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Diarrheic *Escherichia coli*: A Predominant Etiological Agent of Gastroenteritis, a Case Study in Douala, Cameroon

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Abstract

Context: Gastroenteritis remains an infectious disease with high morbidity and mortality particularly in low incomes countries, where the capacity to search all etiological agents, especially pathogenic *Escherichia coli*, is very limited. We investigated the contribution of pathogenic *Escherichia coli* and their antibiotic resistance profiles in cases of gastroenteritis. **Methods**: A cross-sectional study was carried out on human stool samples from October 2021 to June 2022 at Laquintinie Hospital. Samples were received from patients of all age groups and screened for bacteriological and parasitological identification by microscopy, bacterial culture, biochemical identification, and antimicrobial susceptibility tests. **Results**: A total of 296 patients with gastroenteritis complaints, were enrolled in the study with ages ranging from 5 months to 90 years old (Median = 35.5; SD = 20.8). Among the samples analyzed, 1.7% (n = 5/296) were positive for parasites and 27% (n = 80/296) were positive for bacterial pathogens. Parasites were found in mono parasitism, mainly *Entamoeba histolytica* (60%; n = 3/5), followed by *Trichomonas intestinalis* (20%; n = 1/5), and *Giardia intestinalis* (20%; n = 1/5). Three species of bacterial pathogens were identified with no co-infection: diarrheic *Escherichia coli* (DEC), *Salmonella* sp, and *Shigella* sp with respective proportions of 90% (n = 72/80), 6.3% (n = 5/80), and 3.7% (n = 3/80). For antibiotic resistance profiles (ARPs) of the 72 isolates of DEC, high levels of resistance were observed globally with amoxicillin (93.1%; n = 67/72), followed by ciprofloxacin (75%; n = 54/72), and to trimethoprim + sulfamethazole (73.6%; n = 53/72). In contrast, DEC showed low resistance rates with nitrofurans (6.9%; n = 5/72) and imipenem (2.8%; n = 2/72). The strains had 56 distinct ARPs, of which 88.9% (n = 64/72) were MDR. *Salmonella* sp and *Shigella* sp showed

high levels of resistance to amoxicillin and trimethoprim + sulfamethazole. **Conclusion**: These results emphasize the need to consider DEC as the main cause of consultation in cases of gastroenteritis and reiterate the urgent need to rationalize antibiotic use in Cameroon.

Keywords

Gastroenteritis, Enteropathogens, Pathogenic *Escherichia coli*, Antibiotic Resistance, Multidrug-Resistance

1. Introduction

Despite advances in health care, infectious diseases remain a major cause of morbidity and mortality [1]. Control of infectious diseases requires multiple approaches that rely on improved methods of diagnosis and treatment [2]. Gastroenteritis is one of the most infectious diseases with high morbidity, mortality, and serious public health significance particularly in low and middle-income countries [3] [4]. Gastroenteritis can be acute or chronic, caused by viral, bacterial, and more rarely parasitic pathogens [5]. Bacterial pathogens are responsible for 20% - 40% of gastroenteritis with diarrhoeal episodes [6] [7], and the increase of their antimicrobial resistance has become another health challenge for therapy, leading to treatment failures [8] [9] [10].

Generally, one of the management dilemmas in the evaluation of patients with gastroenteritis is deciding when to look for etiological agents and when to initiate antimicrobial therapy [8]. Locally, parasites are researched, and testing for pathogenic bacteria is usually limited to *Salmonella* sp and *Shigella* sp. *Escherichia coli* (*E. coli*) is systematically tested and considered only in children's cases, with non-differentiation of pathogenic or non-pathogenic strains. As commonly known, *E. coli* is a bacterium found in the commensal flora of the gut of humans and warm-blooded animals. However, although most *E. coli* are harmless, some are pathogenic and these species can cause significant gastrointestinal diseases [11] [12]. Indeed, worldwide, the epidemiology is changing with an increasing burden of gastroenteritis associated with diarrheic *Escherichia coli* (DEC) [13]. Pathogenicity is acquired through the capture of genetic elements containing genes coding for virulence factors necessary to cause infection [14]. Pathogenic DEC can be categorized as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and Shiga toxin-producing *E. coli* (STEC) [12]. In addition, *Escherichia coli* can harbor resistance genes using the same capture system [15]. Accordingly, any pathogenic or antimicrobial-resistant *Escherichia coli* can be harmful to its host [16].

