Potential Pathogenicity of *Escherichia coli*Isolated from the Stools of Healthy Children Suffering from Diarrhea Admitted to Hospitals in Southern Benin

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ABSTRACT

Aims: Infant mortality linked to infectious diseases remains a major public health concern, especially in the context of antibiotic resistance. This work aimed to study the potential pathogenicity of *Escherichia coli* strains isolated from the stools of children suffering from diarrhea and healthy from 0 to 15 years old admitted to hospitals in southern Benin.
Methodology: The isolation and characterization of the strains were carried out according to standard microbiology methods. Molecular characterization was carried out by searching for eight virulence factors (Stx1, Stx2, eaeA, bfpB, LT, InVE, IpaH, AggR) and three antibiotic resistance genes (blaTEM, blaSHV, blaCTX -M) by the PCR technique.
Results: This study shows that 47.06% of the children sampled suffered from diarrhea, against 52.94% who were healthy. A total of 9 species of Enterobacteriaceae were identified in different proportions: *Escherichia coli* (66.66%), *Klebsiella ornithinolytica* (13.72%), *Klebsiella pneumonia* (5.88%), *Enterobacter aerogenes* (3.92%), *Escherichia vulneris* (1.96%), *Salmonella arizonae* (1.96%), *Salmonella* spp (1.96%), *Enterobacter sakazakii* (1.96%), *Serratia fonticola* (1.96%). All *E. coli* showed total resistance to amoxicillin, ceftazidime, and cefotaxime. In addition, they showed variable resistance to norfloxacin (95.33%), trimethoprim (83.33%), and sulfamethoxazole (95.24%). 70.83% of E. coli strains isolated from apparently healthy children carry resistance genes blaTEM (12.5%), blaSHV (25%), and blaCTX-M (33.33%). While 66.67% of strains of E. coli isolated from children with diarrhea carry resistance genes blaTEM (12.5%), blaSHV (37.5%), and blaCTX-M (16.67%). Only EPEC was isolated and only in diarrheal children, with a frequency of 20.83%.
Conclusion: The relatively high prevalence of ECD in this study is remarkable, and it highlights the fact that *E. coli* is an important agent of infectious diarrhea and could be the leading cause of gastroenteritis in Benin.

Keywords*: Pathogenic E. coli, antimicrobial resistance, molecular characterization, southern Benin.*

1. INTRODUCTION

"Diarrhea is one of the leading causes of mortality and morbidity in children, especially in developing countries" [1]. "It is the second leading cause of death in children under 5 worldwide" [2]. "Of the 5.9 million child deaths (<5 years) in 2015, 0.53 million were due to diarrhea" [3]. "It is estimated that about 9% of the 1.2 million annual child deaths (<5 years) in India can be attributed to various forms of diarrhea" [3]. "According to the WHO/UNICEF Child Health Epidemiology Reference Group, more than half of diarrhea deaths are caused by rotavirus, calicivirus, and diarrheal *Escherichia coli* (DEC)" [4]. "DEC members, such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC), are responsible for 30-40% of all diarrheal episodes in developing countries"[5].

"ETEC is the leading cause of travelers' diarrhea and is endemic in underdeveloped countries" [6].

"In general, species of *E. coli* are involved as commensal bacteria in the human gastrointestinal tract. Commensal *E. coli* provide several benefits to their human host,This includes protection against epithelial cell damage" [7]. "*E. coli* proteins activate animal and human digestion via neuronal dopamine and the NLP-12-CKR-1 axisand regulatedhost fat storage" [8]. "E. coli induces host immune defence against pathogens, strengthens the intestinal barrier"[9]. "However, by acquiring and combining virulence and antibiotic resistance factors, these ordinarily harmless commensal strains can become pathogenic and cause various diseases. Based on the type of virulence factor present, and the host's clinical symptoms, E. coli strains are classified into pathotypes of zoonotic intestinal pathogenic E. coli (IPEC) or extraintestinal pathogenic E. coli (ExPEC). The strains of Extraintestinal pathogenic *E. coli* (ExPEC) are common pathogens, causing infections of varying severity" [10].

"According to statistics from the Demographic and Health Survey in Benin, the infant mortality rate due to diarrhea remains very high" [11]. Acute diarrhea in children remains a public health problem. Although the microbiological, clinical, and epidemiological aspects of pediatric diarrhea have been monitored in Benin by the national laboratory of the Ministry of Health for years, no attempt has been made to understand the pathogenicity of diarrheal Escherichia coli strains in children. Thus, to improve the management of diarrheal diseases, it is essential to know the virulence potential (levels of antimicrobial resistance, virulence, and capacity for biofilm formation) in strains of diarrheal Escherichia coli. Therefore, this study aimed to investigate the pathogenicity of strains of *E. coli* isolated from the stools of apparently healthy children suffering from acute diarrhea admitted to hospitals in southern Benin.

2. MATERIAL AND METHODS

2.1. Sample collection

Pediatric patients (<15 years old) suffering from acute diarrhea attended/were admitted to the Center Hospitalier Universitaire de la Mère et de l'Enfant-Lagune (CHU-MEL), at the level of the pediatric clinic of Abomey-Calavi and the Center de Santé of Tangbo-Djèvié between January 2022 and May 2022 were included in the study. Fifty-one diarrheal stool samples were collected from patients with and without diarrhea in some private and public medical establishments in Cotonou, Abomey-Calavi, and Zè communes. Patients admitted to the health facilities or those who presented to consultation with complaints of diarrhea were recruited into the study during the single sampling procedure. Patients with symptoms of diarrhea, defined as passing three or more watery or loose stools per day or passing more frequently than usual for the individual, were enrolled in the study. Asymptomatic patients were also recruited in this study. Fresh samples were taken directly from patients who had just passed liquid stools at the time of collection and from the anorectal cavity of some patients who were still passing runny stools but not at the time of collection using sterile swabs. Patients who had started treatment or taken antibiotics within the previous month were excluded from the study.

2.2. Microbial analysis

For isolation of DEC, stool specimens were plated on Chromogenic Medium Agar followed by 18-24 h incubation at 37°C. After 24 h of incubation, the typical colonies of lactose fermentation with a different colony morphology per sample were subcultured on eosin agar with methylene blue (EMB). Three to five distinct lactose fermentation colonies with different colony morphology per sample were selected and subcultured in Mueller Hinton Agar (Becton, Dickinson and Company, Becton Drive Franklin Lakes, NJ 07417-1880. United

States- United). The cultures of this non-selective medium were used for biochemical identification with the API 20E gallery as used by Socohou et al. [12].

