





## Article

# Intestinal Carriage of Extended Spectrum Beta-Lactamase-Producing *Salmonella enterica* from Chickens and Poultry Farmers in Dschang, in the Western Region of Cameroon

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**Abstract:** *Salmonella enterica* is the principal causative agent of salmonellosis, a threat to human health. Because of its high antimicrobial resistance potential, *Salmonella enterica* has become worrisome, mostly in developing countries where hygiene and antimicrobial usage are defective. This study aimed to determine the epidemiology of the intestinal carriage of Extended Spectrum  $\beta$ -Lactamase producing *Salmonella enterica* from chickens and poultry farmers in Dschang, a town in the western region of Cameroon. A total of 416 chickens and 72 farmers were sampled between May and October 2020; and *Salmonella enterica* were isolated and subjected to extended spectrum  $\beta$ -lactamase screening. Logistic regression was used to test for statistical associations using a  $p$ -value of  $\leq 0.05$ . Results from this study revealed that the prevalence of the intestinal carriage of *Salmonella enterica* for chickens and farmers were 55.77% [51.00; 60.54] and 22.22% [12.62; 31.82], respectively. Meanwhile, the intestinal carriage of Extended Spectrum  $\beta$ -Lactamase producing *Salmonella enterica* was 23.08% [13.76; 32.40] and 5.55% [0.26; 10.84] from chickens and poultry farmers, respectively. The risk factor for this carriage was revealed to be lack of knowledge by actors in livestock industries of antibiotic resistance. Chickens, just like poultry farmers, represent the starting point of community salmonellosis, which is difficult to cure; therefore, sensitization of breeders is an effective tool for the mitigation of this burden.

**Keywords:**  $\beta$ -lactamase; antimicrobial resistance; *Salmonella enterica*; salmonellosis; hens; breeders; Cameroon



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## 1. Introduction

Human and animal infection caused by non-typhoidal *Salmonella* (NTS) strains constitutes a very serious pandemic [1–3]. More than 2600 *Salmonella* serotypes have been discovered to date, and most of them are pathogenic to humans, with a variety of animals serving as natural reservoirs (zoonosis) [1–3]. Poultry has been the most reported source for human infections [4–6], and the sub species *Salmonella enterica* (SE) is the most common pathogen, being implicated in 99% of human and warm-blooded animal NTS [2,3,6]. NTS has been reported to be the leading cause of collective foodborne illnesses worldwide [2,7–10].

Approximately 94 million cases of NTS, with 155,000 deaths, are reported annually worldwide [2]. Human NTS is always associated with gastroenteritis, which could therefore require the use of antimicrobials in severe cases, especially in children, the elderly and immunocompromised people. Nonetheless, the development of antimicrobial resistance genes leading to multi-drug-resistant (MDR) strains is much more frequent and contributes greatly to therapeutic failures, thus constituting a danger for humanity [7–14]. One of the

most important resistance gene is that encoding for extended-spectrum beta-lactamases (ESBLs), conferring a remarkable resistance toward  $\beta$ -lactams [1,15–23]. The vertical and horizontal co-transfer of the gene encoding for ESBL via the plasmids between the bacterial entities respectively ensures the duplication and distribution of the resistance gene. Globally, this concerns public health, because infection with a strain of *Salmonella enterica* harboring this resistance gene compromises the treatment directed not only toward this bacterium, but also toward other potentially pathogenic bacteria or opportunists to whom this resistance gene is transferred [3].

In Cameroon, existing *Salmonella*-related threats are mainly attributed to the avian economy, because the consumption of poultry products and direct or indirect contact with chicken feces are frequent. In addition, there is a considerable misuse of antimicrobials through farming practices on one hand and auto consumption of antimicrobials by farmers for curative and prophylactic purposes on the other hand in Dschang [10]. Dschang is considered to be the heart of poultry farming in Cameroon, and thus the country's main chicken suppliers could be a potential source of cross-resistance to antimicrobials by SE and other *Enterobacteriaceae*. The World Health Organisation (WHO) reports on global resistance to third-generation cephalosporins (C3G) and carbapenems by certain *Enterobacteriaceae* show that antimicrobial resistance has reached an alarming level [10]. The WHO reports also indicate that Africa, including Cameroon, suffers from a lot from antibiotic resistance phenomena, and has an enormous lack of data on antimicrobial resistance [4,10]. SE-producing ESBL have been reported in many countries in Africa, but there is a paucity of data available for Cameroon. Therefore, there is an interest in carrying out the present research, which aimed to determine the epidemiology of the intestinal carriage of ESBLs producing SE in chickens and in poultry farmers in Dschang, in the western region of Cameroon, in order to guide health policies related to AMR.

