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Drug Resistance and Susceptibility Profile of Bacterial and Fungal Isolates from Stool Samples in Yaounde, Cameroon

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Abstract **Background:** Intestinal pathologies of bacterial or fungal origin constitute a public health problem all over the world and particularly in developing countries. The diseases associated with these infections, including diarrhea, significantly affect children and continue to be the cause of morbidity and mortality observed in developed countries and even more so in the third world. **Objective:** The objectives of this study were to present the profile of the germs involved in intestinal infections in Yaounde between 2010 and 2020, to analyze the association between these germs with the sex and age groups of the study subjects and to present the profiles of sensitivity and drug resistance of species isolated from stool samples during the same period. **Methods:** This retrospective study was carried out from January 04, 2010 to January 07, 2020 in Yaounde at the Centre Pasteur of Cameroon. After collection of stool samples, laboratory analyzes focused on macroscopic study, isolation and identification of bacterial and fungal species and then performing an antimicrobial susceptibility test by the diffusion method. The automated method using the Vitek 2-compact automaton was also used in order to highlight drug susceptibility and resistance profiles. **Results:** 40,339 stool samples were analyzed during the study period with a prevalence of intestinal infections of 8.1% (3284 samples positive for the presence of a bacterial or fungal species). The male sex was the most represented with 1697 samples (4.2%) against 1484 (3.7%) samples for the female sex. This difference in sex distribution was statistically significant ($p<0.001$). The age of participants positive for infection ranged from 0-100 years with a mean age of 26.9 years ± 21.3 SD. The most contaminated age group was made up of patients aged less than 20 years, ie 1620 (9.3%) samples for an average age of 8.8 years ± 3.8 SD. The difference in the distribution of the samples by age group was significant ($p<0.0001$). The bacterial species most represented in the stool samples were: *Shigella flexneri* (1.16%), *Vibrio cholerae* (1.14%), *Salmonella sp.* (0.74%), *Escherichia coli* (0.62%), *Campylobacter sp.* (0.15%), *Shigella sonnei* (0.13%), *Shigella sp.* (0.11%), *Shigella boydii* (0.11%), *Salmonella enteritidis* (0.09%), *Salmonella typhimurium* (0.09%), *Campylobacter jejuni* (0.08%), *Shigella dysenteriae A2* (0.04%), *Salmonella typhi* (0.03%), *Campylobacter coli* (0.03%), *Campylobacter lari* (0.03%), *Salmonella hadar* (0.03%) and *Shigella dysenteriae A1* (0.03%). Regarding fungi, *Candida albicans* (2.63%) was the most represented followed by other strains of *Candida* (*Candida sp.* with a prevalence of 0.85%). Significant associations of age groups ($p<0.0001$) and sex ($p<0.001$) with the organisms isolated were obtained in this study. About 40 antibiotics and 8 antifungals were used. The greatest resistance was observed for drugs which inhibit the synthesis of bacterial envelopes, nucleic acids, folic acid and proteins. The most resistant species were: *Shigella flexneri* (81.0% resistance to ticarcillin); *Campylobacter coli* (91.7% resistance to cefatrizine); *Campylobacter lari* and *Vibrio cholerae* (100% and 92.4% resistance to nalidixic acid); *Shigella dysenteriae A2* and *Shigella sonnei* (100% and 96.1% resistance to cotrimoxazole); *Vibrio cholerae* (82.1% resistance to Colistin). For fungi, *Candida albicans* was the most represented and showed a high sensitivity for most of the antifungal agents used. **Conclusion:** In the light of the results of this study, it is essential to extend the surveillance of the resistance of strains to antibiotics in other regions of Cameroon to define the therapeutic strategies adapted to the epidemiological data in our context.