Previous studies in Cameroon showed the low implication of *Salmonella* sp, *Shigella* sp, and *Yersinia* sp in cases of gastroenteritis [17] [18] [19]. However, there are very little data in the literature regarding the involvement of DEC in

cases of gastroenteritis due to the limitation of diagnosis methods. Indeed, in the laboratory, phenotypic detection methods by *E. coli* culture use conventional selective media EMB or MacConkey, where the characteristic colonies will be green colonies with a metallic shine and pink lactose fermenting colonies respectively. Although these media are selective for *E. coli*, they showed limits to screening the pathogenic potential species in gastroenteritis cases. Recent studies have revealed that the new chromogenic media, such as CHROMagar™STEC, is a good screening media for DEC detection [20] [21] [22].

Our study was focused on the Littoral region, in the city of Douala, one of the most populated and cosmopolite in Cameroon, and added to that, the absence of existing data concerning the frequency of DEC in cases of gastroenteritis. This study investigated the frequency of common causes of enteric infection with an emphasis on pathogenic DEC in cases of gastroenteritis, and their antibiotic resistance profiles.

2. Methods

2.1. Study Design

Between October 2021 and June 2022, we carried out a cross-sectional study targeting human patients in Douala. With more than 3 million inhabitants, Douala is the economic city of Cameroon; located in Central Africa. This city concentrates almost 20% of the urban population of the country. Laquintinie Hospital has been chosen as a sampling site because it constitutes the regional hospital of the city, where sample stool could be handled in the context of the COVID-19 pandemic. All patients complaining of gastroenteritis were referred there for sample analysis.

2.2. Ethical Considerations

According to the guidelines for human experimental models in clinical research, as stated by the Cameroon Ministry of Public Health, ethical approval was obtained from the Institutional Ethics Committee for Research Human Health of the University of Douala (Reg 2880 IEC-UD/07/2021/T). This was followed by the administrative agreement of the Regional Delegate of the Ministry of Public Health in the Littoral Region and the research authorization of the administrative authority of Laquintinie Hospital.

2.3. Sample Collection

All patients with gastroenteritis complaints consulted by the physician at the hospital which required analysis of stool samples were approached and included consecutively after obtaining their consent. Stools samples were collected using sterile universal containers and then labelled with the gender, age of the patient, and postcode. Samples were transported to the laboratory within 2 hours of collection and analyzed according to the recommendations of the reference in medical microbiology (REMIC) [23].

2.4. Parasite Identification

Stools samples obtained were observed under a microscope (Olympus XSZ-107BN; x40) in fresh state immediately arrive in the laboratory. Parasites were identified based on their morphological characteristics (size, motility, shape) and life stage.

2.5. Bacterial Pathogens Isolation and Identification

We screened different bacterial pathogens: *Campylobacter* species, diarrheic *Escherichia coli*, *Shigella* species, *Salmonella* species, and *Yersinia* species using selective media. Before use, each culture media in this study was subjected to internal quality control using different microorganisms according to the manufacturer's instructions (Table 1).

Screening of *Campylobacter* sp

REMIC protocol was used with minor modifications [23]. We used combination methods of filtration and culture with media Campylobacter Selective Agar (Merck, UK) to optimize the isolation of *Campylobacter* species. Briefly, a Pasteur pipette was used to place eight to ten drops of the sample diluted onto a cellulose triacetate membrane with 0.45 µm pores placed on the surface of the selective agar plate. The membrane was left on the agar surface until all the fluid had passed through; this took approximately 20 to 30 minutes. Plates were incubated microaerobically using GENbag anaer (Biomérieux, France), at 37˚C for two days. Suspect colonies (grey, translucent colonies, sometimes with a silver sheen) were identified to the genus level by a positive oxidase reaction and a typical Gram stain appearance (slender, curved, "seagull wing-shaped", Gram-negative rods).