## 2. Results

### 2.1. Distribution of *Salmonella enterica* Isolates

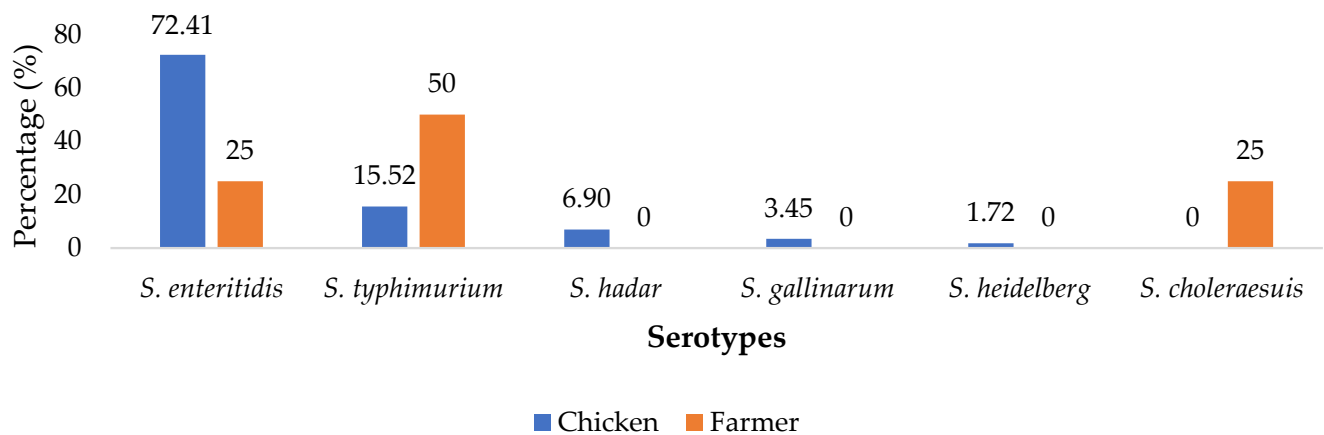
Overall, 47 breeding farms participated in this study. Table 1 shows that the intestinal carriage of SE was prevalent in both chickens (55.77, CI; 51.00–60.54) and farmers (22.22, CI; 12.62–31.82).

**Table 1.** Global distribution of SE according to the participant specie.

Sample Units	Number Sampled	Number of Isolates	Percentage (%)	CI
Chicken	416	232	55.77	51.00, 60.54
Farmers	72	16	22.22	12.62, 31.82

