Chapter

Beta-Lactamase-Producing Genes and Integrons in *Escherichia coli* from Diarrheal Children in Ouagadougou, Burkina Faso

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Abstract

This study aimed to determine the resistance of diarrheagenic *Escherichia coli* (DEC) strains to β-lactams antibiotics and to perform the molecular characterization of extended-spectrum β-lactamases (ESBLs) and integrons genes. It was carried out from August 2013 to October 2015 and involved 31 DEC strains isolated from diarrheal stools samples collected from children less than 5 years. The identification and characterization of DEC strains were done through the standard biochemical tests that were confirmed using API 20E and polymerase chain reaction (PCR). The antibiogram was realized by the disk diffusion method, then an amplification of the β-lactamase resistance genes and integrons by PCR was done. Out of the 419 *E. coli*, 31 isolates (7.4%) harbored the DEC virulence genes. From these DEC, 21 (67.7%) were ESBL-producing *E. coli*. Susceptibility to ESBL-producing *E. coli* showed that the majority of isolates were highly resistant to amoxicillin (77.4%), amoxicillin-clavulanic acid (77.4%), and piperacillin (64.5%). The following antibiotic resistance genes and integron were identified: *bla*TEM (6.5%), *bla*SHV (19.4%), *bla*OXA (38.7%), *bla*CTX-M (9.7%), *Int1* (58.1%), and *Int3* (19.4%). No class 2 integron (*Int2*) was characterized. Because of the high prevalence of multidrug-resistant ESBL organisms found, there is a need of stringent pediatric infection control measures.

Keywords: diarrheagenic, *E. coli*, extended-spectrum β-lactamases, integron, Burkina Faso

1. Introduction

Antimicrobial resistance (AMR) is one of the most serious global public health threats in this century, which is especially urgent regarding antibiotic resistance in bacteria [1], particularly in *Enterobacterales* [2]. This phenomenon has arisen globally in both nosocomial and community settings as a consequence of widespread antibiotics' consumption [3]. *Enterobacterales* are a large order of different types of bacteria including *Escherichia coli* that commonly cause infections both in healthcare settings and in communities [4]. To survive the effects of antibiotics, some *Enterobacterales* can produce enzymes called extended-spectrum β-lactamases (ESBLs) that break down and destroy some commonly used antibiotics, including penicillins and cephalosporins, and make these drugs ineffective for treating infections [4]. Over the last decade, many studies have reported the presence of extended-spectrum β-lactamases (ESBL)-mediated resistance in Gram-negative bacteria causing infections in patients [5–9]. Infections that can be caused by ESBL-producing bacteria include urinary tract infection (UTI), diarrhea, skin infections, and pneumonia [10]. Possible medications used to treat ESBL infection include carbapenems, which are useful against infections caused by *E. coli* or *Klebsiella pneumoniae* bacteria, fosfomycin, β-lactamase inhibitors, non-β-lactam antibiotics, and colistin when other medications have failed to stop the ESBL infection [10]. Unfortunately, the excessive use of antibiotics, in particular β-lactams, leads to the selection of ESBL-producing strains [11]. Because of the emergence and distribution of multidrug-resistant (MDR) *E. coli* is complicating the treatment of various serious infections [12, 13], the World Health Organization (WHO) has long recognized the need for an improved and coordinated global effort to contain AMR [1]. The burden of AMR, including MDR, varies between the regions; however, low- and middle-income countries share a disproportionate burden due to multitude of factors embedded in the characteristics of the health system, policy, and the practice [14].

In Burkina Faso, there is an emergence of β-lactam-resistant enterobacteria, both in rural and urban areas [9, 15–17]. Otherwise, carbapenemase-encoding genes are widespread in many parts of the world [18]. According to a previous study, carbapenemase-producing *Enterobacterales* (CPE) remain one of the most urgent healthcare threats [2]. To this day, the ESBLs and integrons' genes have been poorly characterized in Burkina Faso, particularly in enteric bacteria in children less than 5 years of age. However, it is imperative that bacterial isolates from underdeveloped regions undergo extensive MDR characterization to inform national strategies designed to halt the continuing spread of these dangerous pathogens [19]. Therefore, the aim of this study was to determine the resistance of diarrheagenic *E. coli* strains to β-lactams antibiotics and perform the molecular characterization of extended-spectrum β-lactamases (ESBL) and integrons genes among clinical DEC isolated from stools collected in children less than 5 years of age.