Keywords: *intestinal pathologies, antimicrobial susceptibility test, Vitek 2-compact, surveillance, Cameroon*

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1. Introduction

Gastrointestinal infections of bacterial or fungal origin continue to be the leading cause of mortality and morbidity worldwide, particularly among children in developing countries [1]. Children and young adults are the most affected, especially in areas where resources are limited and hygiene measures are inadequate [1,2]. Among these diseases, diarrheal diseases in the world and endemic developing countries are the second leading cause of death in children under five years of age [2,3,4] with about 550 million people a year becoming ill [4], 760,000 child deaths per year and about 1.7 billion cases of diarrhea each year worldwide [3,5]. In Africa, Asia and Latin America, an estimated 2.5 million child deaths occur each year [6]. According to GebreSilasie *et al. [7]*, diarrhea is the third leading cause of death among children in sub-Saharan Africa and one of the major causes of hospital admissions in rural areas. Reports from the World Health Organization (WHO) show that intestinal infections, particularly foodborne diseases, affect 30% of the population of developing countries each year, with a death rate of 2 million people per year [8,9,10,11]. Moreover, 525,000 deaths per year are recorded among children under 5 years of age who are victims of gastroenteritis [12]. The problem in these same countries is even more severe due to inadequate environmental conditions and inappropriate hygiene measures with about 70% of diarrhea cases associated with the consumption of contaminated food and water with 1.9 million deaths recorded globally in underdeveloped countries [9,11].

Reports from the Center for Disease Control (CDC) reveal that diarrheal diseases, especially foodborne diseases, are responsible for 76 million cases of illness, 325,000 hospitalizations and 5,000 deaths in the United States each year. This costs the United States $6.5-$34.9 billion annually [11]. There are more than 250 foodborne diseases worldwide caused by bacterial, viral and parasitic organisms. These microorganisms release chemical toxins or poisons that are toxic and endotoxins for some bacteria. The bacterial candidates involved are: *Salmonella*, *Campylobacter*, *Listeria*, pathogenic *Escherichia coli* (*E. coli*), *Yersinia*, *Shigella*, *Enterobacter* and *Citrobacter*. More specifically, the germs found in diarrhoea are: *Salmonella enterica*, *Shigella* species, *Vibrio cholera*, *Clostridium difficile*, *Escherichia coli*, *Campylobacter jejuni* and others [4,11].

Salmonella serotype typhi *(S. typhi) and Shigella* are the main agents responsible for foodborne diseases and remain a major public health problem worldwide [9]. *Salmonella* is responsible for Salmonellosis, which can be characterized by diarrhea, fever, vomiting and abdominal pain occurring between 12-72h of infection. *Salmonella typhi* is the causative agent of typhoid fever, a systemic disease with diarrhea responsible for the deaths and morbidities observed worldwide in children under 5 years of age [4]. These salmonellosis are also major zoonoses worldwide with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever, 93.8 million cases of gastroenteritis and 155,000 deaths due to non-typhoidal *Salmonella* (NTS) [13]. *Shigella spp.* are more endemic to temperate and tropical climates and cause approximately 80-165 million cases of morbidity and 600,000 deaths per year [9]. Shigellosis is also known as bacillary dysentery or Marlow's syndrome caused by *Shigella spp.* which can rarely occur in animals compared to humans [4]. Apart from salmonellosis and shigellosis; one of the important agents involved in diarrhoea in developing countries is diarrheagenic *E. coli* (DEC) with six pathogenic types including enterotoxigenic *E. coli.* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterohemoragic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) [14,15,16,17]. Among these pathogens, EAEC, EPEC and ETEC are the most involved in intestinal pathologies and cause 30-40% of acute diarrhea cases in children in developed and developing countries [12]. Apart from the above germs, *Candida* species (*Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, etc.) are also known as intestinal yeasts and constitute a group of organisms living in the gastrointestinal tract whose growth causes the release of chemical toxins into the bloodstream with multiple effects, particularly in children [18].