CI. Confidence interval.

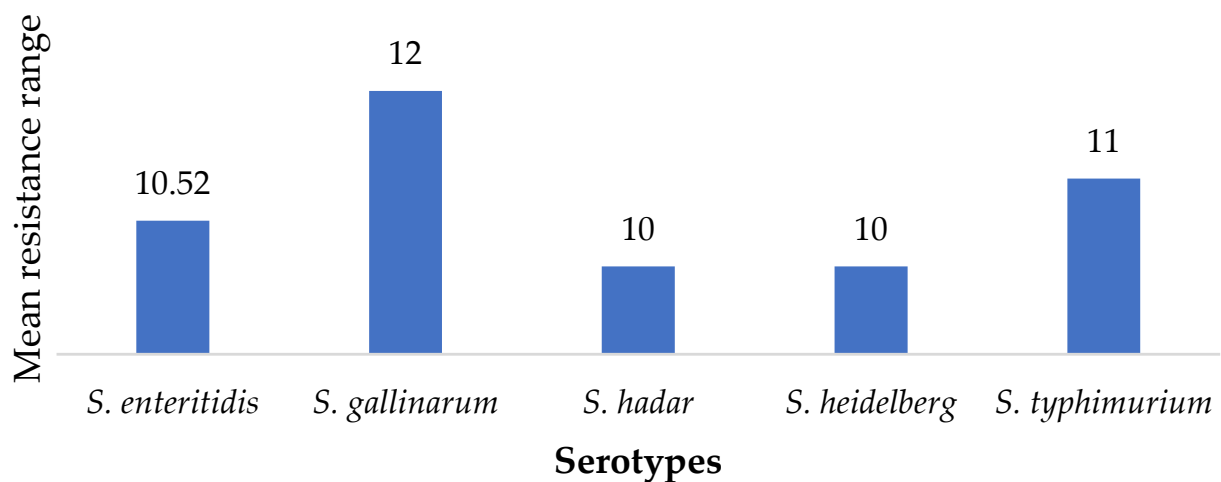
Concerning chickens, five different serotypes were recovered with a predominant *S. enteritidis*; while three serotypes were recovered from farmers with a predominant *S. typhimurium* (Figure 1).



**Figure 1.** Frequency of different SE serotypes recovered from chickens and farmers.

## 2.2. Susceptibility of SE Isolates to Antimicrobials

Most chicken isolates were susceptible to imipenem (220/232), and none were susceptible to tetracycline (0/232) (Table 2). Similarly, breeders' isolates indicated frequent susceptibility to imipenem (12/16) and no susceptibility to tetracycline (0/16), cotrimoxazole (0/16), cefepim (0/16) or ciprofloxacin (0/16). It was noticed that chickens and breeders' isolates had closely related antimicrobial susceptibility profiles. All isolates collected from both chicken and breeders were resistant to tetracycline; a high resistance prevalence was also observed for cefepim (228/232 and 16/16, respectively), ceftiofur (216/232 and 12/16, respectively), ciprofloxacin (208/232 and 16/16, respectively), nalidixic acid (204/232 and 16/16, respectively) and cotrimoxazole (208/232 and 16/16, respectively). Resistance to ceftiofur was high on chicken isolates. The susceptibility profile of the chicken isolates revealed a mean resistance to  $10.60 \pm 0.34$  of the antibiotics tested. Figure 2 presents the serotypes' mean range resistance.



**Figure 2.** Serotype mean range resistance to antimicrobials.

**Table 2.** Susceptibility profile of isolates to antimicrobials.

Antibiotics	Population	Susceptibility to Antimicrobials (%)		
		Susceptible	Intermediate	Resistant
Amoxicilline/clavulanic acid	Chicken	53.45	0	46.55
	Farmers	50	0	50
Amoxicilline	Chicken	24.14	0	75.86
	Farmers	25	0	75
Ceftazidime	Chicken	67.24	3.45	29.31
	Farmers	50	25	25
Cefoxitine	Chicken	1.72	5.17	93.10
	Farmers	25	0	75
Cefepime	Chicken	1.72	0	98.28
	Farmers	0	0	100
Cefotaxime	Chicken	58.62	5.17	36.21
	Farmers	25	0	75
Ticarcilline	Chicken	32.76	5.17	62.07
	Farmers	25	0	75
Imipenem	Chicken	94.83	1.72	3.45
	Farmers	75	0	25
Ceftriaxone	Chicken	67.24	1.72	31.03
	Farmers	50	0	50
Ciprofloxacin	Chicken	3.45	6.90	89.66
	Farmers	0	0	100
Nalidixic acid	Chicken	12.07	0	87.93
	Farmers	0	0	100
Aztreonam	Chicken	53.45	0	46.55
	Farmers	50	0	50
Gentamicine	Chicken	50	0	50
	Farmers	50	0	50
Amikacine	Chicken	63.79	0	36.21
	Farmers	75	0	25
Tetracycline	Chicken	0	0	100
	Farmers	0	0	100
Cotrimoxazole	Chicken	3.45	6.90	89.66
	Farmers	0	0	100
Ceftiofur	Chickens	5.17	8.62	86.21

Red values = highest percentages; blue values = lowest percentages.