2.3. Antibiotic sensitivity test

It was evaluated by the agar diffusion methodinspired by that used by [13]. Each bacterial inoculum was standardized (10^6 CFU/ml) after a 10-2 dilution of Mc Farland's solution and then inoculated by flooding (1 ml) of the surface of the MH agar, already poured and solidified, then a micropipette recovered the excess inoculum. The plates were left to dry at laboratory temperature for 15 min. The antimicrobial agents that were used in this study are Amoxicillin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), norfloxacin (10 µg), ceftazidime (10 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The inhibition diameters were read 24 hours after incubation at 37°C, and the results were interpreted according to the recommendations of the CA-SFM [14].

2.4. Biofilm test

It was carried out according to the in vitro method inspired by that usedby , allowing a semi-quantitative evaluation of the biofilm formation [15]. Thus, from an 18-hour culture in BHI medium (brain heart broth), the wells of a 48-well microplate (polystyrene) are inoculated with 10 µl of diluted suspension (1/10) of bacteria to which are added 150 µl of BHI. The microplates are incubated for 24 hours at 37°C. The wells are washed three times with 0.2 ml of sterile physiological water to eliminate free bacteria (planktonic). The biofilms formed by the adhesion of sessile organisms to the polystyrene support in each of the wells are stained with crystal violet (0.1%) for 10 min. The Crystal Violet was removed by inverting the plate onto paper towels followed by a rinse with saline to remove residuel Crystal Violet and the plates are left at room temperature [16].

2.5. Molecular detection of resistance and virulence genes

2.5.1 Extraction of bacterial DNA from strains

DNA extraction was performed using the method adapted from Rasmussen and Morrissey [17]. The conserved strains were cultured on MH agar and then incubated at 37°C for 24 hours. DNA was extracted from 24-hour bacterial cultures. To do this, a 24-hour preculture was carried out in 1ml of liquid Muller Hinton in an Eppendorf tube. After 24 h, the precultures were centrifuged at 12,000 rpm for 5 min, and the supernatant was discarded. Then, 500 µL of 1X TEB was added to the pellet, and the whole was mixed by several pipetting with a wide-tipped cone (1000 µl blue cone) and heated in a dry bath at 100°C for 30 min. Centrifugation at 12000 rpm for 5 min was performed, followed by recovery of the supernatant in another Eppendorf tube, and 500 µl of 96° alcohol kept at 40 C was added to the mixture. Finally, the mixture was centrifuged at 12,000 rpm for 5 min. After centrifugation, the supernatant was drained and allowed to air dry. After drying, the DNA pellets were suspended in 50 µl of sterile distilled water and then stored at 4°C for immediate use or at -20°C for long-term storage.

2.5.2Detection of genes coding for the ESBL phenotype in *Enterobacteriaceae*

2.5.2.1 Polymerase chain reaction

The polymerase chain reaction (PCR) allows the amplification of many copies of a particular DNA sequence. This polymerization reaction is carried out in a reaction mixture containing small amounts of DNA, two nucleotide primers complementary to the target sequence to be amplified, DNA polymerase (Taq), and a mixture of the four dNTPs (dATP, dTTP, dCTP, and dGTP). Each PCR cycle consists of three steps. It begins with a step of thermal denaturation

of the DNA to be amplified to separate the two complementary strands that compose it. This step is followed by annealing the primers to the ends of the desired sequence. The elongation or elongation of the primers in the 5'--> 3' direction closes the cycle thanks to the action of DNA polymerase (Taq). This cycle is repeated many times to obtain many target DNA sequences at the end of the reaction [18].

The polymerase chain reaction is carried out in a reaction mixture that includes the DNA extract (matrix DNA), Taq DNA polymerase, primers, and the four excess deoxyribonucleoside triphosphates (dNTPs) in a buffer solution. The reaction mixture was carried out in a total volume of 20 µl containing 2 µl of 10X buffer, 2 µl of MgCl (25 mM), 0.5 µl of dNTP (10 mM), 1 µl of each primer, 0.5 µl of Taq polymerase (5 U/µl) and 5 µl of sample DNA. The final volume was adjusted to 20 µl with sterile distilled water.

This same reaction mixture is carried out for each housekeeping gene sought. A conventional PCR reaction was carried out for each gene in 35 cycles in a thermo-cycler (Prime Thermal Cycler). The PCR program is shown as follows in Table 1.

Table 1: PCR program used for amplifications

Step	Temperature	Time	Nb of cycle
Initial denaturation	94°C	5min	1
Denaturation	94°C	45s	**35 cycles**
Hybridation	52°C	30s	
Extension	72°C	30s	
Final extension	72°C	10min	1

2.5.2.2Deposit on 1% agarose gel

Once the agarose gel was prepared, the product from the reaction medium was distributed into the wells. Thus, 7µl of molecular weight marker was deposited in the first well. Then, the remaining wells received a mixture of 10µl of PCR products + 2µl of loading buffer.

2.5.2.3.Agarose gel electrophoresis

The PCR products were migrated by electrophoresis on a 1% agarose gel containing ethidium bromide at the final concentration of 1 µg/ml and buffered with 1X TBE (Tris Borate Ethylene Diamine Tetra-acetic Acid). The migration was carried out in a constant volume of 1X TBE buffer under a constant voltage of 100 V for about 30 min. The gel was visualized using a UV table (Figure 6). A 100-base pair molecular weight marker (GeneRuler) was used. The blaTEM, blaSHV, and blaCTX-M primers were used to detect ESBL-producing Enterobacteriaceae strains. The various primers used are distributed in Table 2 below.

