Advances in Microbiology, 2025, 15(9), 469-483
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Digestive Carriage of Esbl and Shiga-Like Producing Enterobacteriaceae in Outpatients at Saint Jean de Dieu Hospital of Afagnan in Southern Togo

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How to cite this paper: Degbey, C.C., Kouke, R.S.N., Boya, B., Sina, H., Aïkou, N. and Baba-Moussa, L.S. (2025) Digestive Carriage of Esbl and Shiga-Like Producing Enterobacteriaceae in Outpatients at Saint Jean de Dieu Hospital of Afagnan in Southern Togo. *Advances in Microbiology*, **15**, 469-483.
https://doi.org/10.4236/aim.2025.159030

Received: July 19, 2025
Accepted: September 1, 2025
Published: September 4, 2025

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Abstract

The emergence and spread of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae is a significant public health problem. The digestive carriage of ESBL-producing Enterobacteriaceae largely contributes to their diffusion and dissemination within our communities and hospitals. This work aimed to study the digestive carriage of ESBL-producing Enterobacteriaceae in outpatients at Saint Jean de Dieu Hospital in Afagnan in southern Togo. This analytical descriptive cross-sectional study was conducted on 62 stool samples from an outpatient setting. The bacteria were isolated, identified, and an antibiogram was obtained by standard microbiological techniques, followed by molecular characterization in the search for resistance and virulence genes. The results show a predominance of *Escherichia coli* (66.1%), followed by *Klebsiella pneumoniae* (14.5%) and *Klebsiella oxytoca* (9.7%). The strains were resistant to amoxicillin (100%), trimethoprim/sulfamethoxazole (61.3%), and amoxicillin + clavulanic acid (40%). Conventional PCR shows that 12.9% of Enterobacteriaceae isolates possessed blaTEM, blaSHV, and blaCTX-M genes. Enterobacteriaceae possessed the blaCTX-M gene (11.3%), the blaTEM gene (12.9%), and the blaSHV gene (4.8%). None of the *Enterobacteriaceae* isolates possessed the *stx*1 virulence gene. This kind of study is one of the first of this type in southern Togo. It provides evidence on the diffusion and dissemination of resistance genes within the community and their possible importation

into healthcare settings.

Keywords

Enterobacteriaceae, Digestive Carriage, ESBL, Resistance to Antibiotics

1. Introduction

Antimicrobial resistance remains a significant challenge for global health in clinical control and infection prevention. According to a global report, more than 1.2 million people died worldwide in 2019 from infections caused by antibiotic-resistant bacteria [1]. Of particular concern is the rise in antimicrobial resistance associated with extended-spectrum beta-lactamase (ESBL)-producing bacteria, which has seen an increase in hospital and community settings in recent years [2]. The proliferation of antimicrobial resistance is significantly fuelled by the overuse of antibiotics in human medicine and animal husbandry, particularly in sub-Saharan Africa [3]. In the absence of intervention, projections suggest that within 5 years (2030), infections due to antimicrobial resistance will pose a substantial threat to the global economy, with low- and middle-income countries (LMICs), particularly in sub-Saharan Africa, bearing a disproportionate burden [4].

In low-income countries, the lack of enforcement of regulations and guidelines to control the sale and unreasonable use of antibiotics in animals and humans has contributed to the emergence and spread of resistant bacteria [5]. The problem with antimicrobial resistance is that it leads to more extended hospital stays and the need for more expensive drugs, which are often not readily available in resource-limited countries like Togo due to the inability to treat bacteria that are resistant to locally available antimicrobial agents [6] [7].

In Africa, the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae during infectious processes remains high, fluctuating between 30% and 50% [8]. In Ghana, a study carried out at Korle-Bu Teaching Hospital revealed that almost half of all enterobacteria isolated from clinical infections (49.4%) were ESBL producers [9] [10]. In Togo, the few data available come from surveys carried out between 2009 and 2011 to determine the frequency of ESBL strains and assess their antibiotic susceptibility profile. The prevalence of ESBL Enterobacteriaceae was estimated at 22.44%, with a predominance of *Escherichia coli* and *Klebsiella spp*. both showing marked resistance to most antibiotics tested [11]. Initially, these ESBL-producing bacteria were considered purely nosocomial [7]. As causative agents of serious infections, they pose a significant problem for hospitalized patients. However, community-acquired infections and healthy fecal carriers have also been reported [12].