### 2.3. Distribution of ESBL-Producing SE Carriage

The distribution of ESBL-producing SE (SE-ESBL) carriage presented in Table 3 revealed that 96/416 and 4/72 isolates were carried by chickens and farmers, respectively. The SE-ESBL chickens' isolates constituted 56 *S. enteritidis* (58.33%), 28 *S. typhimurium* (29.17%), eight *S. gallinarum* (8.33%) and four *S. heidelberg* (4.17%). *S. hadar* was the only serotype where no isolate exhibited ESBL production. The serotypes that were observed to produce ESBL in the farmers samples were *S. enteritidis* (1/2) and *S. typhimurium* (1/2).

**Table 3.** Distribution of ESBL in the studied population.

Population	Size (N)	ESBL-Producing SE (ESBL-SE)		
		Number (n)	Frequency (%)	Confidence Interval (%)
Chickens	416	96	23.08	[13.76; 32.40]
Farmers	72	4	5.55	[0.26; 10.84]

#### 2.4. Associated Factor Analysis for Intestinal Carriage of SE ESBL Producers

Out of 11 factors analyzed, three were significant ( $p < 0.05$ ) using the univariate logistic regression, namely veterinary doctors follow ups, knowledge of antibioresistance by farmers, and the use of antimicrobials bought from veterinary pharmacies (Table 4). Using multivariate logistic regression, it appeared that ignorance of antibiotic resistance was significant ( $p < 0.05$ ) for the intestinal carriage of SE producing ESBL (Table 5).

**Table 4.** Distribution of associated factors using univariate logistic regression analysis.

Variable		ESBL (%)	OR	OR (CI 95%)	p-Value
Feed	No	24 (15.00)	1		Ref
	Yes	72 (10.71)	0.68	0.17–2.78	0.59
Veterinary doctor	No	64 (20.51)	1		Ref
	Yes	32 (6.15)	0.25	0.07–0.91	0.03
Knowledge of antibioresistance	No	88 (18.64)	1		Ref
	Yes	8 (2.22)	0.10	0.01–0.80	0.03
Crawlspace	No	56 (15.91)	1		Ref
	Yes	40 (8.33)	0.27	0.14–1.63	0.06
Veterinary pharmacy	No	56 (14.00)	1		Ref
	Yes	40 (9.26)	0.19	0.03–0.27	0.00
Litter aspect	Dried	24 (7.50)	1		Ref
	Humid	72 (14.06)	2.02	0.51–7.96	0.31
Mean of antimicrobial administration	Food	48 (31.58)	1		Ref
	Water	48 (11.54)	0.54	0.09–3.12	0.49
Formation in aviculture	No	56 (10.94)	1		Ref
	Yes	40 (12.50)	1.16	0.34–3.95	0.80
Formation in biosecurity	No	72 (14.06)	1		Ref
	Yes	24 (7.50)	0.50	0.08–0.33	0.31
Water	Drilling	48 (31.58)	1		Ref
	Well	48 (10)	0.51	0.05–5.53	0.57
Use of antibiotics as growth factors	No	40 (12.50)	1		Ref
	Yes	56 (10.94)	0.86	0.25–2.92	0.05

OR, odds ratio; CI, confidence interval.

**Table 5.** Distribution of associated factors using multivariate logistic regression analysis.

Variable	No/Yes	Adj OR	OR (CI 95%)	p-Value
Veterinary Doctors	No	1		
	Yes	1.20	0.17–8.27	0.85
Knowledge of Antibioresistance	No	1		
	Yes	0.06	$3.80 \times 10^{-3}$ –0.86	0.04
Veterinary pharmacy	No	1		
	Yes	0.21	0.04–1.17	0.07

Adj OR, adjusted odds ratio; CI, confidence interval.