Table 2: Primers used for the polymerase chain reaction

Genes	Primer	Sequences	Reference
***bla*$_{TEM}$**	OT-F	5'-ATTGGGTGCACGAGTGGGTTAC-3'	[19]
	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'	

2.5.3Search for virulence factors in *E. coli*

The delineation of isolates of *E. coli* confirmed in respective pathotypes was performed by PCR using specific primer pairs (Table 3) targeting the relevant virulence genes of EHEC,

EPEC, ETEC, EIEC, EAEC, and DAEC pathotypes, as shown in Table 3. The reaction mixture (20µL) contained 2 µl of 10X buffer, 2 µl of MgCl (25 mM), 0.5 µl of dNTP (10 mM), 1 µl of each primer, 0.5 µl of Taq polymerase (5 U/µl) and 5 µl of the DNA sample. The final volume was adjusted to 20 µl with sterile distilled water. The cycling conditions were as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles at 95°C in 60 sec, Hybridization (68°c for stx2, 66°c for stx1 and LT, 70°C for InVE and IpaH, 65°C for bfpB and aggR finally 62°C for eae) as indicated in Table 1 for the respective primers for 30 sec, 72°C (extension) for 60 sec, and a final elongation step at 72°C for 5 mins. PCR products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium bromide and visualized using a UV stable.

Table 3: Sequences of the specific primers for the search for the virulence genes of *E. coli*

Pathogens	genes	Primer sequences (5'→3')	Size	References
Shiga toxin-producing enterohemorrhagic *E. coli (*EHEC*)*	*eaeA*	F: CACACGAATAAACTGACTAAAATG R: AAAAACGCTGACCCGCACCTAAAT	376	[20, 21]
	Stx1	F: CGATGTTACGGTTTGTTACTGTGACAGC R: AATGCCACGCTTCCCAGAATTG	244	
	Stx2	F: GTTTTGACCATCTTCGTCTGATTATTGAG R: AGCGTAAGGCTTCTGCTGTGAC	324	
Enteropathogenic *E. coli* (EPEC)	*Eae*	F: TCAATGCAGTTCCGTTATCAGTT R: GTAAAGTCCGTTACCCCAACCTG	482	
	bfpB	F: GACACCTCATTGCTGAAGTCG R: CCAGAACACCTCCGTTATGC	910	
Enterotoxigenic *E. coli* (ETEC)	*LT*	F: GAACAGGAGGTTTCTGCGTTAGGTG R: CTTTCAATGGCTTTTTTTTGGGAGTC	655	
Enteroinvasive *E. coli* (EIEC)	*InvE*	F: CGATAGATGGCGAGAAATTATATCCCG R: CGATCAAGAATCCCTAACAGAAGAATCAC	766	
	ipaH	F: GAAAACCCTCCTGGTCCATCAGG R: GCCGGTCAGCCACCCTCTGAGAGTAC	437	
Enteroaggregative *E. coli* (EAEC)	*AggR*	F: ACGCAGAGTTGCCTGATAAAG R: AATACAGAATCGTCAGCATCAGC	400	

2.6. Data processing and analysis

Data encoding was performed with Microsoft Excel 2013 spreadsheet software. Graph Pad Prism 8 software was used to determine significant differences between the calculated means at the 5% threshold ($p < 0.05$).

Diarrheal effusions in children were analyzed according to the characteristics of resistance genes, virulence factors, and biofilm production. Cross tables and the Chi2 statistical test were used to analyze the existing relationships between these variables and those representing the occurrence of diarrhea.

This study made it possible to explain the influence of resistance genes, virulence factors, and biofilm production in the occurrence of diarrhea in children. The dependent variable in this study is the occurrence of diarrhea. The correlation between the variables was studied by calculating the Spearman correlation coefficient. When two variables were strongly correlated with each other, one of them was eliminated from the list of predictors. The variables selected were used as predictors in the logistic regression procedure to identify the probable causes of diarrhea in children.

The relationship between the dependent and independent variables is not a linear function but rather the Logit transformation of y and is expressed as follows: $\text{Logit}[y(x)] = \alpha + \beta 1x1 + \beta 2x2 + \ldots + \beta ixi$ Where α = the constant of the equation and β = the coefficient of the independent variables.

Analyses were performed using R version 4.2.1 software.

3. RESULTS AND DISCUSSION

3.1.Results

3.1.1. Sociodemographic characteristics of the children sampled

Children aged 5 years are the most recorded (17.64%) in this study, while those of 1, 2, 4, 5.8, and 9 months are the least represented. The most advanced age is 7 years (i.e., 3.92%). 56.86% of the samples was collected from boys against 43.14% from girls, i.e., a male/female sex ratio of 1.32. Figure 1 below shows the children sampled according to age distribution.