Screening of DEC

Samples were enriched in Trypticase Soya Broth (Oxoid, UK) at 37˚C for 24 h to optimize the recovery of pathogenic *E. coli* [20]. Subsequently, a loop of 10 µl of the enrichment was plated on CHROMagar™STEC and incubated for 24 h at 37˚C. Next, one to three mauve colonies were purified in PCA and incubated overnight at 37˚C for 18 hours. The isolates were confirmed as *E. coli* using morphological characteristics such as motility, Gram staining, and biochemical characteristics from media such as Kliger's iron agar, Simmon's citrate agar, and urea-indole media according to the manufacturer's instructions.

Screening of *Salmonella* sp and *Shigella* sp

Traditional detection methods involve enrichment in a selective liquid culture medium followed by isolation using selective and differential agar [23]. Briefly, stool samples were inoculated into 9 ml of Selenite broth (Biolab, Hungary) and incubated at 37˚C for 24 hours. Subsequently, 10 µL of the culture was plated onto Salmonella-Shigella agar and incubated for 24 hours at 37˚C. Based on the morphology and appearance of the colonies, presumptive *Salmonella* colonies (colorless with a black center) and *Shigella* colonies (colorless), a subculture was made by plating onto Plate Count agar (PCA) (Oxoid, UK) and incubated at

Table 1. Internal control quality of media used in the study.

	Test performed before using media	
	Fertility test/ Specificity test	**Selectivity test**
CHROMagar™STEC agar	*Escherichia coli* O157H7	*Escherichia coli* ATCC 25922 *Klebsiella pneumoniae*[a] *Citrobacter freundi*[a] *Enterobacter aerogenes*[a] *Proteus mirabilis*/ *vulgaris*[a]
MacConkey agar	*Escherichia coli* ATCC 25922 *Yersinia enterocolitica*[a]	*Staphylococcus aureus* NCTC 12493
Mueller Hinton Agar	*Escherichia coli* ATCC 25922	-
Plate Count Agar	*Escherichia coli* ATCC 25922	-
Salmonella-Shigella agar	*Salmonella typhimirium*[a] *Shigella flexneri*[a]	*Staphylococcus aureus* NCTC 12493
Selenite broth	*Salmonella typhimirium*[a] *Salmonella typhi*[a] *Shigella flexneri*[b]	-

a. species were identified using biochemical characters in API20^{E}. b. species identification was performed using API20^{E} and serotyping.

37˚C for 24 hours. Colony species were biochemically identified using API 20E (Biomérieux, France) according to the manufacturer's instructions.

Screening of *Yersinia* sp

Stool samples were diluted at 10^{-1} in distilled sterile water, and subsequently, a loop of 10 µL of the suspension was plated onto MacConkey agar and incubated for 24 - 48 hours at 37˚C. Lactose-negative small colonies (1 - 2 mm diameter) colorless or pale pink colonies, and flat were selected [24]. A subculture of these colonies was made by plating onto PCA and incubating at 37˚C for 24 hours. Finally, colony species were biochemically identified using API 20E (Biomérieux, France) according to the manufacturer's instructions.

2.6. *In-Vitro* Antimicrobial Susceptibility Testing of Bacterial Pathogens

All isolated strains were subjected to susceptibility testing and evaluated to commonly used antibiotics using the Kirby–Bauer disc diffusion method according to the European Committee of Antimicrobial Susceptibility Testing criteria (EUCAST) [25]. **Table 2** presents the different antibiotics used with their concentrations and their breakdown used to categorize results as Sensible or Resistant. With a bacterial cell culture of 24 h on PCA, a loop of each isolate was emulsified in a sterile physiological water solution in a test tube and the density was measured with a McFarland densitometer to obtain 0.5 McFarland

Table 2. Antibiotics tested (and the respective antibiotic classes) and interpretation of zone of inhibition (mm), from EUCAST 2021.

		Breakpoints Used (Ø mm)		
Drug class	**Antibiotic**	**Disc Content µg**	**Susceptible**	**Resistant**
β-lactam antibiotic	Amoxicillin-clavulanic acid	20/10	Ø ≥ 19	Ø < 19
Penicillin	Amoxicillin	10	Ø ≥ 19	Ø < 19
Cephalosporin	Cefotaxime	30	Ø ≥ 20	Ø < 17
Cephamycins	Cefoxitin	30	Ø ≥ 18	Ø < 18
Carbapenems	Imipenem	10	Ø ≥ 22	Ø < 19
Fluoroquinolone	Ciprofloxacin	10	Ø ≥ 25	Ø < 22
Aminoglycoside	Amikacin	25	Ø ≥ 18	Ø < 18
Phenicol	Chloramphenicol	30	Ø ≥ 17	Ø < 17
Nitrofurans	Nitrofurantoin	300	Ø ≥ 11	Ø < 11
Macrolides	Azithromycin	15	Ø ≥ 17	Ø < 17
Folate pathway/acid inhibitor	Trimethoprim + Sulfamethoxazole	1, 23 - 25, 75	Ø ≥ 14	Ø < 11
Tetracycline	Doxycycline	30	Ø ≥ 14	Ø < 10