2. Methodology

2.1 Study design, area, and sample population

It is a cross-sectional study conducted in two hospitals of Ouagadougou, Burkina Faso (Paul VI and Schiphra), during August 2013 to October 2015 (**Figure 1**). The Paul VI hospital is located in peripheral area and the Schiphra's hospital in the city

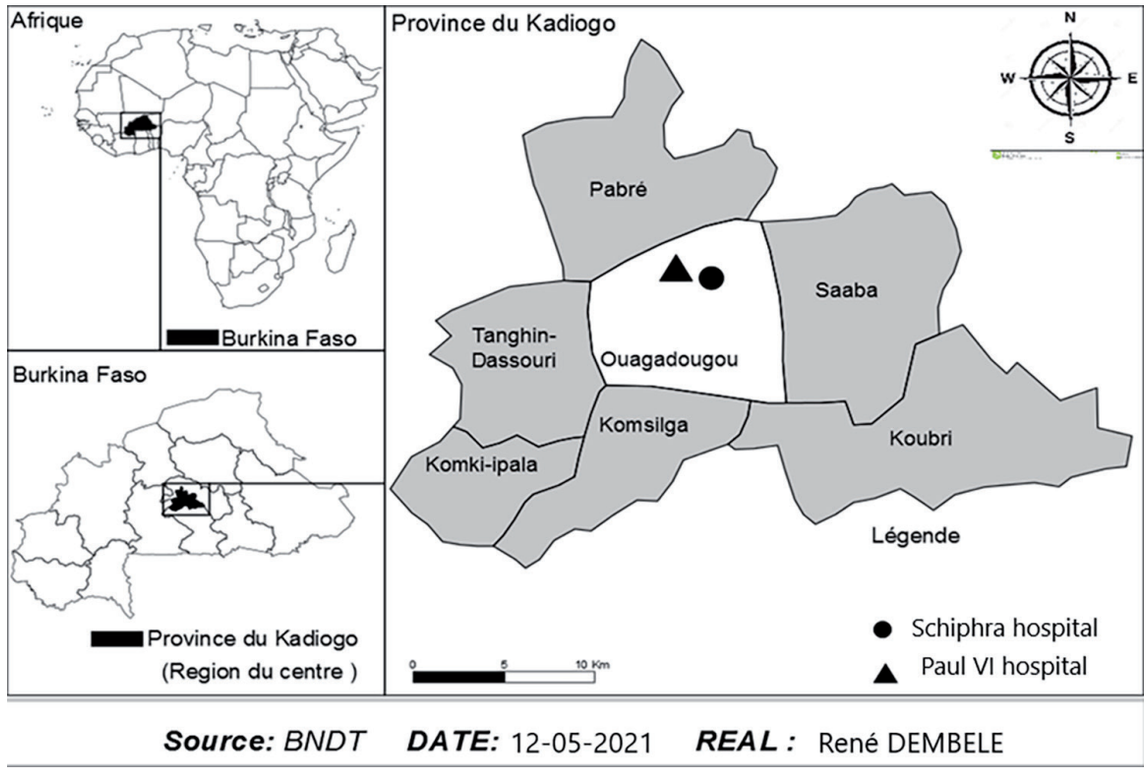

Figure 1.
Sampling sites in Ouagadougou.

center at the dam edge of Ouagadougou. Many patients from Ouagadougou and its surroundings attend these two healthcare centers because of the good level of healthcare. The study population comprised children below 5 years attending the hospital for treatment.

The specimens were collected adhering to a standard protocol from pediatric patients below 5 years of age with acute diarrhea and who were hospitalized or visited the health centers as outpatient. Thus, children who attended the hospitals for treatment and provided assent (from parents) or consent for the study were included in the study. Any child over the age of 5 years was excluded from the study.

2.2 Sample collection and transport

Three hundred and fifteen stool samples were collected in sterile containers and transported to the laboratory of molecular biology, epidemiology, and surveillance of bacteria and viruses transmitted by food, center for research in biological, food, and nutritional sciences at the Joseph KI-ZERBO University of Ouagadougou within 24 h in a cool box at +4°C for immediate analysis.

2.3 Bacterial isolates

Isolation of *E. coli* was carried out onto eosin methylene blue agar (Liofilchem, Italy), and the plates were incubated at +37°C for 18–24 h. After this stage, the suspected *E. coli* colonies were selected and streaked onto Mueller-Hinton agar plate (Liofilchem, Italy). Confirmation was carried out by a biochemical microbiology method based on negative urease (Bio-Rad, France), negative citrate (Liofilchem, Italy), positive indole (Bio-Rad, France), positive

lactose (Liofilchem, Italy), and positive orthonitrophenyl-β-D-galactopyranoside (ONPG) (bioMerieux, France). *E. coli* strains isolated were confirmed by API 20E (bioMérieux, France).