### 3. Discussion

This study aimed to determine the epidemiology of SE ESBL producers among chickens and poultry farmers on poultry farms in Dschang. The prevalence of intestinal carriage of SE of 55.77% is similar to the results reported by Lina et al. [1], who reported a prevalence of 63.6%. Frequent contamination of chickens was reported by Andoh et al. (44%) [24] and Orum et al. (21.4%) [25]. This fact is not surprising, because hens are known to mostly carry asymptomatic SE strains, while at the same time representing a reservoir of the above-mentioned bacteria for humans [2]. Therefore, the presence of these bacteria in poultry is

highly problematic, being responsible for mild to severe human infections. The asymptomatic intestinal carriage by farmers (22.22%), as observed in this study, must constitute an alert, since inter-human contaminations, as opposed to interspecies contaminations, are much more evident, and occur frequently through contact with people, contact with drinking water, contact with cooked and undercooked or raw foods, with asymptomatic carriers having been confirmed as the principal disseminators [2]. Similar findings were also observed by Nzouankeu et al. [3], who described the frequent contamination of poultry farmers in Cameroon with SE. Hence, poultry farmers may represent a potential point of origin for the dissemination of SE that is ignored in the community and more fastidious than hens.

Two SE serotypes, *S. enteritidis* and *S. typhimurium*, were recovered from both chickens and farmers, suggesting possible interspecies transmission. The co-carriage of *S. enteritidis* was similarly observed by Nzouankeu et al. [3]. *S. typhimurium*, *S. heidelberg* and *S. gallinarum* isolates, known to be chicken serotypes, were not found in chicken samples by Nzouankeu et al. [3]; this may be due to differences in climatic conditions, human treatment, and environmental composition. Not even *S. choleraesuis* isolated from breeders was found by these authors. The fact that this serotype is related to pigs [2] may suggest that the farmers were instead contaminated by pigs. However, *S. heidelberg* was also identified by Adel et al. [26] and Reseala et al. [27], thereby implying that this serotype is common in Africa. *S. enteritidis* and *S. typhimurium* were the most prevalent serotypes, with finding being similar to that reported by Nzouankeu et al. [3], which indicates that these two serotypes are the most widespread in Cameroon. Igbinosa et al. reported similar findings in a locality in Nigeria. This observation reinforces the findings regarding serovar distribution in the current study, since the areas are geographically close [28]. The relatively high occurrence of *S. typhimurium* in the farmer samples may be due to the fact that this serotype is more significantly adaptive to humans than to other animal species [2].

The low resistance to imipenem, as observed in numerous studies [1,3,10,29], may be due to the fact that this antimicrobial is not susceptible to the hydrolytic action of a wide variety of ESBLs, except those of class B of Ambler carbapenemases, which to date remain narrowly disseminated compared to other ESBLs [21]. These factors allow *enterobacteria* to resist antimicrobials, with the genes encoding them not yet being widespread in bacteria populations [13]. The high resistance to cefepim and cefoxitin was different from what has been reported in other studies [1,3], which may be due to the production of cephalosporinase being capable of lysing these antimicrobials. The high resistance of the chicken isolates to tetracycline, cotrimoxazole, ciprofloxacin, nalidixic acid and ceftiofur is probably due to the fact that, as veterinary antimicrobials, they are misused on poultry farms [1,10]. The study by Nzouankeu et al. [3] in Cameroon and that of Adel et al. [26] in Egypt similarly described the observation of a high resistance rate to tetracycline, cotrimoxazole and nalidixic acid. Accordingly, this phenomenon requires further study in order to obtain a better understanding. Globally, the close similarity in terms of resistance pattern observed in both farmer and chicken isolates in our study may suggest that transmission from chickens to farmers took place, though this remains to be confirmed by molecular biology techniques. The increased resistance pattern observed in the farmer isolates may be due to self-medication when attempting to prevent infections, which was declared by farmers during the administration of the questionnaire. This may have led the MDR *Salmonella* strains with which the farmers were contaminated to select more resistant genes. The high resistance rate observed in the serotype *S. gallinarum* is quite novel, since this serotype has only rarely been isolated in most previous studies. Close to *S. gallinarum*, *S. typhimurium* exhibited the most resistant profile, which is in agreement with the study by Nzouankeu et al. [3], who qualified *S. typhimurium* as being among the most resistant *Salmonella* serotypes.