The emergence of multiple drug resistance is a public health problem and complicates the treatment of enteric bacterial pathogens, particularly the antimicrobial agents used such as ampicillin, tetracyclin or trimethoprim sulfamethoxazole [6]. Moreover, the development of resistance by pathogens such as *Shigella*, *Salmonella spp.* and *Campylobacter is* becoming a major problem worldwide, especially in underdeveloped countries. In Ethiopia, for example, studies have shown that *Shigella* species are resistant to erythromycin, tetracyclin, cephalothin, ampicillin, chloramphenicol, sulfonamide, cotrimoxazole etc. In the United States of America, *Shigella* species are resistant to erythromycin, tetracyclin, cephalothin, ampicillin, chloramphenicol, sulfonamide, cotrimoxazole etc. In the same country, *Salmonella* species were resistant to ampicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, tetracyclin, sulfonamide and trimethoprim-sulfamethoxazole. *Campylobacter* was resistant to ampicillin and trimethoprim-sulfamethoxazole [2]. Konaté *et al.* [19], in the study conducted in Burkina Faso reveal that the choice of antibiotic drugs is becoming more and more difficult due to the observed resistance to first-line antibiotics such as chloramphenicol, trimethoprim-sulfamethoxazole, tetracyclin and penicillin A and when seen in this light, the treatment of *Escherichia coli* gastroenteritis is still based on the use of 3rd generation cephalosporins, aminoglycosides and quinolones. In addition, *Escherichia coli* strains not only constitute a health problem, but also complicate the treatment of a wide variety of infections, particularly in pediatrics with the spread of Extended spectrum β-lactamases (ESBL), which remains remarkable worldwide [12]. This outbreak of multi-resistant strains producing extended-spectrum β-lactamases (ESBL) has also been observed in China with the prevalence of carbapenemase-producing *Enterobacteriaceae* increasing in recent years [5].

In Cameroon, childhood diarrhoea is one of the major causes of death in children under five years of age, after malaria, measles and respiratory diseases [3]. Studies carried out in the West Cameroon region by Marbou and

Kuete [20] showed that *Escherichia coli* was the pathogenic species most represented in the intestinal tract of people living with HIV (PLHIV) (85.3%) compared to people without HIV (81.1%), with *Klebsiella* infection predominating among HIV-positive subjects (29.4%). This second species was significantly resistant to ceftriaxone, gentamicin, chloramphenicol, ciprofloxacin and doxycyclin in HIV+ patients compared to HIV- patients. Another study conducted in the same region by Ngalani *et al. [21]* also showed that *Salmonella sp., Escherichia coli* and *Klebsiella pneumoniae* species were the most represented in HIV+ patients compared to HIV- patients with significant *Enterobacter aerogenes* and *Shigella sp*. resistance to amoxicillin + clavulanic acid and cephixime. The resistance problem was also found for methicillin resistant *Staphylococcus aureus* (MRSA) due to the prevalence of the *mecA* gene by Marbou and Kuete at Mbouda Hospital in the Western Region [22] in patients with metabolic syndrome. In order to provide accurate information on multi-drug resistant germs in our context, the objectives of this study conducted between 2010 and 2020 in the Yaounde region at the Centre Pasteur of Cameroon were to present the microbiological profile of germs responsible for intestinal infections in our context, to assess whether there is an association between these germs and the sex and age of the study participants, and to present the sensitivity and resistance profiles of organisms isolated from stool samples to the drugs tested during the same period.

2. Materials and Methods

2.1. Place and Period of Study

This study was conducted from January 04, 2010 to January 07, 2020 in Yaounde, capital of the Central region at the Centre Pasteur of Cameroon (CPC) which is a technical body of the Ministry of Public Health of Cameroon and member of the International Network of Pasteur Institutes (RIIP).

2.2. Type of Study and Samples

This was an observational and retrospective study which focused on stool samples from patients from several health institutions in the city of Yaounde presenting a clinical picture of an intestinal infection and who came to the Centre Pasteur of Cameroon for a bacteriological and mycological examination of the digestive sphere.