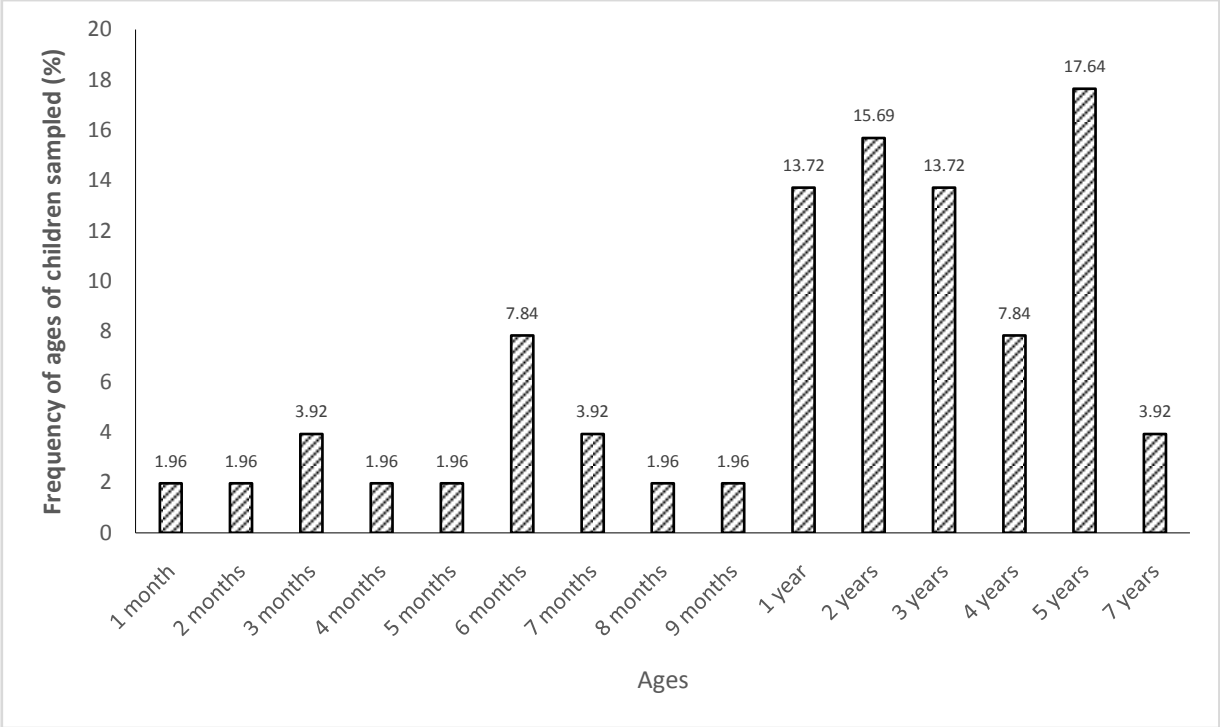

Figure 1: Distribution of children sampled according to their ages

3.1.2. Medical-clinical aspects

On the medico-clinical level, a rate of 47.06% of the children sampled suffered from diarrhea against a proportion of 52.94% who were healthy. Figure 2 below shows the distribution of the children tested according to their health aspect.

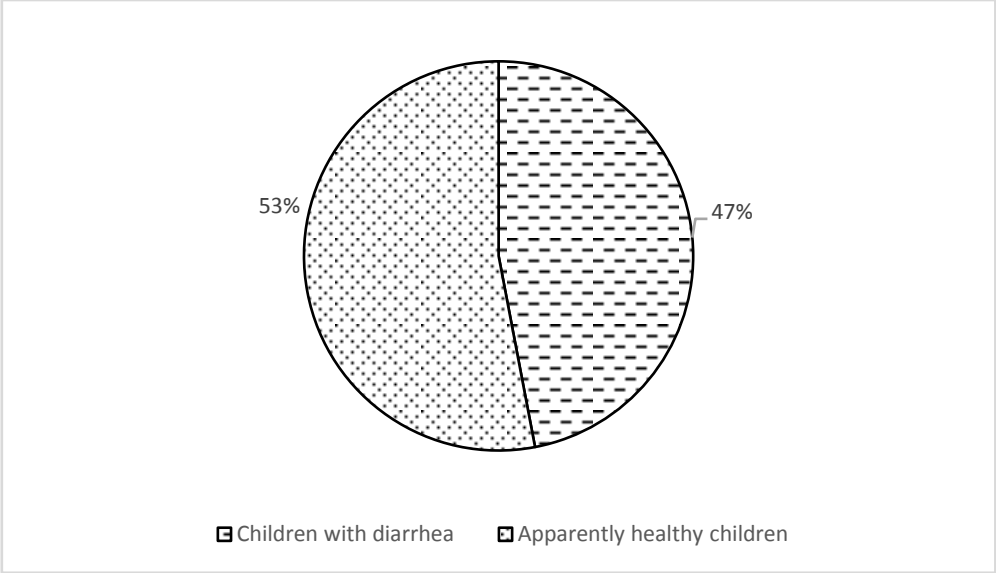

Figure 2: Distribution of children sampled according to their health aspect

3.1.3. Microbiological quality of stool

A diversity of microbial species was observed in the stool samples analyzed. Nine species of *Enterobacteriaceae* have been identified in different proportions. *Escherichia coli* (66.66%), *Klebsiella ornithinolytica* (13.72%), *Escherichia vulneris* (1.96%), *Klebsiella pneumoniae* (5.96%), *Enterobacter aerogene* (3.92%), *Salmonella* spp (1 .96%), *Salmonella arizonae* (1.96%), *Enterobacter sakazakii* (1.96%), *Enterobacter aerogenes* (1.96%), *Serratia fonticola* (1.96%). Figure 3 below shows the frequency of *Enterobacteriaceae* isolated from stool.

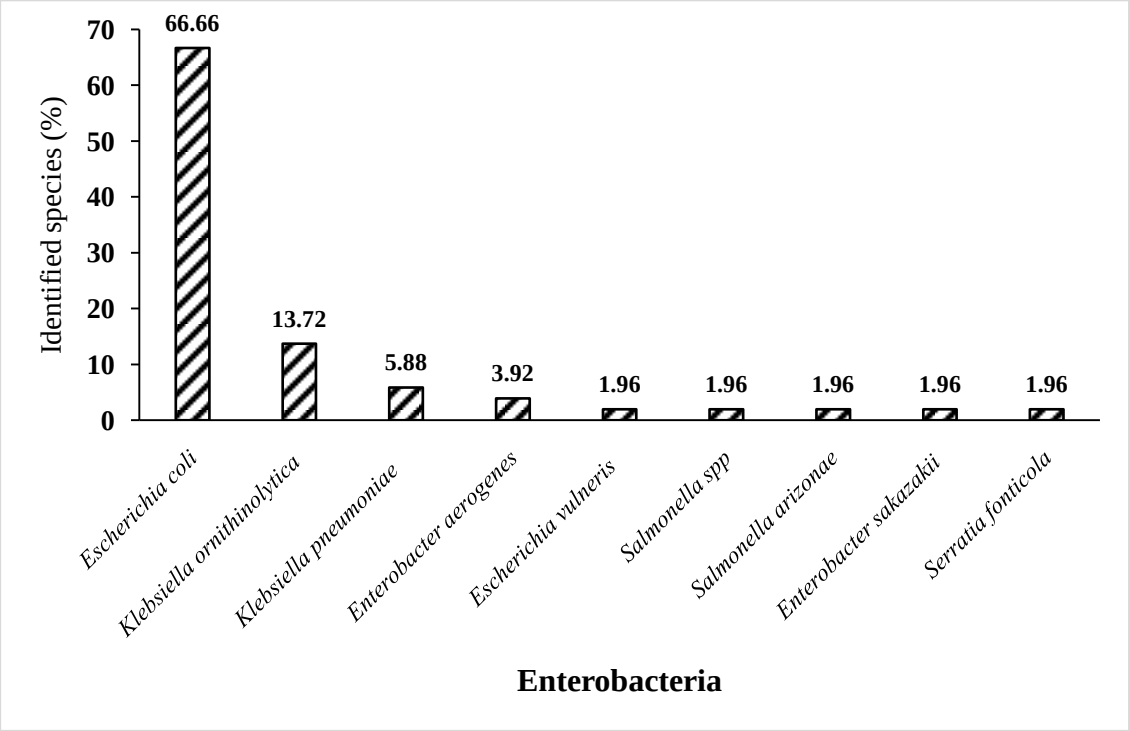

Figure 3: Frequency of enterobacteria isolated from stools

3.1.4. Microbiological quality of stool

The strains of *E. coli* isolated from the stools of apparently healthy children (100%) showed a strong potential for biofilm formation presented against 53.33% of the strains isolated from the stools of children suffering from diarrhea. Figure 4 below shows the potential for biofilm formation by the different strains of *E. coli.*