The five main pathogroups of *E. coli* (Enteroaggregative *E. coli:* EAEC, Enteropathogenic *E. coli*: EPEC, Enteroinvasive *E. coli*: EIEC, Enterohemorrhagic *E. coli*: EHEC, and Enterotoxigenic *E. coli*: ETEC) were characterized by the 16-plex polymerase chain reaction (PCR) as described by Antikainen et al. [20].

2.4 Antimicrobial susceptibility testing

All identified isolates of *E. coli* were treated for susceptibility testing against amoxicillin (25 μg), amoxicillin-clavulanic acid (20/10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cefepime (30 μg), cefixime (10 μg), piperacillin (75 μg), piperacillin-tazobactam (100 + 10 μg), imipenem (10 μg), and aztreonam (30 μg) (Bio-Rad, France) following disk diffusion method on Mueller-Hinton Agar (Liofilchem, Italy). Results were interpreted based on the European Committee of Antimicrobial Susceptibility Testing (EUCAST) guidelines [21]. These isolates, which were not susceptible (either resistant or intermediate) to three or more antibiotics classes, were considered as MDR [22].

2.5 Screening and confirmation of ESBL and integrons producers

A double synergy test was used for ESBL-producing strains testing. This consisted of placing disks (2–3 cm diameter) of ceftriaxone and cefotaxime around an amoxicillin-clavulanic acid disk on the bacterial plate.

For molecular characterization, DNA extraction was performed using heating method [23]. A loopful of bacterial growth from Mueller-Hinton agar (Liofilchem, Italy) plate was suspended in 1 ml of sterilized water. The mixture was boiled for 10 min at +100°C and centrifuged for 10 min at 12000 rpm at +4°C. Supernatant was then collected and used for the PCR reactions as DNA matrices. Multiplex PCR assays were performed for detecting EBLS-encoding genes (bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{CTX\text{-}M}$) and the presence of the class 1, class 2, and class 3 integrons from the β-lactams-resistant DEC strains. Primers (GeneCust, France) used for these amplifications are described in **Table 1**.

Thermocycling conditions were as follows: 5 min at +94°C, followed by 35 amplification cycles of +94°C for 30 s, 59 ± 4°C for 60 s, and +72°C for 60 s with a final extension of +72°C for 10 min on a thermal cycler (Gene Amp 9700, Applied Biosystems). PCR products were revealed on 1.5% stained Redsaf agarose gel (Prolabo, France), after electrophoresis under UV light (Gel Logic 200).

The PCR assays were carried out in a 25-ml reaction mixture, which consisted of 2.5 μl of the supernatant added to 22.5 μl of reaction mixture. This mixture contained 5 U of Taq DNA polymerase (Accu Power, South Korea), deoxyribonucleic triphosphate (10 mM), buffer GC (10X), $MgCl_2$ (25 mM), and PCR primers (10 μM). Thermocycling conditions were as follows: 5 min at +94°C, followed by 35 amplification cycles at +94°C for 30 s, + 59 ± 4°C for 60 s, and +72°C for 60 s with a final extension of +72°C for 10 min on a thermal cycler (AB Applied Biosystems). Following PCR, the reaction products were separated using electrophoresis in 1.5% agarose gel (weight/volume), stained with Redsaf solution (Prolabo, France), and visualized under UV light (Gel Logic 200) [23].

Genetic resistance supports	Genes	Primers sequence (5′ to 3′)	Product size (bp)
β-Lactams resistance gene	bla_{TEM}	F: ATG AGT ATT CAA CAT TTC CG	1080
		R: CCA ATG CTT ATT CAG TGA GG	
	bla_{SHV}	F: TTA TCT CCC TGT TAG CCA CC	768
		R: GAT TTG CTG ATT TCG CTC GG	
	bla_{OXA}	F: ATG AAA AAC ACA ATA CAT ATC	813
		R: AAT TTA GTG TGT TTA GAA TGG	
	bla_{CTX-M}	F: -ATG TGC AGY ACC AGT AAR GT	544
		R: -TGG GTR AAR TAR GTS ACC AGA	
Integrons	*Int1*	F: ATT TCT GTC CTG GCT GGC GA	600
		R: ACA TGT GAT GGC GAC GCA CGA	
	Int2	F: CAC GGA TAT GCG ACA AAA AGG T	806
		R: GTA GCA AAC GAC TGA CGA AAT G	
	Int3	F: GCC CCG GCA GCG ACT TTC AG	600
		R: ACG GCT CTG CCA AAC CTG ACT	

Table 1.
List of all primers used for antibiotic ESBL genes and integrons detection.

2.6 Statistical analysis

The Fisher's exact test with two-tailed p of Open Epi version 7.1.2.0 was used to determine the statistical significance of the results. A p value of <0.05 was considered statistically significant.