Although transmission of Enterobacteriaceae producing extended-spectrum-β-lactamase has been studied extensively in clinical settings [12], little is known about the risk and routes of transmission in the general population. This trans-

mission is favored by poor hygiene (fecal hazard). Because latrines do not exist in some parts of our developing world, fecal matter enters the environment and can contaminate water, food, and plants. Animal and human populations, therefore, remain at high risk of contamination by Enterobacteriaceae that produce broad-spectrum-β-lactamases. Because the hospital environment is also conducive to the spread of multidrug-resistant bacteria, the admission of patients with ESBLs is a significant contributor to the spread of these antimicrobial resistance genes.

Thus, the present work aimed to investigate the transmission of ESBL-producing Enterobacteriaceae in the digestive tract of ambulatory patients and the factors associated with this transmission and indirectly to determine the presence of a community reservoir of ESBL-producing Enterobacteriaceae in this region of southern Togo.

2. Materials and Methods

2.1. Study Design

This analytical descriptive cross-sectional study was carried out between October and December 2020 at the Saint Jean de Dieu Afagnan Hospital. Founded in 1961, Saint Jean de Dieu Afagnan Hospital is located in the maritime region, Bas-Mono prefecture in Togo. This hospital provides comprehensive health coverage considering several prefectures in southern Togo. These are mainly the prefectures of Lacs, Bas-Mono, Yoto, and Vo.

2.2. Study Population, Sample Size, and Sample

The study included a total of 62 ambulatory patients, aged between 15 and 74 years, who consulted at Saint Jean de Dieu Hospital in Afagnan during the study period. These adult patients were selected based on a clinician's request for coprological analysis, issued in the context of various consultation reasons such as general asthenia (AEG), abdominal pain, or pregnancy assessment. All proven diagnostic stool samples received at the Saint Jean de Dieu Afagnan hospital laboratory during the study period from patients consulting on an outpatient basis aged 15 or over are included in the sample. However, all samples other than stools, from health care services or children (age < 15 years), and any isolated germ other than Enterobacteriaceae are excluded from our study. The sample size, set at 62 patients, was not determined through prior statistical calculation but was based on the number of cases available during the study period.

2.3. Data Collection

The sampling method was non-probabilistic, with the technique of reasoned choice, based on the criteria: external adult consultants and requests for coprological examination. The stool samples collected were transported to the Microbiology Laboratory of Saint Jean de Dieu Afagnan Hospital (Togo) for the isolation and identification of bacteria and to the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM) of the Faculty of Sciences et Techniques of the

University of Abomey-Calavi (Benin) for the research of the bla and sxt1 genes by PCR. The data collection techniques used are documentary review, survey by questionnaire, and collection of samples. Clinical and demographic data such as diagnosis, age, sex, and origin were recorded on the analysis request forms presented to the laboratory. A questionnaire was used at the time of receipt of the samples, including information such as the use of antibiotics during the last three months, history of hospitalization during the last three months, and the presence of pregnancy. In parallel with the coprological analyzes requested, the doctors made requests for HIV serology for each of the patients included in the study. The serology results were listed in the laboratory's HIV serology register.

2.4. Isolation, Identification of Bacteria, and Antibiotic Sensitivity

Once at the Saint Jean de Dieu Afagnan Hospital Microbiology Laboratory, the stool samples were seeded on Mac Conkey agar.

2.5. Isolation and Identification of Enterobacteriaceae Strains

Once collected, the stool samples were cultured on Mac Conkey agar and incubated at 37˚C for 24 h. After incubation, suspicious colonies were stained using the Gram stain method. In addition, the colonies' shapes, colors, and arrangements were observed [13]. The identification of Enterobacteriaceae was carried out using 23 biochemical tests (0-nitrophenyl-fi-D-galactosidase, arginine dihydrolase, lysine, and ornithine decarboxylase, use of citrate, hydrogen sulfide, urease, tryptophan deaminase, indole, Voges-Proskauer, gelatin liquefaction, glucose fermentation, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose, nitrate reduction and nitrogen gas production and catalase production) on an API 20E strip (BioMérieux SA, Marcy-l'Etoile, France).