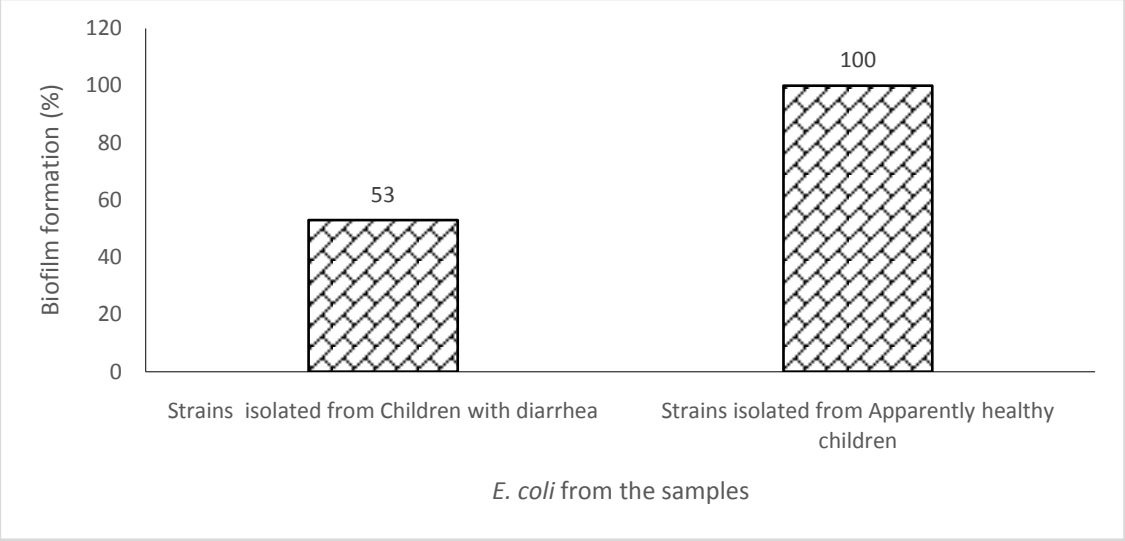

Figure 4: Potential for biofilm formation at the level of different strains of *E. coli*

3.1.5. Susceptibility of strains of *E. coli* to antibiotics

The susceptibility of strains to antibiotics shows that all (100%) of *E. coli* strains isolated from apparently healthy children suffering from diarrhea showed resistance to amoxicillin, ceftazidime, and cefotaxime. The strains of *E. coli* isolated from children suffering from diarrhea showed variable resistance to certain antibiotics such as ceftriaxone (100%), norfloxacin (95.33%), and trimethoprim-sulfamethoxazole (83.33%). In addition, the strains isolated from the stools of apparently healthy children are resistant to ceftriaxone (95.24%), norfloxacin (20%), and trimethoprim-sulfamethoxazole (50%). Resistance to imipenem was meager in both cases, i.e., 1% of *E. coli* isolated from children suffering from diarrhea and 0% from apparently healthy children. Figure 5 below shows the resistance profile of strains of *E. coli* isolated in children.

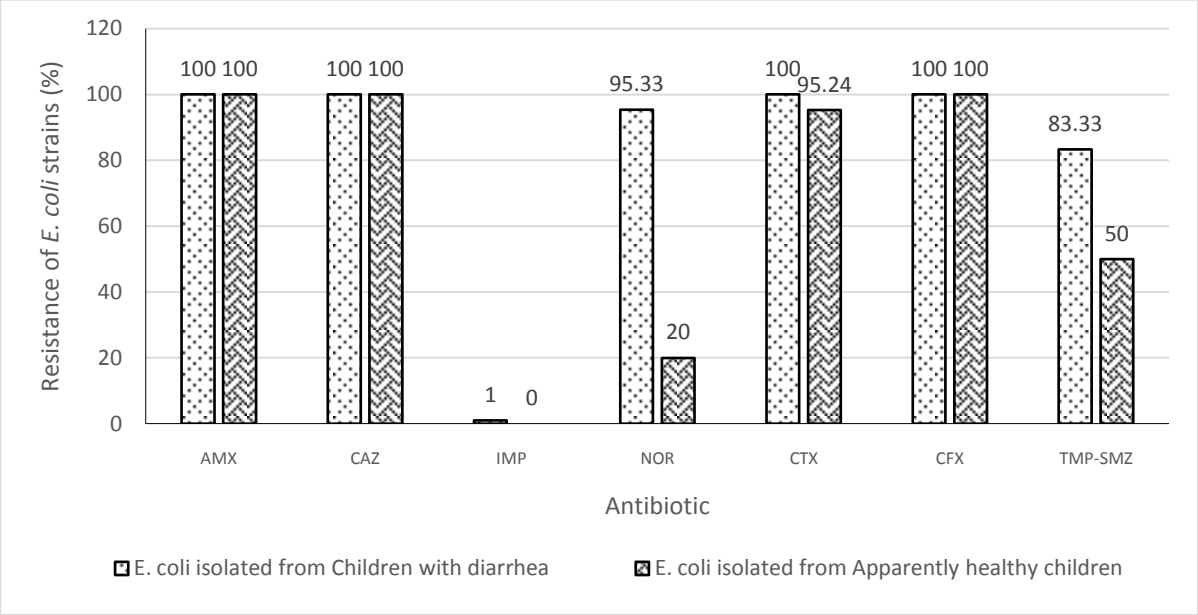

Figure 5: Resistance profile of *E. coli* strains isolated from children
AMX: Amoxicillin; CAZ: Ceftazidime; IMP: Imipenem; NOR: Norfloxacin; CTX: Ceftriaxone; CFX: Cefotaxime; TMP-SMZ: Trimethoprim-Sulfamethoxazole

3.1.6. Molecular analysis of strains of *E. coli* isolated

Detection of resistance genes by conventional PCR using specific primers blaTEM, blaSHV, and blaCTX-M was carried out on each DNA of the strains isolated. Figure 6 shows the appearance of the electrophoresis gel after migration.