3. Results

3.1 Prevalence of bacterial isolates

From 315 children with diarrhea, 192 stool samples were positive to one suspected *E. coli* detection (60.9%). Four hundred and nineteen (419) strains of *E. coli* were isolated, from which 31 DEC (7.4%) were characterized. From these DEC, 21 DEC were ESBL-producing *E. coli* (67.7%).

3.2 Antimicrobial susceptibility

All the DEC strains tested for the 10 β-lactams antibiotics showed important resistances to the aminopenicillins. However, few cephalosporins and carbapenems were yet active on some pathotypes (**Table 2**).

3.3 Correlation between resistance phenotype and resistance genetic supports

Nineteen (19) out of the 21 ESBLs-producing *E. coli* (90.5%) had ESBLs genes. The following resistance genes were characterized: 12 bla_{OXA} (38.7%), 6 bla_{SHV} (19.4%), 3

β-Lactams subfamilies	Antibiotics	Prevalence of antibiotic susceptibility N (%)		DEC resistance prevalence N (%)				
		Resistant	Sensitive	EPEC (*n* = 8)	EHEC (*n* = 3)	EIEC (*n* = 4)	EAEC (*n* = 15)	ETEC (*n* = 1)
Penicillins	Amoxicillin	24 (77.4)	7 (22.6)	6 (76)	2 (66.6)	4 (100)	11 (73.3)	1 (100)
	Amoxicillin-clavulanic acid	24 (77.4)	7 (22.6)	6 (76)	2 (66.6)	4 (100)	11 (73.3)	1 (100)
	Piperacillin	20 (64.5)	11 (35.5)	5 (62.5)	2 (66.6)	3 (75)	9 (60)	1 (100)
	Piperacillin-tazobactam	12 (38.7)	19 (61.3)	3 (37.5)	2 (66.6)	1 (25)	5 (33.3)	1 (100)
Cephalosporins	Ceftriaxone	13 (41.9)	18 (58.1)	2 (25)	1 (33.3)	2 (50)	7 (46.6)	1 (100)
	Cefixime	13 (41.9)	18 (58.1)	2 (25)	1 (33.3)	2 (50)	7 (46.6)	1 (100)
	Cefotaxim	14 (45.2)	17 (54.8)	2 (25)	2 (66.6)	2 (50)	7 (46.6)	1 (100)
	Cefepim	14 (45.2)	17 (54.8)	2 (25)	2 (66.6)	2 (50)	7 (46.6)	1 (100)
Monobactam	Aztreonam	14 (45.2)	17 (54.8)	2 (25)	2 (66.6)	2 (50)	7 (46.6)	1 (100)
Carbapenems	Imipenem	5 (16.1)	26 (83.9)	1 (12.5)	1 (33.3)	0 (0)	3 (20)	0 (0)

Table 2.
Antimicrobials susceptibility of the studied isolates to β-lactams.

DEC strains	Resistance phenotypes	Antibiotics										Genetic resistance supports						
												Resistance genes				Integrons		
		AMC	AMX	CTX	ATM	IPM	CRO	FEP	CFM	TZP	PIP	*bla*$_{TEM}$	*bla*$_{SHV}$	*bla*$_{OXA}$	*bla*$_{CTX-M}$	*IntI1*	*IntI2*	*IntI3*
Schiphra's hospital (*n* = 9/18; 50%)																		
EHEC	ESBL, Carbapenemase	R	R	R	R	R	-	R	-	R	R	-	+	+	+	+	-	-
aEPEC	ESBL	R	R	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
tEPEC	ESBL	R	R	R	R	-	R	R	R	R	R	-	-	+	-	+	-	-
tEPEC	ESBL	R	R	-	-	-	-	-	-	R	R	-	+	-	-	+	-	-
EIEC	ESBL	R	R	-	-	-	-	-	-	-	R	-	+	-	-	+	-	+
EAEC	ESBL	R	R	-	-	-	-	-	-	-	R	-	+	-	-	+	-	-
EAEC	ESBL	R	R	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
EAEC	ESBL, Carbapenemase	R	R	R	R	R	R	R	R	R	R	-	-	-	+	+	-	-
EAEC	ESBL	R	R	R	R	-	R	R	R	R	R	-	-	+	-	+	-	-
Paul VI hospital (n = 10/13; 76.9%)																		
aEPEC	ESBL, PHN	R	R	-	-	-	-	-	-	-	R	-	-	+	-	+	-	-
aEPEC	ESBL, Carbapenemase	R	R	R	R	R	R	R	R	R	R	-	-	+	-	+	-	-
aEPEC	ESBL, PHN	R	R	-	-	-	-	-	-	-	R	-	-	+	-	+	-	-
EIEC	ESBL, PHN	R	R	R	R	-	R	R	R	R	R	-	+	+	-	-	-	+
EAEC	ESBL, PHN	R	R	-	-	-	-	-	-	-	R	-	-	+	-	+	-	-
ETEC	CASE, PHN	R	R	R	R	-	R	R	R	R	R	-	-	+	-	+	-	-
EAEC	ESBL, PHN	R	R	-	-	-	-	-	-	-	R	-	-	+	-	+	-	-