Ø = diameter of inhibition zone in mm.

standards. Using a sterile cotton swab, the suspension was emulsified onto a Mueller Hinton agar plate and incubated at 37˚C for 18 h.

The zone of inhibition was measured, and the results were interpreted. *Escherichia coli* ATCC 25922 was used as quality control. Isolates observed resistant to at least three classes of antimicrobials were considered Multidrug Resistant (MDR). Pansusceptible was defined as isolates susceptible to all antibiotics tested.

2.7. Data Analysis

All data were recorded into an Excel spreadsheet and used the sheet for descriptive statistical analysis (frequencies, proportions, and Chi-square test) with SPSS 23.0. A McNemar of Chi-square test was performed to compare the frequency of enteric pathogenic species found in positive samples. We considered an association statistically significant if P-values < 0.05.

3. Results

3.1. Study Population

We collected samples from 296 patients included in the study with ages ranging between 5 months to 90 years old (Median = 35.5; SD = 20.8). The sex ratio was 0.73 with more females (57.8%) than males.

3.2. Parasitic Pathogens

Parasites were identified in 1.7% (n = 5/296) patients as the etiological agent of gastroenteritis. Parasites were identified in monoparasitism and included *Entamoeba histolytica* (60%; n = 3/5), *Trichomonas intestinalis* (20%; n = 1/5), and *Giardia intestinalis* (20%; n = 1/5).

3.3. Bacterial Pathogens

Out of the 296 samples, 27% (n = 80/296) of the samples were positive for bacterial pathogens. The pathogens identified in the samples were DEC (90%; n = 72/80) followed by, *Salmonella* sp (6.3%; n = 5/80) and *Shigella* sp (3.7%; n = 3/80) (Figure 1). No *Yersinia* sp and *Campylobacter* sp were found in stool samples in this study. Also, no co-infections were identified.

The diarrheic *E. coli* were frequently isolated in population studies with an age range between 5 to 50 years old (Table 3) and was not detected in patients with an age range between 0 to 5 years old. There was no statistical difference between females and males across bacterial pathogens.

3.4. Antibiotic Resistance Profiles of Bacterial Pathogens Isolates

Strains of diarrheic *E. coli* were most resistant to AMO (93.1%; n = 67/72), followed by CIP (75%, n = 54/72), SXT (73.6%, n = 53/72), DOX (68.1%, n = 49/72), AMC (52.8%, n = 38/72), FOX (47.2%, n = 34/72), CTX (45.8%, n = 33/72), AZM (38.9%, n = 28/72), AMC (38.9%, n = 28/72), AKN (34.7%, n = 24/72) and CHL (34.7%, n = 24/72) (Figure 2). In contrast, strains showed low resistance to NIT and IMI, with respective rates of 6.9% (n = 5/72) and 2.8% (n = 2/72).

Fifty-six distinctive antimicrobial resistance profiles (ARPs) were recorded with resistance levels ranging from one to ten antibiotics from the twelve antibiotics tested. The most common resistance levels were recorded in five classes of antibiotics, and the common phenotype of resistance was AMO-AMC-CIP-SXT-DOX. Among these ARPs, we found 88.9% MDR strains (n = 64/72) and no pan-susceptible isolates as presented in Table 4.

Salmonella sp strains were resistant to only three drugs: AMO (100%; n = 5/5), SXT (80%, n = 4/5), and DOX (60%, n = 3/5). *Shigella* sp isolates were resistant to SXT (66.7%, n = 2/3), AMO (33.3%, n = 1/3), and CIP (33.3%, n = 1/3). MDR strains were found in *Salmonella* sp (80%, n = 4/5) (Table 5).