2.6. Antibiotic Susceptibility of Isolates

The susceptibility of isolated Enterobacteriaceae to 11 antibiotics was tested by the disk diffusion method on Mueller Hinton agar medium, following the recommendations of the Antibiogram Committee of the French Society of Microbiology [14]. The antibiotics studied are amoxicillin (AMO), amoxicillin + clavulanic acid (AMC), ceftazidime (CDZ), cefotaxime (CTX), cefixime (CFM), cefuroxime (CXM), imipenem (IPM), gentamicin (GMN), levofloxacin (LVX), trimethoprim/sulfamethoxazole (SXT) and tobramycin (TOB). The bacterial suspension was standardized using the McFarland 0.5 control. To ensure the reliability and reproducibility of the results, quality-control measures were implemented for antimicrobial susceptibility testing (AST), including the use of the reference strain *Escherichia coli* ATCC 25922.

2.7. ESBL Production Detection Tests

The extended-spectrum beta-lactamase (ESBL) production test was carried out with 3rd generation cephalosporins, namely cefotaxime (CTX) and Ceftazidime

(CDZ) in the presence of amoxicillin + clavulanic acid (AMC) placed at the center of two cephalosporin discs. The result was considered positive if a potentiation of the corkscrew-shaped zone of inhibition between the discs of CTX and AMC, and that of AMC and CDZ, was observed [11].

2.8. Search for Genes Coding for the ESBL Phenotype

DNA extraction was performed according to the method of Rasmussen and Morrissey (2008), and multiplex PCR was used to detect virulence genes. Amplification was performed in a 20 µL mixture containing 2.0 µL buffer (10×), 0.4 µL MgCl2 (25 mM), 0.2 µL dNTP (10 mM), 1 µL primer F (10 mM), 1 µL of primer R (10 mM), 0.2 µL of Taq DNA polymerase and DNA (5 µL) under the effect of heat. 2 µl of Taq DNA polymerase and DNA (5 µl) under initial denaturation conditions of 94˚C for 5 minutes, followed by 35 cycles of denaturation (45 s at 94˚C), annealing (30 s at 50˚C) and elongation (30 s at 72˚C), followed by final elongation at 72˚C for 10 minutes. After amplification, the PCR products migrated on a 1.2% agarose gel for approximately 30 min at 100 V. A 100 bp molecular weight marker (Gene Ruler) was used. The primer sequences used to target the detection of virulence factors are shown in **Table 1** [15]. To ensure the reliability and reproducibility of the results, quality-control measures were applied to PCR, including the use of positive and negative control strains described in the study by Moussé *et al.* [16] on antibiotic resistance and extended-spectrum *β*-lactamase production in clinical Gram-negative bacteria in Benin.

Table 1. Sequences of primers used for the polymerase chain reaction.

Genes	Primers	Sequences
bla_{TEM}	OT-F	5’-ATTGGGTGCACGAGTGGGTTAC-3’
	OT-R	5’-ATAATTGTTGCCGGGAAGCTAG-3’
bla_{SHV}	SHV-F	5’-CGCCGGGTTATTCTTATTTGTCGC-3’
	SHV-R	5’-TCTTTCCGATGCCGCCGCCAGTCA-3’
bla_{CTX-M}	CTX-F	5’-CGCTTTGCGATGTGCAG-3’
	CTX-R	5’-ACCGCGATATCGTTGGTAAT-3’
SLTI (stx1)	SLTI-F	5’-GAAGAGTCCGTGGGATTACG-3’
	SLTI-R	5’-AGCGATGCAGCTATTAATAA-3’

2.9. Data Analysis

Means and standard deviations were calculated using an Excel 2013 spreadsheet from the experimental results. R 3.5.2 (R Core Team, 2019) was used to determine significant differences between calculated means at the threshold of 5% ($p < 0.05$). Multivariate logistic regression was used to identify factors associated with the presence of ESBL-producing Enterobacteriaceae from univariate analysis. The univariate logistic regression made it possible to search for associations between the variable of interest and the independent variables, such as age, sex, pregnancy,

HIV serological status, antibiotic intake, and hospitalization in the last three months, with the calculation of the Odds Ratio (OR), p. value and their 95% confidence intervals.

3. Results

3.1. Sociodemographic Characteristics of Patients

In this study, samples positive for Enterobacteriaceae were mainly isolated from female patients (62.9%) with a sex ratio M/F of 0.59. The average age of the population was 40.05 years ± 1.87, with extremes of 15 and 74 years. Within this study population, 8.1% of patients had been hospitalized in the past three months, 9.7% had received antibiotic therapy in the past three months, 8.1% were pregnant, and 1.6% were HIV positive. The characteristics of the study population are presented in Table 2.