DEC strains	Resistance phenotypes	Antibiotics										Genetic resistance supports						
												Resistance genes				Integrons		
		AMC	AMX	CTX	ATM	IPM	CRO	FEP	CFM	TZP	PIP	*bla*$_{TEM}$	*bla*$_{SHV}$	*bla*$_{OXA}$	*bla*$_{CTX-M}$	*IntI1*	*IntI2*	*IntI3*
EAEC	ESBL, PBN	R	R	R	R	-	R	R	R	-	S	+	-	-	-	+	-	-
EAEC	ESBL, Carbapenemase	R	R	R	R	R	R	R	R	R	R	-	-	+	+	+	-	-
EAEC	ESBL	R	R	R	R	-	R	R	R	-	-	-	-	+	-	+	-	-

EAEC = Enteroaggregative E. coli, aEPEC = Atypical Enteropathogenic E. coli, tEPEC = Typical Enteropathogenic E. coli, EIEC = Enteroinvasive E. coli, EHEC = Enterohemorrhagic E. coli, ETEC = Enterotoxigenic E. coli, PHN = High-level penicillinases, CASE = Cephalosporinases, ESBL = Extended-spectrum β-lactamases, AMC = Amoxicillin-Clavulanic Acid, AMX = Amoxicillin, CTX = Cefotaxime, ATM = Aztreonam, IPM = Imipenem, CRO = Ceftriaxone, FEP = Cefepime, CFM = Cefixime, TZP = Piperacillin-tazobactam, PIP = Piperacillin, R = Resistant, − = Absence, + = Presence.

Table 3.
Correlation between E. coli pathotypes, antibiotics resistance, and genetic resistance supports.

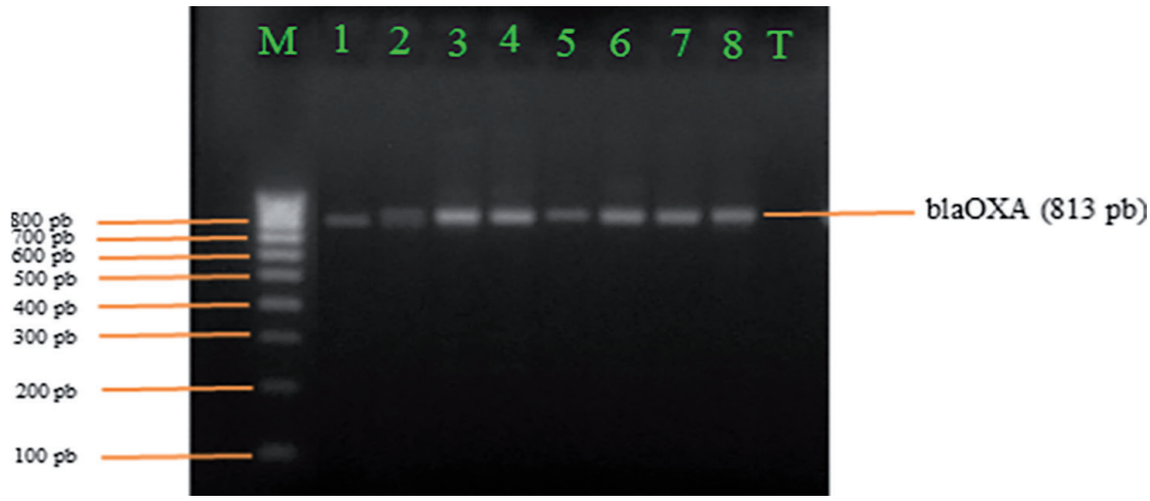

Figure 2.
bla_{OXA} gene on agarose gel electrophoresis (1.5%). Lane M: molecular size marker (100 bp), 1: bla_{OXA1}: positive control (813 pb), lanes: 2–8 are positive for bla_{OXA} gene, lane T: negative control.