Globally, resistance to AMO, CIP, and SXT was common in *Salmonella* sp, *Shigella* sp, and, diarrheic *Escherichia coli* (Figure 2).

4. Discussion

The main objective of this work was to investigate the contribution of pathogenic *Escherichia coli* and their antibiotic resistance profiles in gastroenteritis. While the research of pathogens is usually limited to parasites and two bacterial pathogens namely *Salmonella* sp and *Shigella* sp in the context of Cameroon, the

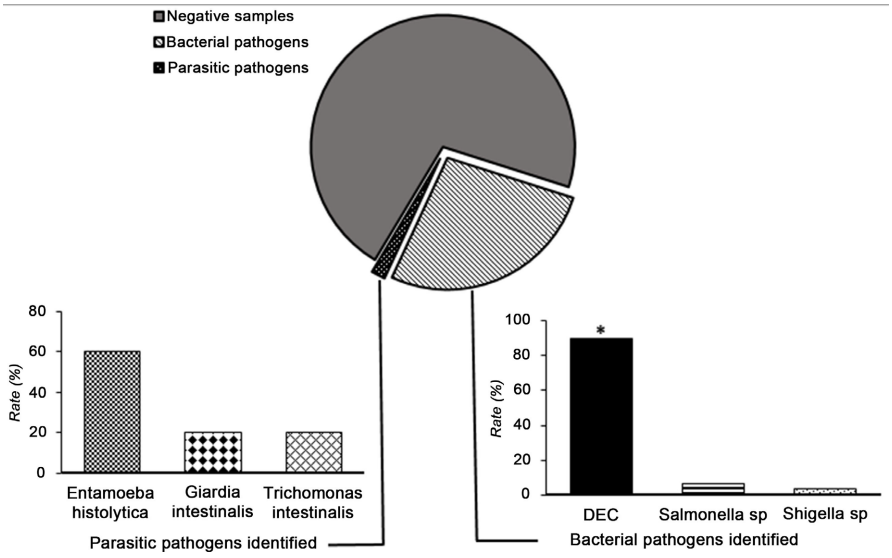

Figure 1. Distribution of enteropathogens identified in samples analyzed. Asterisk denotes a significant difference between the proportion of this pathogen across bacterial pathogens identified, p-value < 0.001 was obtained using McNemar of Chi-square test.

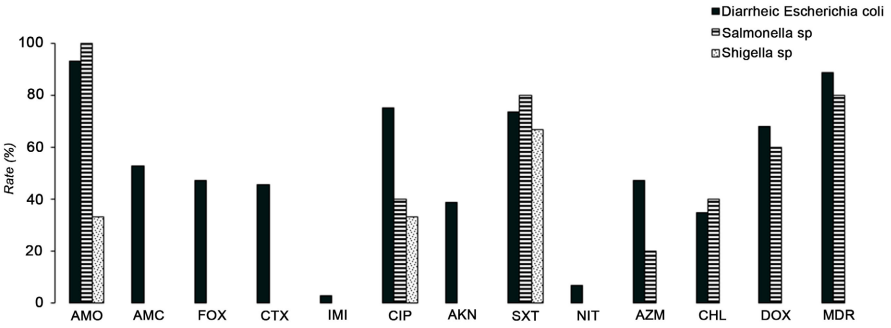

Figure 2. Resistance profiles of different antibiotics tested on enteric bacteria pathogen isolates. Abbreviations: AMO, amoxicillin; AMC, amoxicillin + clavulanic acid; FOX, cefoxitin; CTX, cefotaxime; IPM, imipenem; CIP, ciprofloxacin; AKN, amikacin; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; AZM, azitromycin; CHL, chloramphenicol; DOX, doxycycline; MDR, Multidrug resistant.

Table 3. Distribution of diarrheic *E. coli*, *Salmonella* sp, and *Shigella* sp per sex and age of the population study.