The identification of SE-ESBL in the current study is contrary to One Health and calls for an integrated approach for tackling AMR. Obviously, the prevalence of SE-ESBL in chickens (23.08%) and farmers (5.55%) suggests the contamination of the environment,



most significantly soil surfaces, as identified by Raseala et al. (34.6%) [27]. The presence of ESBL on poultry farms in Dschang requires the attention of the public health sector, because the ESBL enzyme confers resistance not only to a high variety of  $\beta$ -lactams, but also co-resistance to quinolones, fluoroquinolone, aminosides and cotrimoxazole, as previously observed by Djuikoue et al. [15]. The selection of ESBL by *Salmonella* strains may be due to the misuse of antimicrobials on poultry farms. SE producing ESBL were also isolated in a study by Lina et al. [1] in China, but at a much lower rate (3.41%). This could be due to the implementation of legislation regulating the usage of antimicrobials in China. Similarly, Igbinosa et al. reported a low prevalence of SE-ESBL carriage (6.8%), which equally suggests better AMR control in this locality in Nigeria [28]. The prevalence of SE-ESBL was relatively higher in the study by Adel et al. (41.2%) [26]. This may be due to the nature of the sample, which consisted of slaughtered chicken meat, which is obviously exposed to further contamination [27]. Nevertheless, this indicates a greater burden of resistant SE in African countries.

The overall observations in this study describe poultry farms in Dschang as harboring ESBL-producing SE, making these strains resistant to a wide range of antimicrobials. These findings explain the therapeutic difficulties and failures observed for most infectious diseases in general and salmonellosis in particular, owing to the fact that the genes encoding for ESBL are often located on mobile genetic elements. In addition to this, the “One Health” concept is not known, and is consequently not considered by farmers in Dschang, with drastic consequences [30]. These farmers are only interested in the health status of their living chickens, which is their sole source of income. The fact that the “One Health” notion is ignored by farmers may also be the origin of highly prevalent salmonellosis cases in the area, which are difficult to cure because of the effectiveness of internal and external interspecies contaminations on farms. The growth of the bacterial population through the horizontal (spreading) and vertical (duplication) transfer of ESBL genes solidifies its defense against antimicrobials administered to save human lives. Therefore, in this challenge facing humanity posed by bacteria capable of causing infectious diseases, humanity is weakened by evil human practices, mainly the abusive use, misuse and non-standard use of antibiotics.

Following the analysis of the associated factors, it appeared that the ignorance regarding antibiotic resistance among some farmers was significantly associated with the intestinal carriage of SE ESBL producers by chickens ( $p$ -value < 0.05). This may indicate that farmers in Dschang misuse antimicrobials because they are ignorant of the capacity of bacteria to change in the presence of antibiotics to no longer respond to the latter, as well as the existing barriers to antimicrobial effects of bacteria. Therefore, they consider antimicrobials to be the ultimate solution to infections, for which reason these drugs are abused. This hence contributes to the emergence of SE ESBL in Dschang poultry farms. If they were aware of the antimicrobial resistance phenomenon and its direct influence on the selection of resistant SE, they may improve their use of antimicrobials in order to avoid both sanitary (on hens and themselves) and economic consequences.

#### 4. Materials and Methods

##### 4.1. Study Description, Sampling Method and Selection Criteria

A cross-sectional descriptive and analytical study was carried out in Dschang city over a period of six months, from May to October 2020. A cluster sampling technique was used, with many steps through random and successive selection of quarters and farms. All poultry farms of the town of Dschang with authorization issued by the owners were included in the study. Farms where the breeders refused to participate were excluded from the study. The number of hens randomly sampled per farm was a function of the farm size (number of chickens in the farm) using the proportionality principle via the following formula:  $n = \frac{b}{B} * N$ , where  $b$  = the number of chickens in the farm;  $B$  = the total number of chickens in all participating farms; and  $N$  = sample size.