Table 2. Characteristics of the study population.

Variable		Frequency (%)
Sex	Home	37.1
	Femme	62.9
Pregnancy	No	91.9
	Yes	8.1
Use of ATB	No	90.3
	Yes	9.7
Hospitalization	No	91.9
	Yes	8.1
HIV + status	Negative	98.4
	Positif	1.6

3.2. Frequency of Enterobacteriaceae Strain Isolation

Of the 62 stool samples collected, five species of Enterobacteriaceae were identified with a predominance of *Escherichia coli* (66.1%), followed by *Klebsiella pneumoniae* (14.5%) and *Klebsiella oxytoca* (9.7%). The least represented species were *Enterobacter* spp (6.5%) and *Citrobacter* spp. (3.2%). A remarkable diversity was observed among these strains of Enterobacteriaceae (Table 3).

Table 3. Number and frequency of strains isolated.

Strains	Frequency (%)
Escherichia coli	66.1
Klebsiella pneumoniae	14.5
Klebsiella oxytoca	9.7
Enterobacter spp	6.5
Citrobacter spp	3.2

3.3. Susceptibility of Enterobacteriaceae Strains to Antibiotics

The antibiotic resistance of Enterobacteriaceae isolates is shown in Figure 1. The results showed variable sensitivity (between 0% and 100%) of the isolated Enterobacteriaceae strains to the antibiotics tested. Indeed, the highest strain resistance rates were observed with antibiotics such as amoxicillin (100%) and Trimethoprim/sulfamethoxazole (61.3%). Additionally, the results indicated that *Escherichia coli* (51.2%), *Serratia* spp (50%), and *Klebsiella oxytoca* (33.33%) strains are resistant to Amoxicillin + clavulanic acid. On the other hand, all strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp, and *Serratia* spp are sensitive to imipenem and Tobramycin (TOB) (Table 4). The variance analysis showed a significant difference between species resistance and antibiotics ($p < 0.0001$).

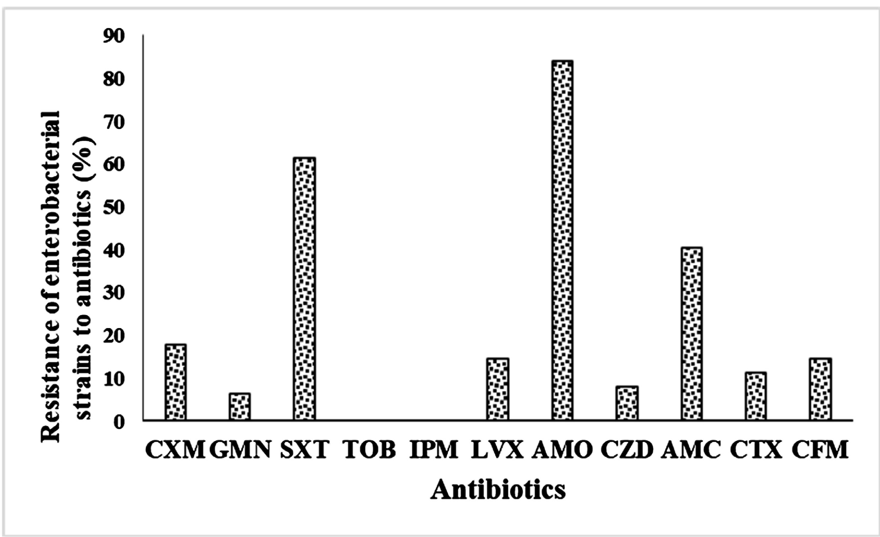

Figure 1. Resistance profile of isolated strains of Enterobacteriaceae to antibiotics. Amoxicillin (AMO), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CDZ), Cefotaxime (CTX), Cefixime (CFM), Cefuroxime (CXM), Imipenem (IPM), Gentamicin (GMN), Levofloxacin (LVX), Trimethoprim/sulfamethoxazole (SXT) and Tobramycin (TOB).

Table 4. Antibiotic resistance of species isolated from Enterobacteriaceae.