			Bacterial pathogens identified (n = 80)								
			Diarrheic *E. coli* (n = 72)			*Salmonella* sp (n = 5)			*Shigella* sp (n = 3)		
		N[a]	N[b]	%	P-value[c]	N[b]	%	P-value[c]	N[b]	%	P-value[c]
Sex	Female	171	46	26.9	0.27	03	1.7	0.919	03	1.7	0.131
	Male	125	26	20.8		02	1.6		00	0	
Age (year old)	[0 - 5]	27	00	00	-	00	00	-	00	00	-
	[5 - 20]	42	12	28.6		00	00		00	00	
	[20 - 35]	70	21	30		02	2.9		01	1.4	

Continued

Age (year old)	[35 - 50]	83	26	31.3	02	2.4	00	00
	[50 - 65]	46	09	19.6	01	2.2	01	2.2
	≥65	28	04	14.3	00	00	01	3.6

a. represents the number of samples analyzed. b. represents the number of positive samples population. c. P-value was calculated using the Pearson Chi-square test and the significance level was considered with $p < 0.05$.

Table 4. Antibiotic resistance profiles (ARPs) of diarrheic *E. coli* isolates on CHRO Magar™ STEC.

ARPs	Number of isolates
Ten class	**3**
AMO AMC FOX CTX IMI CIP AKN SXT AZM DOX	1
AMO AMC FOX CTX CIP AKN SXT AZM CHL DOX	2
Nine class	**8**
AMO AMC FOX CIP AKN SXT AZM CHL DOX	1
AMO AMC FOX CTX CIP AKN SXT AZM DOX	1
AMO AMC FOX CTX CIP SXT AZM CHL DOX	3
AMO FOX CTX CIP SXT NIT AZM CHL DOX	1
AMO AMC FOX CTX CIP SXT NIT CHL DOX	1
AMO AMC FOX CTX IMI CIP AKN AZM DOX	1
Eight class	**9**
AMO AMC CTX CIP AKN SXT CHL DOX	1
AMO AMC FOX CTX AKN SXT AZM DOX	1
AMO AMC FOX CTX CIP SXT NIT CHL	2
AMO AMC FOX CTX CIP SXT CHL DOX	1
AMO AMC FOX CTX CIP SXT AZM DOX	2
AMO FOX CTX CIP SXT NIT AZM DOX	1
AMO CTX CIP AKN SXT AZM CHL DOX	1
Seven class	**9**
AMO AMC CIP AKN SXT AZM CHL	3
AMO AMC FOX CIP AKN SXT DOX	1
AMO AMC CTX CIP SXT AZM DOX	1
AMO AMC FOX CTX CIP SXT AZM	1
AMO AMC FOX CTX CIP SXT CHL	1
AMO CTX CIP AKN SXT AZM DOX	1
AMO CTX CIP AKN SXT AZM CHL	1
Six class	**8**
AMO AMC CIP SXT CHL DOX	1

Continued

AMO AMC FOX CIP AKN AZM	1
AMO AMC FOX CIP SXT DOX	1
AMO AMC FOX CTX CIP AZM	1
AMO CTX CIP SXT AZM DOX	1
AMO FOX CIP SXT NIT CHL	1
AMO CIP AKN SXT AZM DOX	1
AMO CTX AKN SXT AZM DOX	1
Five class	**14**
AMO AMC CIP SXT AZM	1
AMO AMC CIP SXT DOX	4
AMO AMC FOX AZM DOX	2
AMO AMC FOX CIP SXT	1
AMO AMC FOX CTX CIP	1
AMO CIP AKN SXT DOX	1
AMO CTX CIP SXT AZM	1
AMO FOX CIP AZM DOX	1
AMO FOX CIP SXT DOX	1
AMO CTX CIP SXT DOX	1
Four class	**10**
AMO AKN AZM DOX	1
AMO AMC CIP SXT	1
AMO AMC FOX AKN	1
AMO AMC SXT DOX	2
AMO CIP SXT DOX	1
AMO SXT CHL DOX	3
AMO FOX CIP AKN	1
Three class	**3**
AMO CIP DOX	1
AMO CIP SXT	1
AMO FOX AKN	1
Two class	**3**
AKN AZM	1
AMO AKN	1
CIP SXT	1
One class	**5**
AMO	2
CIP	1
DOX	2
Pan susceptible	**0**
Total	**56 profiles**	**72 isolates**

Table 5. Antibiotic resistance profiles of *Salmonella* sp and *Shigella* sp isolates from cases of gastroenteritis.