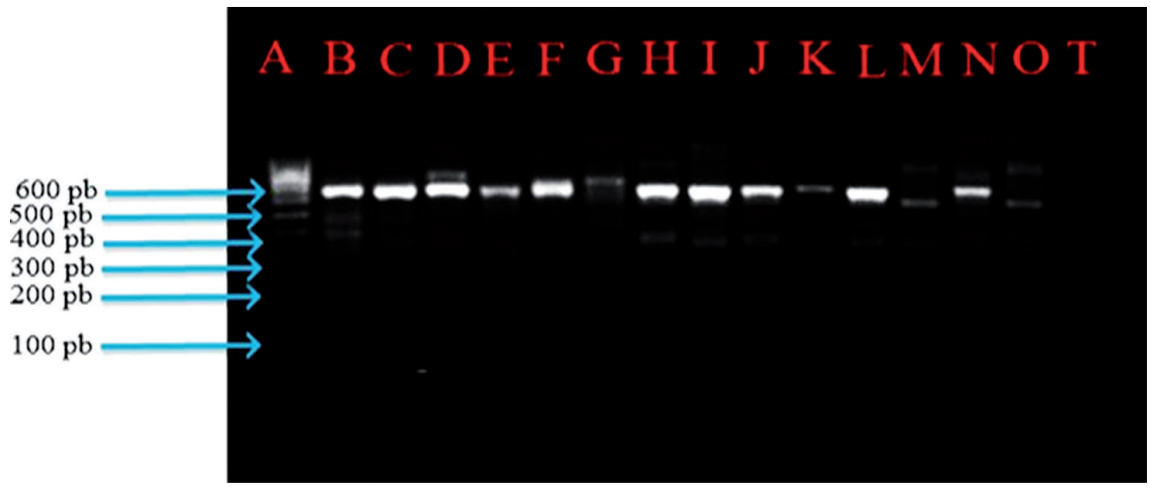

Figure 3.
Int1 gene on agarose gel electrophoresis (1.5%). Lane A: molecular size marker (100 bp), B: Int1: positive control (600 pb), lanes: C-O are positive for Int1 gene, lane T: negative control.

$bla_{CTX\text{-}M}$ (9.7%), and 2 bla_{TEM} (6.5%). Our results showed that the genes responsible for the production of bla_{OXA} β-lactamases 12/31 (38.7%) were more prevalent in comparison to the genes encoding bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ β-lactamases (**Table 3**). From the three classes of integrons (*Int1, Int2*, and *Int3*) assessed among the resistant strains carrying ESBL genes, only 18 *Int1* (58.1%) and 2 *Int3* (19.4%) were detected. The class 3 integron was detected in only EIEC. No class 2 integrons (*Int2*) were characterized from the resistant strains. The coexistence of the three resistance genes (bla_{SHV}, bla_{OXA}, and $bla_{CTX\text{-}M}$) and *Int1* was found in one EHEC (**Table 3**). The bla_{OXA} gene (**Figure 2**) was associated with *Int1* (**Figure 3**) in 11 cases ($p = 0.001$), while the bla_{SHV} gene was associated with *Int1* in 5 cases ($p = 0.100$).

4. Discussion

The emergence and spread of multidrug-resistant (MDR) bacteria are major public health threats worldwide. Particularly, DEC that produce ESBL are of great

concern, because their resistance to penicillins and narrow extended-spectrum cephalosporins reduces considerably the treatment options. The prevalence of ESBL in *Enterobacteriaceae* has been detected at local levels in various African countries; moreover, a study was conducted in 2014 on the prevalence of ESBL and what type of genes are involved in its occurrence [24]. The frequency of ESBL-producing *E. coli* was 67.7% in our study. Similar prevalence was reported in Egypt (69.6%) [25] and Palestine (66.7%) [26]. Nevertheless, our prevalence was higher than those in Burkina Faso (58%) [6], Iran (40.8%) [27], Saudi Arabia (30.6%) [28], Japan (20.4%) [29], Colombia (11.7%) [30], and Nepal (22.7%) [31]. Otherwise, our result is lower than the ESBL production in clinical isolates of *E. coli* reported somewhere else in Iran [32]. The prevalence of ESBL resistance in *E. coli* isolates in European countries is reported to be around 3.9% with variations between countries [33]. Overall, these percentages are lower than those found in middle-income countries like Thailand (71.25%) [34] and China (50.5%) [35]. This difference between ESBLs' prevalence might be due to patient's age, the type of samples, and the country health facilities in the management of diarrheal infections regarding antibiotics use. Indeed, in developing countries, most patients received antibiotics treatment without prescription [36, 37]; such common practices in nearly all developing countries cause a selective pressure on *E. coli*, whereas in more developed countries effective strategies for the control of antimicrobial are present, which effectively prevents the emergence of ESBLs [36].