Spices	Antibiotics (%)										
	CXM	AMO	CZD	AMC	CTX	CFM	IPM	SXT	TOB	GMN	LVX
Escherichia coli	19.5	75.6	9.7	51.2	14.6	19.5	0	70.7	0	7.3	22
Klebsiella pneumoniae	11.1	100	0	11.1	11.1	11.1	0	44	0	11.1	0
Klebsiella oxytoca	33.3	100	16.7	33.3	0	0	0	33.3	0	0	0
Enterobacter spp	0	100	0	0	0	0	0	50	0	0	0
Serratia spp	0	100	0	50	0	0	0	50	0	0	0

Amoxicillin (AMO), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CDZ), Cefotaxime (CTX), Cefixime (CFM), Cefuroxime (CXM), Imipenem (IPM), Gentamicin (GMN), Levofloxacin (LVX), Trimethoprim/sulfamethoxazole (SXT) and Tobramycin (TOB).

3.4. ESBL Production by Isolated Enterobacteriaceae Strains

Of the Enterobacteriaceae tested, none of the strains produced ESBL. However, eight strains were either resistant or intermediate to the three CG3s used in the study.

3.4.1. Detection of Resistance Genes

Detection of the blaTEM, blaSHV, and blaCTX-M genes by conventional PCR shows that 12.9% of Enterobacteriaceae isolates possessed blaTEM, blaSHV, and blaCTX-M genes. The variance analysis showed a significant difference between the presence of resistance genes and Enterobacteriaceae ($p < 0.001$).

Considering by resistance gene, 12.9% of Enterobacteriaceae strains possessed blaTEM genes. 11.3% of Enterobacteriaceae strains possessed the blaCTX-M gene, and 4.8% possessed the blaSHV gene. Considering by species, 2.43% of *E. coli* strains and 16.67% of *Klebsiella oxytoca* strains possessed resistant genes. Among the *E. coli* strains with resistant genes, 100% had blaCTX-M genes, 87.5% blaTEM genes, and 66.7% blaSHV genes. *Klebsiella oxytoca* strains possessed 12.5% blaTEM genes and 33.3% blaSHV genes. Among the *E. coli* strains possessing resistance genes, 71.42% had the blaTEM and blaCTX-M genes simultaneously, and 28.57% had the blaTEM, blaSHV, and blaCTX-M genes simultaneously. The *Klebsiella oxytoca* strain simultaneously possessed the blaTEM and blaSHV genes.

3.4.2. Analysis of Predictive Factors of EPBLSE Carriage

The univariate analysis shows that only the pregnancy ($p = 0.08$), antibiotic use ($p = 0.14$), and hospitalization ($p = 0.14$) factors were significantly associated with ESBL at the 20% threshold (Table 5).

Table 5. Univariate logistic regression of predictive variables of EPBLSE carriage.

Variables	OR	IC	P
Age	0.99	[0.94; 1.04]	0.81
Sex			
Women	1		
Male	0.52	[0.07; 2.52]	0.45
Pregnancy			
No	1		
Yes	5.67	[0.65; 41.71]	0.08
Use of ATB			
No	1		
Yes	4.17	[0.50; 26.82]	0.14
Hospitalization			
No	1		
Yes	1.43	[0.97; 1.47]	0.14
HIV status			
Negative	1		
Positive	1.20E+08	[6.47e−205; Na]	0.99

Table 6 shows that pregnancy and hospitalization were associated with the carriage of Enterobacteriaceae possessing ESBL enzymes. Pregnant patients are 10.7 times more at risk of being carriers of Enterobacteriaceae with ESBL genes than those who are not (OR = 10.7; 95% CI = [1.10; 96.77]); those who have been hospitalized are 10.7 times more at risk of carrying Enterobacteriaceae with ESBL genes than those who have not been (OR = 10.7; 95% CI = [1.10; 96.77]).

Table 6. Multivariate logistic regression of predictive factors of EPBLSE carriage.

Variables	**OR**	**IC**	**P**
	Pregnancy		
No	1		
Yes	1.07E+01	[1.10; 96.77]	0.029*
	Hospitalization		
No	1		
Yes	1.07E+01	[1.10; 96.77]	0.029*

*P < 0.05.

Search for sxt1 genes in *E. coli* strains

Not all *E. coli* strains harbored the stx1 gene (100%) encoding Shiga-like toxin.

4. Discussion

Resistance of bacteria, particularly Enterobacteriaceae, to antibiotics is a real public health problem. This resistance affects both developed and especially developing countries, where self-medication and the anarchic sale of drugs outside legal structures coexist [17]. In West African countries, the endemicity of diarrheal diseases and other infectious diseases has increased the consumption of antibiotics [18]. In this study, we aimed to determine the prevalence of ESBL-producing Enterobacteriaceae in the digestive tract of non-hospitalized adult patients treated at Saint Jean de Dieu Afagnan Hospital.