	ARPs	Number of isolates
***Salmonella* sp**	AMO CIP SXT DOX	1
	AMO CIP SXT CHL	1
	AMO SXT CHL DOX	1
	AMO SXT DOX	1
	AMO AZM	1
***Shigella* sp**	AMO SXT	1
	CIP	1
	AMO	1

current worldwide trend shows an important involvement of diarrheic *E. coli* in cases of diarrhea in countries with more advanced methods of pathogen identification [13].

Although our results are online with the conclusion of Riddle *et al.* [5] that parasites are rarely found in case of diarrhea, our low values found are not similar to the results of Belay *et al.* [26] which found a high prevalence of intestinal parasites in human samples. In this study, parasite pathogens were found at a low frequency, representing 1.7% of cases of gastroenteritis. Regarding the situation in Cameroon, the frequency of intestinal parasites in this study are contrary to previous studies in other regions, which found 8.4%, 15.4%, and 21.9% respectively [27] [28] [29]. Similarly, regarding trends in other countries of the world, our frequency values obtained are much lower concerning the carriage of intestinal parasites [30] [31] [32] [33]. Various sizes of samples analyzed in these previous studies ranging from at least 500 to 50,000 cases, could be strongly associated with this difference in our value obtained.

If this study reports the first results of a screening of parasites and bacterial pathogens in cases of gastroenteritis in Douala, the frequency of detection of parasites (1.7%) was very low than the detection of bacterial pathogens (27%) among cases. Our results are online with the conclusion of Moro *et al.* [3] which showed in a minireview presenting the causes of infectious gastroenteritis, that parasites are less commonly implicated in gastroenteritis than bacterial pathogens. However, Belay *et al.* [26] in Ethiopia, found a high prevalence of intestinal parasites (20.7%) than bacterial pathogens (6.6%) in human samples in Ethiopia. The low rate of detection of parasites as observed in the present study might be due to the increasing awareness of the people about personal and environmental hygiene and, as well Douala is an urban zone. It should be noted that all parasites found were protozoans with *Entamoeba histolytica* mainly detected in positive cases. This may be justified by the fact that this parasite remains one of the top three parasitic implicates in cases of gastroenteritis and causes of mortality worldwide [34].

Among the 27% positives samples to bacterial pathogens, *Salmonella* sp and *Shigella* sp were detected at very low frequencies (6.3% and 1.7% respectively) than diarrheic *Escherichia coli*. These findings are in line with the previous study which found relatively low frequency in the city of Douala, Littoral region (10.3% of *Salmonella* sp and 3.99 of *Shigella sp*) [18], and in the city of Buea, North West Region of Cameroon (8.7% of *Salmonella* sp) [17]. *Salmonella* sp and *Shigella* sp are bacterial pathogens that represent major public health problems in terms of mortality and morbidity for both developed and undeveloped countries. The possible explanation for the low prevalence of these pathogens could be due to the fact that the health center has initiated treatment of patient referral to this hospital, or associated with an increase in self-medication which has been described previously [35].

Diarrheic *E. coli* was isolated with a significantly higher proportion than other enteric pathogens. If here we reported a first analysis of common enteric pathogens in other to show the real contribution of each pathogen in cases of gastroenteritis in Douala, especially pathogenic *Escherichia coli*, a recent study highlighted the important involvement of diarrheic *E. coli* associated with gastroenteritis in the city of Mbouda, West region in Cameroon (19.7% of cases) [36]. If *Escherichia coli* is a commensal bacterium representing 80% of digestive flora, it is easily found in high proportions using classical media as in some studies, which unfortunately did not provide specific data about the proportion of pathogenic strains. In addition to the framework of this current study, no co-infections were found in the analysis of samples. So, our findings suggest that diarrheic strains of *E. coli* could be one of the main causes of consultation for gastroenteritis in hospitals, and should be taken into account when suspecting enteric pathogens.

In the study population, patients' age ranging from 5 to 50 years old showed a high frequency of isolation of diarrheic *E. coli*. Our result is similar to that obtained for patients of ages ranging between 20 to 50 years old (20 to 30 years old, 26.89%; 30 to 40 years old, 18.49%; 40 to 50 years old, 30.25%) by Marbou *et al.* [36]. However, in this study, no isolates were obtained from children aged between 0 to 5 years old. This is contrary to the results of a previous study in the Littoral region, where diarrheic *E. coli* were identified in children at a low rate [37] [38]. This contrast could be explained by the fact that identification methods were different from this study. In addition, worldwide the major cause of childhood diarrhea is Rotavirus as an infectious agent. Our result highlighted that the use of antibiotics in children aged between 0 - 5 years old should be better controlled, as children routinely receive antibiotics when *Escherichia coli* has been isolated from their stool samples in cases of gastroenteritis in Douala.