#### 4.2. Sample Collection and Processing

The analysis of the samples collected from the poultry farms was performed in the microbiology laboratories of Dschang District Hospital (Menoua division, Dschang). Prior to sample collection, a questionnaire was issued to the farmers to gather possible associated risk factors. That questionnaire included the following questions: 1. Do you feed chickens with provender? 2. Do you provide a crawlspace in between timely separated chicken flocks? 3. Do you know what is meant by antimicrobial resistance? 4. Is your farm followed up by a veterinary doctor? 5. Do you always buy antibiotics from veterinary pharmacies? 6. Do you use personal protective equipment? 7. What is the origin of the chickens' drinking water? 8. Have you been certified in breeding? 9. Have you been certified in biosecurity? 10. Do you use antibiotics as growth promoters?

A cloacal swab was performed on poultry and fecal samples were collected from the farmers. The samples were conveyed in coolers containing icepacks. Isolates were obtained based on the recommendations of REMIC (Référentiel en Microbiologie Médicale) 2019 using selenite broth and Hektoen enteric agar successively [31]. The isolates were biochemically identified using the API 20 E test strips (BioMerieux, France) and subsequently serotyped according to the Kauffman–White Le Minor scheme [32]. Slide agglutination with specific antiserum was used to identify the Vi capsular antigen of *Salmonella* antisera [33].

The antimicrobial susceptibility testing was realized by the disk diffusion method or Kirby Bauer method [34] on Mueller–Hinton agar medium using a panel of 17 antibiotics (Table 6). This assay was performed according to the 2020 recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2020) [35,36]. ESBL production was detected via the double-disk synergy testing following the recommendations of the EUCAST 2020 using a Mueller–Hinton agar medium [35]. The presence of ESBL was concluded when the inhibition zone around cefotaxime, ceftazidime, cefepime, or aztreonam antibiotic disks was enhanced on the side of the clavulanate-containing disk, resulting in a characteristically shaped zone referred to as a “champagne cork”. *Escherichia coli* ATCC 25922 and 35218 were respectively used as quality control strains for the antimicrobial susceptibility testing and the double disk synergy testing.

**Table 6.** Table of antimicrobials used (EUCAST 2020).

Antimicrobial	Family	Abbr	Charge (µg)	Diameter	
				Critical	
				S ≥	R <
Amoxicilline/clavulanic acid	Oxapenam	AMC	20–10	19	19
Amoxicilline	Amino-penicillin	AMX	20	19	19
Ceftazidime	C3G	CAZ	10	22	19
Cefoxitine	C2G	FOX	30	19	15
Cefepime	C3G	FEP	30	27	24
Cefotaxime	C3G	CTX	5	20	17
Ticarcilline	Carboxy-penicillin	TIC	75	23	20
Imipenem	Carbapenem	IMP	10	22	17
Ceftriaxone	C3G	CRO	30	25	22
Ciprofloxacin	Fluro-quinolones	CIP	5	25	22
Acide nalidixique	Quinolone	NA	30	14	14
Aztreonam	Monobactam	ATM	30	26	21
Gentamicine	Aminoside	GN	10	17	17
Amikacine	Aminoside	AK	10	18	18
Tetracycline	Cycline	TE	30	25	22
Cotrimoxazole	Diaminopyri-midine	SXT	1.25–23.75	14	11

Abbr. abbreviation; C2G. second-generation cephalosporine; C3G. third-generation cephalosporine.



#### 4.3. Statistical Analysis

Statistical analysis was performed using Epi-Info 7 software. The confidence interval was calculated when necessary. Logistic regression was used to identify predictive factors of avian carriage of ESBL-producing SE, a  $p \leq 0.05$  was considered statistically significant.

#### 5. Conclusions

This study indicated that SE strains are a threat to poultry farms, and are highly transmissible. *S. enteritidis* was predominantly observed in chicken isolates, whereas *S. typhimurium* was predominantly found in farmer isolates. All SE strains were MDR. It was observed that 3/13 chickens carried SE ESBL producers, compared to 1/18 of farmers, indicating an important proportion of ESBL-producing SE carriage among hens and breeders in Dschang. Overall, factors associated with the intestinal carriage of SE ESBL producers lies in the ignorance regarding antibiotic resistance among farmers. These interesting findings warrant further study on the molecular biology of the isolates to identify different classes of ESBL that exist in Cameroon.

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**Data Availability Statement:** All data are available upon request.

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**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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