2.3. Sample Collection and Analysis

The freshly emitted stools (3 to 4 grams for solid stools and 2 to 3 ml for liquid stools) by each participant were collected in a properly identified vial and transferred to the Bacteriology/Mycology laboratory for analysis. Once in the laboratory, the stool culture was performed in order to search for microorganisms involved in digestive pathologies (watery diarrhea or dysenteric syndrome, etc.). Macroscopic examination was performed to assess the consistency of the stool samples and then a microscopic examination was carried out (fresh state, gram staining). A culture step was performed according to the patient clinical data (age, clinical signs such as fever, abdominal pain, vomiting, etc.). Selective isolation media and enrichment media were used depending on the clinical context [23]. The identification of bacterial strains or species was based on the study of their morphological, cultural and biochemical characteristics (fermentation of sugars, reduction of nitrate, search for enzymes such as oxidase, catalase etc.) using API 20E galleries [24] and by automated method on the Vitek 2-compact apparatus [25,26]. The antibiotic susceptibility test was performed by the method of diffusion of antibiotic discs on Mueller-Hinton agar (MH Agar) and Mueller-Hinton agar with defibrinated horse blood and the addition of β-NAD (MH-F Agar) [27]. The Vitek 2-compact automaton was also used in the search for antibiotic susceptibility and resistance profiles [26].

The mycological analyses were carried out using slide and coverglass microscopy with the 10X objective and then with the 40X objective. The culture was carried out on Sabouraud Chloramphenicol medium for the detection of yeasts in the different samples. Blast assays, the API-20C gallery and the Vitek 2-compact automaton were used to identify the yeasts and to study morphological, cultural and biochemical characteristics [28]. The antifungal susceptibility test was performed using the method by diffusion on Sabouraud medium.

The antimicrobial susceptibility test, the control of the performance of the antibiotic and antifungal discs, the measurement of the diameters of the inhibition zones observed and the interpretation of the results were carried out in accordance with the recommendations of the authorities in force (CLSI [29], AFST/EUCAST [27]).

2.4. Data Collection and Statistical Analysis

The data were taken from the CPC GLIMS data management software. The data were collected taking into account the variables days and years of collection, sex, age (divided into classes or groups), the germs identified, their classifications, and the three-letter code corresponding to the antibiotics and antifungals used. After extraction of data from the GLIMS system, the database was cleaned with Microsoft Office Excel 2019 and statistical analyses were performed using R language version 3.6.1 (2019-07-05) [30]. The *finalfit* package of the R software was used to create the various analysis tables [31]. The statistical tests used in this research were: The Pearson Chi-square and Fisher's exact test for comparing differences in proportions between qualitative variables, the Kruskal Wallis test for comparing differences in means between the ages of participants according to age groups and the logistic regression model for determining Odds-ratio (OR) values and confidence intervals between identified germs according to the sex variable. The significance level was set at $p<0.05$.

3. Results

3.1. Characteristics of Study Population

During the study period, 40,339 stool samples were analysed with a prevalence of infection of 8.1% (3,284

samples positive for the presence of a bacterial species or fungus). The male sex was the most represented with 1697 samples (4.2%) versus 1484 (3.7%) samples for the female sex for a sex ratio of 1.14. This difference in sex distribution was statistically significant (p<0.001) (Table 1). The age of participants positive for infection ranged from 0-100 years with a mean age of 26.9 years ±21.3 SD. The most contaminated age group consisted of patients under 20 years of age (<20 years), i.e. 1620 (9.3%) samples for a mean age of 8.8 years ± 3.8 SD. In addition, in this age group, children were mostly male with 890 (4.9%) samples for boys and 712 (4.1%) samples for girls. The difference in sample distribution by age group was significant (p<0.0001) (Table 1). The year 2011 was the most represented in terms of contaminated samples followed by 2013 and 2010 for a significant difference in sample distribution by age group (p<0.0001) (Table 1).