Enterobacteriaceae were isolated from predominantly female patients (62.9%) during our study. This observation can be justified because they are often responsible for the sick and household chores. In our study, five different species of Enterobacteriaceae were isolated, with a predominance of *Escherichia coli* (66.1%), followed by *Klebsiella* pneumoniae (14.5%) and *Klebsiella oxytoca* (9.7%). On the other hand, the species poorly represented were *Enterobacter* spp (6.5%) and *Citrobacter* spp (3.2%). The same observation was made by Onduru *et al.* [19]. These authors showed that the species most frequently isolated from outpatients attending community health centers in Blantyre, Malawi was *Escherichia coli* (66%), followed by *Klebsiella* spp (8%). These results could be explained by the fact that *E. coli* strains, being a commensal species and an opportunistic pathogen commonly found in the intestinal tract of animals and humans [20], are considered an indicator organism of antibiotic resistance for many bacteria [2].

The resistance of Enterobacteriaceae isolates varied among antibiotics. The antibiotic susceptibility profile showed that isolates of *E. coli* were susceptible to tobramycin and imipenem and very resistant to amoxicillin. This result agrees with previous studies showing that isolates from urine samples were susceptible to imipenem [21]. In this study, tobramycin and imipenem proved to be the most effective antimicrobials against isolates of Enterobacteriaceae from stools in outpatients at Saint Jean Hospital. At the same time, these strains of Enterobacteriaceae showed high resistance to trimethoprim/sulfamethoxazole and amoxicillin + clavulanic acid. Our results are alarming and call for urgent measures to control the threatening development of antibacterial resistance, particularly to Amoxicillin, Trimethoprim/sulfamethoxazole, and Amoxicillin + clavulanic acid, in the region. As for the cephalosporins, 17.7%, 8.1%, 11.3%, and 14.5% of the strains were resistant to Cefuroxime, Ceftazidime, respectively, Cefotaxime, and Cefixime. Our results are contrary to those obtained by Li *et al.* [22]. These authors showed that bacteria isolated from fecal samples of patients with diarrhea were highly resistant to cefazolin, ceftriaxone, and cefuroxime. These differences in the antibiotic susceptibility pattern could be attributed to the time difference between the two studies, population variations, and significant differences in sample sizes and types. Levofloxacin, the only fluoroquinolone tested in the study, was 100% sensitive to strains isolated from *E. coli*, of which 22% were resistant. Fluoroquinolones are a widely used family of antibiotics. This family has not been spared from bacterial resistance either, the levels of which are becoming worrying. Several mechanisms may explain the resistance of EPBLSEs to fluoroquinolones. This resistance can be either chromosomal or plasmid. Chromosomal resistance is mainly manifested by the modification of the enzymatic target of the fluoroquinolones, which are DNA-gyrase and topoisomerase IV, by the reduction in the intracellular concentration of the antibiotic which is done by reducing the production of porins or by modification of the activity of various efflux pumps [22]. One of the genetic determinants of the plasmid resistance of Enterobacteriaceae to Fluoroquinolones is the qnr gene, the main characteristic of which is to be carried by a class 1 integron which is highly mobile between different plasmids [22] [23].

On the other hand, 6.45% of the Enterobacteriaceae strains were resistant to gentamicin. This record can be attributed to the use and abuse of antibiotics in the area studied. In our societies where drugs continue to be sold on street corners, where the hospital is generally the last resort after risky and sometimes disastrous self-medication, inevitably, 90% of the population has already had access to antibiotics such as amoxicillin, which would explain the acquired resistance to the latter within the population of *E. coli*. The excessive and uncontrolled use of Cotrimoxazole would also be at the origin of the resistance of 61.3% observed within the population. Indeed, sulfonamides and trimethoprim have been used for several decades as effective and inexpensive antibacterial agents in animals and humans [24]. This observation would explain the resistance to these two agents, which has spread widely and rapidly.