It has been reported that bacteria such as *E. coli* and *K. pneumoniae* are major ESBL producers resulting in serious threat to the treatment regimen [38]. Indeed, ESBL enzymes are becoming increasingly expressed by many strains of pathogenic bacteria presenting diagnostic challenges to the clinical microbiology laboratories [39, 40]. Until recently, antimicrobial therapy has played an important role in the treatment of human bacterial infections. However, the drug resistance has emerged in the treatment of bacterial infections due to ESBL enzymes [39]. Indeed, these enzymes can degrade all β-lactam antibiotics leading to multidrug-resistant bacteria. Therefore, reporting of ESBL-producing isolates from clinical samples is critical for the clinicians. It constitutes the guidelines to select appropriate antibiotics for the treatment, including to take proper precaution to prevent the spread of these resistant organisms to other patients [31].

The present study shows 19 ESBLs genes (90.5%) out of the 21 ESBLs-producing *E. coli*. Analysis of the ESBL-encoding genes indicated that the majority of the ESBL-positive isolates harbored bla_{OXA} (38.7%), followed by bla_{SHV} (19.4%), $bla_{\text{CTX-M}}$ (9.7%), and bla_{TEM} (6.5%). The emergence of β-lactam resistance in *Enterobacteriaceae* is related primarily to the production of enzymes such as TEM and SHV variant, which were the most common ESBLs during the past decade. However, OXA and CTX-M β-lactamases have emerged as prevalent ESBL worldwide type compared with the TEM and SHV genotypes [41].

In the present study, OXA-type ESBL-producing DEC strains (38.7%) were the most frequently detected ESBL gene. This prevalence is lower than that reported in our previous study in rural area of Burkina Faso: 100% [9], also lower comparatively to 52% reported in Pakistan [42]. However, a recent study in young children reported 3% of commensal *E. coli* bearing the bla_{OXA} gene in Bangladesh [41]. Thus, it appears that the emergence of ESBLs-producing bacteria among gut bacteria of young children can transfer resistance and related genes horizontally across pathogenic *E. coli*, and commensal *E. coli* leading to a public health concern. Most of the OXA-type ESBL-producing *E. coli* isolates (29%) in our study were detected from the Paul VI hospital ($p = 0.002$). This hospital is located in peripheral area

of Ouagadougou, and most of the people living in the slums with poor sanitation conditions attend it for healthcare sought. Moreover, the provision of confessional care has less difficult accessibility for the peripheral neighborhoods and the population with low socioeconomic level. Otherwise, people in Burkina Faso do not consult a healthcare agent in the case of diseases such as gastrointestinal infections and use self-medication instead [37]. Our results showed 19.4% of SHV-type ESBL-producing *E. coli* which is a little similar to 21% detected in Pakistan [42]. By cons, this prevalence is higher than 0% [9] and 5.9% [17], previously reported in Burkina Faso but lower than 45% reported in Iran [27]. The $bla_{\text{CTX-M}}$ gene (**Figure 4**) has been detected in three *E. coli* isolates, while its prevalence was 25% in our earlier report [9] and 40.1% by a study conducted in *Enterobacteriaceae* from Burkinabe patients [17]. Moreover, few studies from other parts of world have shown different prevalence of $bla_{\text{CTX-M}}$ gene among isolates, including 98.8% (China), 84.7% (Chile), 13.6% (Tanzania), 76% (Pakistan), 97.8% (Chad), and 81.6% (Egypt) [25, 42–46]. Indeed, CTX-M β-lactamases are recognized as the most widespread extended-spectrum β-lactamases (ESBLs) among clinical isolates of *Enterobacteriaceae* [47]. Besides, an earlier report from Nigeria has shown the predominance of CTX-M15 in wild birds and cattle in Nigeria [48] suggesting that this gene could be transferred to humans by animals. Finally, our study revealed 6.5% of TEM-type ESBL-producing *E. coli*, while no bla_{TEM} gene has been detected in our previous study [9]. However, this value is lower than 26.2 and 28% reported in Burkina Faso and Pakistan, respectively [17, 42]. The resistance to amoxicillin/amoxicillin-clavulanic acid observed in the two *E. coli* strains (6.5%) may be mainly mediated by the production of these plasmid-encoded TEM enzymes.