Regarding the antibiotic resistances profiles, if good activity was observed among isolates of diarrheic *E. coli* against imipenem and nitrofurans in this study, high levels of resistance were observed against amoxicillin (91%), ciprofloxacin (75%) and trimetoprim + sulfamethazole (73.5%). Analysis of the correlation between diarrheic *E. coli* antimicrobial resistance and virulence profiles

can help physicians avoid treatment failure. Indeed, the choice of antimicrobial therapies depends on the type of diarrheic *E. coli* as well as its virulence and resistance profiles [39]. Similar high rates of resistance have been described previously on diarrheic *E. coli* in the case of Mbouda, West region which was found with amoxicillin and trimetoprim + sulfamethazole [36]. In a recent review of human health in Cameroon, these same resistances have been described in *E. coli* from extra digestive infections [40].

Of the diarrheic *E. coli* isolates, 47.2% showed resistance to azithromycin. While studies in Cameroon on *E. coli* have not described resistance to this antibiotic in humans, our results are contrary to a recent study in Congo, which found a low level of resistance in *E. coli* strains of fecal origin [41]. It would be important to note that this lack of data regarding azithromycin resistance in our context, could be related to the reference used in the laboratory which recommends systematically testing azithromycin in particular for *Salmonella* sp and *Shigella* sp [25]. However, azithromycin is a promising alternative with excellent activity against the most common enteric pathogens including diarrheic *E. coli* [42] [43]. Our results could be related to the use of azithromycin for treatment during the COVID-19 pandemic, where in our area self-medication was a common phenomenon and has already been described. The expression of this resistance to antibiotics represents a serious problem worldwide. Indeed, *E. coli* may harbor resistance genes that may be transferred to pathogenic or opportunistic bacteria. For these reasons, *E. coli* has been classified by the World Health Organization as a priority pathogen due to its widespread resistance to antibiotics [44].

Among *Salmonella* sp, *Shigella* sp, and diarrheic *Escherichia coli* isolates, resistance to amoxicillin, ciprofloxacin, and trimetoprim + sulfamethazole was commonly observed. These three medicines are on the national list of essential medicines in Cameroon [45]. While high resistance to amoxicillin is commonly described, the WHO has recently reported high resistance levels for *Escherichia coli* and *Salmonella* sp to ciprofloxacin [46]. Ciprofloxacin is an antibiotic substance usually prescribed for the treatment of salmonellosis. Trimetoprim + sulfamethazole (known as Metronidazole) is an antibiotic and antiparasitic substance widely used in the treatment of several infections caused by bacteria and some types of protozoa. The resistance especially in this case of them could be associated with the poor quality of these two drugs in the market of Cameroon [47], which promotes antibiotic resistance, and finally can lead to the reduction or absence of effectiveness of first-line therapies [48].

Based on antibiotic resistance profiles, diarrheic *E. coli* showed 56 distinctive resistance profiles with resistance levels ranging from one to ten antibiotic classes, which allowed us to find that 88.9% (n = 64/72) of isolates were MDR. Similar results have been described in diarrheic *E. coli* in Egypt, which found 90% MDR among isolates [39]. These results could be linked to a carriage of genetic elements such as integrons, genetic structures that will allow the bacteria to capture many antibiotic resistance genes.

This study was limited by the lack of identification of viral enteropathogens among stool samples, which could allow us to give complete profiles of etiological agents responsible for gastroenteritis cases. In further studies, a molecular analysis of diarrheic *Escherichia coli* obtained could be necessary to identify the different pathotypes and virulence genes among the isolates obtained, and genes of resistance associated with the resistance observed.

5. Conclusion

These results emphasize the need to consider diarrheic *Escherichia coli* as the main cause of consultation in cases of gastroenteritis in our hospitals in Douala and reiterate the urgent need to rationalize antibiotic use.

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Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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