In this study, the prevalence of Enterobacteriaceae strains possessing the study ESBL genes was 12.9%. This prevalence is close to that found in a study conducted in Iran by Agha Mohammad *et al.* [25]. These authors showed that the prevalence rate of ESBL fecal carriage was 18.3% among patients in intensive care units and outpatients. The majority of studies have focused on humans in the context of infectious diseases and antimicrobial resistance, and we have observed a human prevalence of 20.23% in sub-Saharan Africa [26]. Fecal carriage of ESBL in patients with urinary tract infections was reported to be 35% in another study by Bazargani *et al.* [27] The incidence of fecal carriage was estimated to be high in these patients due to frequent hospital visits, frequent contact with medical personnel and devices, food intake, and antibiotics [28]. According to the literature, *E. coli* represents the most isolated ESBL-producing species. Our study confirms that *E. coli* carried 87.5% of ESBL genes. Since the 2000s, the number of publications concerning the emergence of *E. coli* possessing BlaCTX-M, BlaSHV, and BlaTEM genes has exploded [29]. Our results are contrary to those obtained by Gadou (2019) [23]. The author showed that the β-lactam resistance genes detected were the BlaCTX-M-1 (96.7%), blaTEM (67.8%), blaSHV (27.8%), and blaCTX-M-9 (5.56%). This difference in the proportions of genes between the studies may be explained by the fact that our strains are not producers of the ESBL used in each of them. The observations of a low level of resistance to Cefuroxime, Ceftazidime, Cefotaxime, and Cefixime among the strains analyzed in the present study explain this difference.

The absence of synergy on the antibiogram of the 12.9% strains carrying the blaCTX-M, blaTEM, and blaSHV genes suggests the presence of hyperproduced cephalosporins of the chromosomal or plasmid type. According to the antibiogram committee of the SFM, ESBL-producing strains would have other mechanisms of resistance to beta-lactams, such as the hyperproduction of cephalosporins; in this case, the image of synergy could have been facilitated by bringing together the cephalosporin discs and that of the clavulanic acid disc or by carrying out a standard antibiogram on Mueller-Hinton agar supplemented with 250 mg/L of Cloxacillin (inhibitor of cephalosporins) (French Society of Microbiology, 2020) [14]. Preventing and implementing measures to limit the spread of EPBLSE requires correct detection of these bacteria. Unfortunately, this detection can be complex, mainly due to the complexity of the resistance mechanisms that accumulate within the same microorganism and the variability in the expression of the corresponding resistance factors. According to Chassagne, the phenotypic detection of the mechanisms in question is increasingly complex, especially since the same resistance can be due to different associated mechanisms [19]. Our study shows the limits of the double disc synergy test for detecting ESBL production. According to some studies, this synergy test can also give false positives [13]. All this, therefore, makes the task of a medical biology laboratory easier, as it usually only has a limited number of phenotypic methods. The phenotypic characterization seems insufficient to apprehend the problem insofar as the cause of these re-

sistances resides at the level of the genes. The coexistence of blaCTX-M, blaTEM and blaSHV genes with a negative synergy test may be explained by low or silent expression of these genes, or by their functional inhibition by other resistance mechanisms [30] [31]. This discrepancy between genotype and phenotype underlines the need for an integrated approach combining molecular and phenotypic methods for reliable ESBL detection [30].

Another part of our study focused on the presence of virulence factors stx1 in strains of *E. coli* in Patients in Outpatient Consultation at Saint Jean de Dieu Hospital in Afagnan in southern Togo. The absence of this factor in strains of *E. coli* has shown their low pathogenicity for humans and, in particular, for patients. Our results are contrary to those obtained by Ranjbar *et al.* [32]. These authors showed a strong presence of the stx1 genes in the strains of *E. coli* from hospital foods. This difference could be explained by the fact that the samples are different, and our strains do not express the enzymes blaCTX-M, blaTEM, and blaSHV.

5. Conclusion

This study confirms the existence of a community reservoir of ESBL-producing Enterobacteriaceae in southern Togo. Our results reveal an absence of ESBL phenotypes in Enterobacteriaceae strains of Patients in Outpatient Consultation at Saint Jean de Dieu Hospital in Afagnan in southern Togo. Some of its Enterobacteriaceae possess the genes coding for the production of ESBLs. These results suggest community acquisition of ESBL Enterobacteriaceae at Saint Jean de Dieu Hospital in Afagnan. Suppose these carrier patients in the community are not treated. In that case, they may serve as a community reservoir of resistant pathogen potential for transmission and the spread of acquired ESBLs in the community. The low prevalence of ESBL Enterobacteriaceae in community settings makes it necessary to monitor the prevalence and transmission of Enterobacteriaceae, which possess the genes coding for ESBL production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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