-trimoxazole was not significantly different compared to amikacin. But the inhibition diameter of co-trimoxazole was significantly different to chloramphenicol (d). T test to a theoretical mean was used to compare the TID of each antibiotic to the measured ID-average of the same antibiotic.

Democratic Republic of the Congo (DRC), where *E. coli* isolates were resistant to ciprofloxacin (75%), co-trimoxazole (73.5%), amikacin (91.2%) and nalidixic acid (100%) [28], and 85% of Enterobacteriaceae species have been reported to be resistant to antibiotics [12]. Mbuyi-Kalonji *et al.* [29] reported that *S. Typhi* (37.8%), *S. Typhimurium* (90.2%) and *S. Enteritidis* (79.7%) were antibiotic-resistant. However, there are some variations in the findings of the current study from other studies, which could be attributed to the origin of the analyzed isolates. In the current study, *S. enterica* and *E. coli* were isolated from food, milk and water samples whereas in majority of the previous studies conducted in DRC, bacteria were isolated from human samples. It has been reported that in human samples such as blood, stool and urine, bacteria undergo interactions that could promote the transfer of AMR genes and bacterial mutations against antibiotics [30] [31]. In the global context of ongoing emerging and re-emerging diseases, the environment remains an understudied link in the spread of the AMR bacteria. There is therefore need for national and international commitment to develop strategies and methods to address the environmental dimensions of AMR [1]-[3].

4.2. Evaluation of the Distribution of Multidrug Resistance in Food, Water and Milk

90.5% of isolates were multidrug-resistant to the tested antibiotics; particularly *S. enterica* from food and milk, and *E. coli* from water samples. Previous studies in

the Democratic Republic of the Congo reported a high multidrug resistance (87.5%) among non-typhoidal *Salmonella* [25]. Another study reported that *E. coli* isolates (23.3%) were multidrug-resistant due to the presence of the extended-spectrum beta-lactamase in the isolates [28]. The *S. enterica* and *E. coli* isolates analyzed in the current study were multidrug-resistant against the three first-line antibiotics (ampicillin, chloramphenicol, co-trimoxazole) [24]. The high rate of multidrug resistance (MDR) observed in *S. enterica* and *E. coli* isolates during the current study could be explained by the poor sanitation and hygiene conditions in Bukavu city as reported by Bisimwa *et al.* [18] favoring the spread of multidrug-resistant bacteria contaminating food and water. The other factors could be misuse and overuse of antibiotics in animal production. A study conducted in Bukavu city reported that high meat and milk consumption was associated with the high rate of MDR among patients treated for *Salmonella* infection [13]. Considering that the analyzed *S. enterica* and *E. coli* were isolated from beef, fish, pork, and milk; the high rate of MDR could be associated with the type of food analyzed. Finally, the MDR observed could be influenced by certain genetic or epigenetic conditions conferring antibiotic resistance to bacteria [32] [33], and the production of beta-lactamase [7] [30] leading bacteria to resist to a large family of antibiotics.

4.3. Effect of *S. enterica* and *E. coli* Cohabitation on the Antibiotic Resistance of *S. enterica*

Cohabitation of antibiotic-susceptible *S. enterica* and antibiotic-resistant *E. coli* in the same environment increased the rate of antibiotic resistance of *S. enterica* from 30% to 89.5%. By sharing the same environment, genes of antimicrobial resistance or multidrug resistance can be transferred from antibiotic-resistant bacteria to susceptible bacteria as demonstrated by the transfer of the gene encoding cefixime resistance from *S. enterica* to *E. coli* [34]. In Democratic Republic of the Congo, there are limited studies on the association between bacterial cohabitation and antimicrobial resistance. The current study is one of the few that have investigated this aspect. At global level, limited studies have investigated the effect of bacterial cohabitation on antibiotic resistance. A study conducted in France by Faure *et al.* [17] showed that the blaCTX-M-9 gene was transferred from *S. enterica* to *E. coli* with a low frequency in an *in vitro* experiment but with a high frequency in an *in vivo* experiment in axenic mice. Consequently, the antibiotic-susceptible *E. coli* became antibiotic-resistant after cohabiting with antibiotic-resistant *S. enterica* [17]. Despite our study being an *in vitro* experiment, the results corroborate the findings by Faure *et al.* [17] confirming that the probability of transfer of antibiotic resistance could become higher when bacteria share the same *in vitro* or *in vivo* environment. Another study confirmed that horizontal or vertical gene transfer could involve the transmission of antimicrobial resistance genes between bacterial species sharing the same niche [35]. It is therefore necessary to address the environmental dimension of antimicrobial resistance since water and foods can harbor antibiotic-resistant bacteria that could be transmitted to humans

and animals [34] [36]. There is need to operationalize the One Health approach in Democratic Republic of the Congo to mitigate the contamination of food, milk and water by antimicrobial-resistant bacteria.

5. Conclusion and Recommendations

This study showed high rate of antimicrobial resistance and multidrug resistance in Bukavu city. Multidrug-resistant *S. enterica* was distributed mainly in food and milk, while multidrug-resistant *E. coli* was predominant in water. The cohabitation of *S. enterica* and *E. coli* in the same environment favored the transmission of antibiotic resistance capacity from an antibiotic-resistant *E. coli* to an antibiotic-susceptible *S. enterica* which led to the latter acquiring antibiotic resistance. Since *S. enterica* and *E. coli* usually share the same environment, there is a risk of transmission of multidrug resistance from a pathogen to a commensal bacterium which subsequently becomes pathogenic. There is a need to change the approach in diagnosis of bacterial infections by isolating at least 3 species or serotypes of bacteria before administering antibiotic treatment to a patient, and also to develop surveillance strategies for detection of antibiotic-resistant bacteria in food, water, and other environmental components to mitigate cases of antimicrobial resistance in Bukavu city, Democratic Republic of the Congo.

6. Limitations

This study focused on food, water and milk samples from Bukavu city. It could be better to integrate data from other cities of Democratic Republic of the Congo, and to replicate the same experience on isolates from human samples. The transformation of antibiotic-susceptible *S. enterica* into antibiotic-resistant could have other explanations such as the transfer of antibiotic resistance genes. However, the assessment of these aspects was not a focus for the current study. Therefore, future investigations could focus on tracking the transfer of antibiotic resistance genes particularly in the genome of the transformed *S. enterica*. It is also important to evaluate the influence of nutrients such as carbohydrates, lipids and proteins on the antibiotic resistance capacity of *S. enterica*.

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Conflicts of Interest

The authors declare there is no conflict of interest concerning this publication.

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