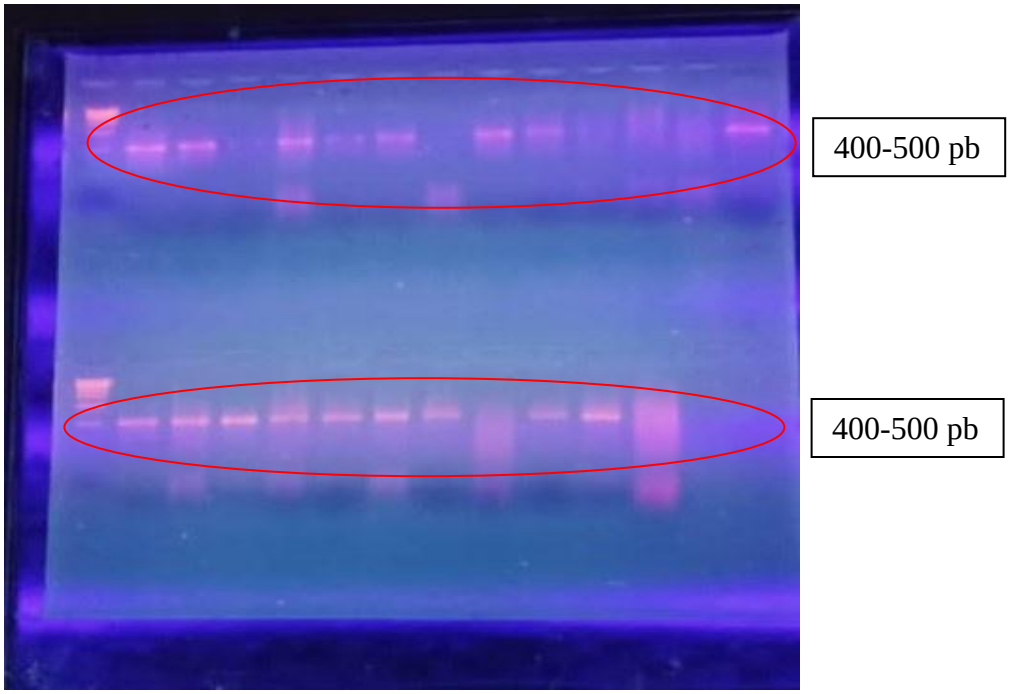

Figure 6: Image showing visualization of a gel using a UV table

After electrophoresis (Figure 6), the 70.83 strains of *E. coli* isolated from healthy children carry resistance genes blaTEM (12.5%), blaSHV (25%), and blaCTX-M (33.33%). While 66.67% of strains of E. coli isolated from children with diarrhea carry resistance genes blaTEM (12.5%), blaSHV (37.5%), and blaCTX-M (16.67%). The figure 7 below shows the frequency of antibiotic-resistance genes in *E. coli* strains isolated from children.

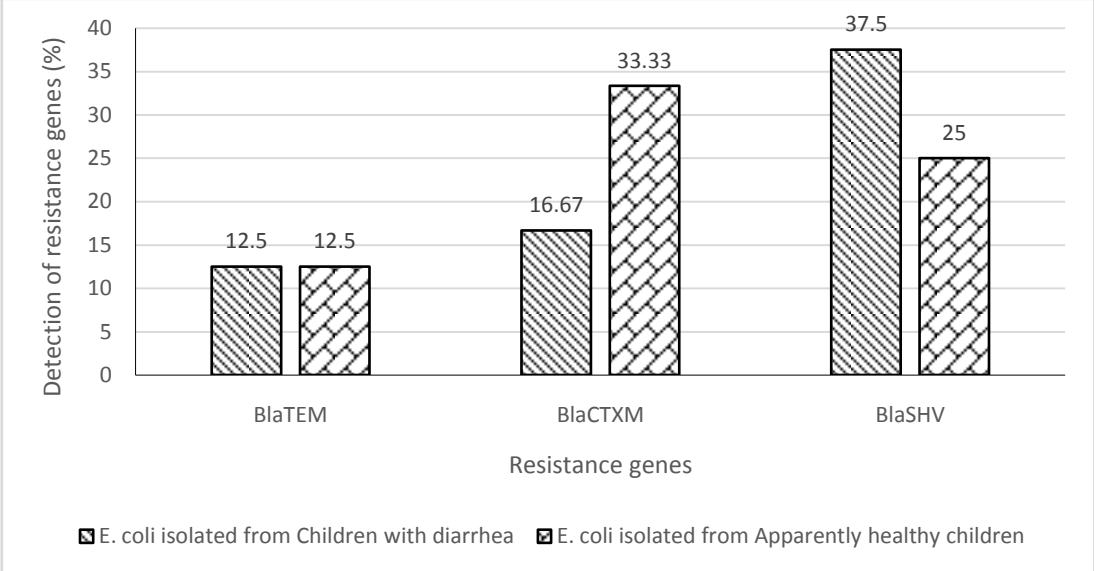

Figure 7: Frequency of antibiotic resistance genes in E. coli isolated from children

Detection of virulence genes by conventional PCR using specific primers Stx1, Stx2, eaeA, bfpB, LT, InVE, IpaH, and AggR was carried out on each DNA of the isolated strains. After electrophoresis, 100% of *E. coli* strains isolated from healthy children do not carry any of

these virulence genes. The same is true for strains of *E. coli* isolated in children suffering from diarrhea, with the difference that 20.83% are carriers of the bfpB gene (Table 4).

Table 4: Frequency of virulence genes in strains of *E. coli* isolated in children

***E. coli* strains**	**Virulence genes (%)**							
	Stx1	*Stx2*	*eaeA*	*bfpB*	*LT*	*InVE*	*IpaH*	*AggR*
***E. coli* isolated from Children with diarrhea**	0	0	0	20,83	0	0	0	0
***E. coli* isolated from Apparently healthy children**	0	0	0	0	0	0	0	0

3.1.7. Determination of the relationship between diarrheal individuals and the taking or not of antibiotics

It was noted that only blaCTX-M, blaSHV, and Trimethoprim/Sulfamethoxazole showed a significant effect in the occurrence of diarrheal effusions in children (Table 5).