Among the three class of integron, class 1 integron (58.1%) was majority characterized from the resistant strains in accordance with 56% reported in Bangladesh [41]. This result confirms those of previous studies showing that class 1 integron was predominantly represented in *Enterobacteriaceae* [49, 50]. However, a previous report in Burkina Faso has shown a lower prevalence (44.4%) of *Int1* [51]. On the other hand, studies reported a high prevalence of *Int1* (80%) in *E. coli* isolated from dairy products consumed in Burkina Faso [52] and in human, animal, and food in Spain [53]. This could increase the risk of emergence and spread of MDR *E. coli*, since humans are always in contact with these

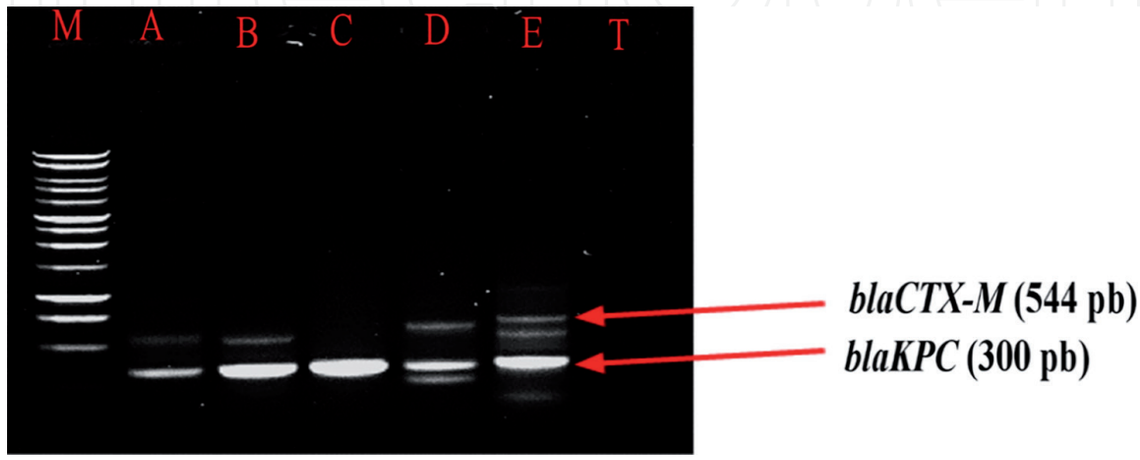

Figure 4.
$bla_{\text{CTX-M}}$ gene on agarose gel electrophoresis (1.5%). Lane M: molecular size marker (100 bp), A: $bla_{\text{CTX-M}}$ positive control (544 pb), lanes: B, D, and E are positive for $bla_{\text{CTX-M}}$ gene (544 pb), lane T: negative control.

different ecosystems, especially when there is a lack of food hygiene and sanitation. Moreover, class 1 integrons can facilitate the spread of antibiotic-resistant genes meaning that it could have public health consequences [54].

The class 3 integron was detected in only EIEC. No class 2 integron (*Int2*) was characterized from the resistant strains. By cons, 22.2% of *Int2* was detected in our previous study [51]. Moreover, a study also found the presence of *Int2* gene in Senegalese *Shigella* spp. isolates [49].

Two strains of EIEC harbored both class 1 and 3 integrons. However, a previous study showed that *E. coli* harbored class 1 and 2 integrons simultaneously [50]. Otherwise, in the present study, one EIEC strain was resistant to aztreonam and imipenem and possesses ESBL-carbapenemase phenotype. This strain was resistant to all subfamilies (penicillins, cephalosporins, monobactam, and carbapenems) of β-lactams antibiotic tested and also showed simultaneous presence of bla_{SHV}, $bla_{OXA,}$ bla_{CTX-M}, and *Int1*. Indeed, strains that had this aztreonam-resistant phenotype possessed both the resistance gene [27]. Resistance to this antibiotic could be explained by genetic mutations [43]. It has been described that the coexistence of these two classes of integrons [42] and/or several genes suggests that they have integrated the same gene and give these strains a high level of resistance. However, bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CTX-M} as well as integrons (*Int1, Int2*, *and Int3*) are involved in the antibiotic resistance of DEC, but the presence of resistant strains producing ESBL and lacking ESBL gene (bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{CTX-M)}$ and integron suggests that there are other mechanisms for the dissemination of antibiotic resistance in DEC strains.

5. Conclusion

This study highlights the important involvement of genes and integrons into multidrug resistance strains of *E. coli* in two main hospitals of Ouagadougou. The most important finding was the detection of four *E. coli* multiresistant strains producing ESBL that were resistant to imipenem, aztreonam, and harbored class 1 integrons. Another important observation was the detection of two *E. coli* multiresistant strains producing ESBL but lacking a resistance gene and/or integrons. Our results have demonstrated the emergence and dissemination of multidrug-resistant *E. coli* strains hosting several genes responsible for the production of ESBL in clinical isolates. Ultimately, to fight effectively against the emergence of antimicrobial resistance, an integrated surveillance network should be set up, which would be of great benefit to national antimicrobial resistance control programs.

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