Table 5: Prediction of resistance or not of resistance genes and antibiotics during diarrheal effusions in children

Predictors	B	S.E.	Wald	Df	Sig.	Exp(β)	95% C.I. for EXP(B)	
							Lower	Upper
***bla*$_{CTX-M}$** (Presence)	-1,596	0,691	5,34	1	0,021	0,203	0,052	0,785
***bla*$_{SHV}$** (Presence)	1,008	0,693	2,115	1	0,146	2,741	0,704	10,671
Trimethoprim (Resistance)	1,595	0,713	5,005	1	0,025	4,928	1,218	19,934
Constant	-0,003	0,697	0	1	0,996	0,997		
TESTS								
Omnibus Tests of Model Coefficients			14,533	3	0,002			
-2 Log likelihood = 57,247								
Cox & Snell R Square =0,244								
Nagelkerke R Square =0,326								

The logistic regression analysis results are displayed in Tables 5 and 6. With the full model test and the constant, only the model was significant, indicating that the predictors, blaCTX-M (p = 0.021) and Trimethoprim/Sulfamethoxazole (p = 0.025), were found to reliably distinguish between the occurrence of diarrheal effusions in children (x2=14.533, p=0.002, df= 3).

Table 6: Classification table of the predictions used in the study of the determinants of diarrhea in children

Observed		**Prediction**		
		Diarrhea		Correct percentage
		Yes	No	
Diarrhea	**Yes**	19	5	79,2
	No	9	19	67,9

Global percentage	73,1

Thus, conversely, the presence of the blaCTX-M gene (β = -1.596) and resistance to the antibiotic Trimethoprim/Sulfamethoxazole (β = 1.595) in E. coli are significantly linked to the occurrence of diarrhea in children (Table 6 and Figure 8).
On the other hand, the presence or absence of the blaSHV gene is not significantly (p=0.146) linked to diarrhea in children (Table 5 and Figure 8). The value of Nagelkerke's R square (0.326) indicates a moderately strong relationship between the predictor variables and the occurrence or not of diarrhea (Table 6). The overall success of the predictions was 73.1%, with 79.2% for the occurrence of diarrhea and 67.9% for the non-occurrence of diarrhea (Table 6).

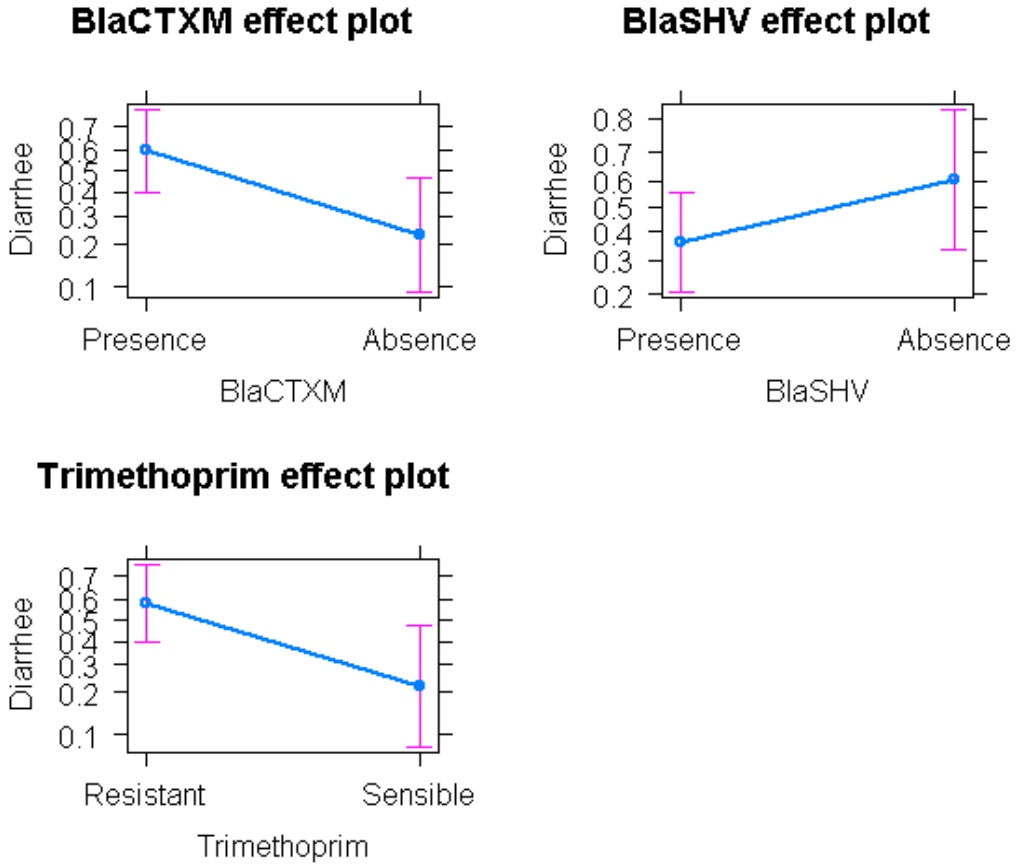

Figure 8: Figures showing the relationship between the presence of the blaCTX-M gene, resistance to Trimethoprim/Sulfamethoxazole in *E. coli,* and the occurrence of diarrhea

3.2.Discussion

This study focused on the pathogenicity of *E. coli* strains isolated from the stools of apparently healthy children suffering from diarrhea admitted to hospitals in southern Benin. This study observed that 17.64% of the children were 5 years old. Our results are contrary to those obtained by Zohra et al. [22] in Algeria during a study on acute gastroenteritis in infants, where they reported 61% of patients aged less than 12 months with a male predominance.

Bacterial diversity has been considered a characteristic related to health status and multiple diseases. Previous studies have frequently reported that when the host is in pathological conditions, the diversity of fecal bacteria is often reduced or significantly altered [23]. The profile of bacteria associated with diarrhea is essential information to guide antibiotic prescriptions when a pathogenic bacterium is suspected to cause diarrhea. In the present study, *Escherichia coli, Klebsiella ornithinolytica, Escherichia vulneris, Klebsiella pneumonia, Salmonella spp, Salmonella arizonae, Enterobacter sakazakii, Enterobacter aerogenes, Serratia fonticola* are suspected pathogenic bacteria that were identified in the participants of the present study. As mentioned above, the context of the present study did not allow the causal relationship between these cases of diarrhea and the isolated bacteria to be established. Studies conducted in other settings have described a different bacteria profile than the present study [24]. The differences in the patients' environments, behavior, and biology can explain this difference.

Antibiotic sensitivity needs to be monitored in an environment where the prescription of antibiotics is usually made presumptively. The present study tested the susceptibility of the predominant strain (*E. coli*) to antibiotics commonly used to treat diarrheal disease. The results show that 100% of the *E. coli* strains isolated from apparently healthy children suffering from diarrhea showed total resistance to Amoxicillin, Ceftazidime, and Cefotaxime. *E. coli* isolated from children suffering from diarrhea also showed resistance to Ceftriaxone, Norfloxacin, and Trimethoprim-Sulfamethoxazole, respectively of 100%, 95.33%, and 83.33% against 95.24%, 20 %, and 50% in apparently healthy children. Weak resistance to imipenem has been observed in strains of E. coli isolated from children with diarrhea and healthy children. In our study, imipenem is an antibiotic for treating *E. coli* infections. These results in apparently healthy children are like those obtained by Sina et al. [25] for Cefotaxime, Norfloxacin, and Imipenem. On the other hand, these results are contrary to those obtained by Linefiene et al. [26] in Tchad, who found a sensitivity of 53.12% to Cefotaxime and Ceftriaxone. This may be due to strain selection pressure from one area to another. The low susceptibility of strains of *E. coli* isolated to most of the antibiotics usually prescribed pleads in favor of setting up an epidemiological surveillance system to guide periodically and by area the antibiotic to be prescribed when it is suspected that a bacterium is the cause of diarrhea [27]. Since this sensitivity likely varies according to the strains of the different germs, it can also vary by geographical area, age group, and season.

Despite biofilm formation not being correlated with increased AMR, it is well established that this growth state is important for other aspects of persistence and survival [28]. Biofilms provide a survival strategy for bacteria by positioning them to efficiently use available nutrients and prevent access to antimicrobial agents. In our study, 100% of *E. coli* isolated from the stools of apparently healthy children could form biofilm against 53.33% of *E. coli* strains isolated from children suffering from diarrhea. This may, therefore, be one of the causes that explain this high rate of resistance to antibiotics in *E. coli* isolated from apparently healthy children. This difference could be explained by the fact that the adhesins of strains of *E. coli* isolated from the stools of children suffering from diarrhea did not all participate in the colonization or the maturation of biofilms. Some biofilm-forming species possess specific adhesins that only function in the biofilm context, while other species use the same molecules for adhesion of both individual cells and biofilm-dwelling cells [29].Interestingly, there is an inherent “avidity” effect [30] for biofilm adhesion: Adhesion molecules in the biofilm matrix can bind to foreign surfaces simultaneously and increase the adhesive energy collectively; meanwhile, adhesins that function for individual cells can be amplified on the scale of the entire biofilm [29]. Few, if any, of these adhesion factors are necessary for biofilm formation; other adhesion factors can replace them. The expression of protein adhesins and the production of this matrix are essential for the maturation of the biofilm structure[29].

Molecular analysis of isolates resistant to third-generation cephalosporins has indicated the presence of at least two genes that code for resistance. In our study, 70.83% of strains of E.

coli isolated from healthy children carry resistance genes blaTEM (12.5%), blaSHV (25%), and blaCTX-M (33.33%). While 66.67% of strains of E. coli isolated from children with diarrhea carry resistance genes blaTEM (12.5%), blaSHV (37.5%), and blaCTX-M (16.67%). The presence of one of these genes does not predict the association of resistance to quinolones and aminoglycosides. These results are like those obtained by Guindo et al. [31] for blaSHV (21.6%) and much lower for blaTEM (40.5%).

Detection of virulence genes by conventional PCR using specific primers Stx1, Stx2, eaeA, bfpB, LT, InVE, IpaH, and AggR was carried out on each DNA of the isolated strains. After electrophoresis, 100% of strains of E. coli isolated from healthy children do not carry any of these virulence genes. The same is true for strains of *E. coli* isolated in children suffering from diarrhea, with the difference that 20.83% are carriers of the bfpB gene. These results are different from the reports from Maputo in Mozambique, in which the frequency of DAEC was higher than that of EPEC, ETEC, EIEC, or EAEC in diarrheal stool samples [32]. Studies have shown that DAECs are very common and may be more common in HIV-positive patients [33]. Interestingly, in a human challenge study, some strains of DAEC were found not to cause diarrhea in healthy people; however, as this category of bacteria is heterogeneous, the fact that it cannot cause diarrhea in the elderly does not mean that there is no virulence in more susceptible people [33]. These results are significantly lower than those obtained by Guindo et al. [31] for Aagg (10.8%), bfp (18.9%), eae (18.9%), ipaH (16.2%), slt1 (29.7%) genes. This difference in results can be explained by the eating habits of the Beninese population characterized by the high-temperature cooking of food consumption.

4. CONCLUSION

The relatively high prevalence of ECD in this study is remarkable, and it highlights the fact that *E. coli* is an essential agent of infectious diarrhea and could be the leading cause of gastroenteritis in Benin. Furthermore, our results indicate a high rate of antimicrobial resistance in ECD strains, which necessitates the development of regulatory programs and reporting systems for antimicrobial resistance in ECD and other bacteria associated with acute gastroenteritis. to ensure effective control of diarrheal diseases. The results of our study could be used to develop recommendations for treating infections by ECD bacteria, especially in pediatric populations. Knowledge of ECD antimicrobial resistance is vital for choosing the appropriate treatment for severe diarrheal infections and formulating local antimicrobial guidelines. The ECD bacteria in our study possessed bfpB virulence genes.

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AUTHORS' CONTRIBUTIONS

D.D-N, H.S., and S.A. designed the study, performed the statistical analysis, wrote the protocol, andwrote the first draft of the manuscript. 'A.Y., B.B.,C.N., A.D.P.N.,andL.B-M. managed the analyses ofthe study. F.F.A., A.A., D.D-N., S.A.,and H.S. managed the literature searches. All authors read and approved the final manuscript.

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