Table 1. Distribution of sociodemographic variables according to age groups

Variables	Age (years) <20 (n=17.513) No. (%)	20-39 (n=8.167) No. (%)	40-59 (n=8.792) No. (%)	60-79 (n=4.839) No. (%)	≥80 (n=784) No. (%)	Total (n=40.339) No. (%)	P-value
Age							
Mean (SD)	8.8 (3.8)	30.4 (5.6)	48.2 (5.8)	67.1 (5.3)	85.1 (5.2)	26.9 (21.3)	**<0.0001***
Sex							
Men	860 (4.9)	317 (3.9)	391 (4.4)	103 (2.1)	16 (2.0)	1687 (4.2)	**<0.0001**
Women	712 (4.1)	338 (4.1)	262 (3.0)	137 (2.8)	30 (3.8)	1479 (3.7)	
Years							
2010	210 (1.2)	55 (0.7)	73 (0.8)	29 (0.6)	3 (0.4)	370 (0.9)	**<0.0001**
2011	207 (1.2)	221 (2.7)	165 (1.9)	52 (1.1)	7 (0.9)	652 (1.6)	
2012	192 (1.1)	38 (0.5)	38 (0.4)	16 (0.3)	4 (0.5)	288 (0.7)	
2013	240 (1.4)	60 (0.7)	66 (0.8)	33 (0.7)	3 (0.3)	402 (1.0)	
2014	142 (0.8)	43 (0.5)	51 (0.6)	21 (0.4)	8 (1.0)	265 (0.7)	
2015	180 (1.0)	65 (0.8)	63 (0.7)	23 (0.5)	8 (1.0)	339 (0.8)	
2016	151 (0.9)	55 (0.7)	71 (0.8)	30 (0.6)	3 (0.4)	310 (0.8)	
2017	119 (0.7)	66 (0.0)	70 (0.8)	21 (0.4)	7 (0.9)	283 (0.7)	
2018	102 (0.6)	57 (0.7)	47 (0.5)	18 (0.3)	3 (0.4)	227 (0.6)	
2019	76 (0.4)	18 (0.2)	28 (0.3)	2 (0.0)	0 (0.0)	124 (0.3)	
2020	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	
Overall prevalence	1620 (9.3)	678 (8.3)	672 (7.6)	245 (5.1)	46 (5.9)	3284 (8.1)	**<0.0001**

P-value: p-value of Pearson's Chi-squared test
*: p-value of Kruskal-Wallis rank sum test.

Table 2. The 19 most common organisms isolated from CPC during the study period

Ranking	Organisms	No. of isolates	% of total
	Bacteria		
1	*Shigella flexneri*	468	1.16
2	*Vibrio cholerae*	459	1.14
3	*Salmonella sp*	298	0.74
4	*Escherichia coli*	250	0.62
5	*Campylobacter sp*	59	0.15
6	*Shigella sonnei*	51	0.13
7	*Shigella sp*	46	0.11
8	*Shigella boydii*	45	0.11
9	*Salmonella enteritidis*	36	0.09
10	*Salmonella typhimurium*	36	0.09
11	*Campylobacter jejuni*	34	0.08
12	*Shigella dysenteriae A2*	15	0.04
13	*Salmonella typhi*	13	0.03
14	*Campylobacter coli*	12	0.03
15	*Campylobacter lari*	12	0.03
16	*Salmonella hadar*	11	0.03
17	*Shigella dysenteriae A1*	11	0.03
	Total	**1856**	**4.61**
	Fungi		
18	*Candida albicans*	1062	2.63
19	*Candida sp*	342	0.85
	Total	**1404**	**3.48**
	Other*	24	0.06

*Other organisms included the following (No.): *Campylobacter upsaliensis* (4); *Salmonella infantis* (4); *Salmonella stanley* (3); *Salmonella anatum* (2); *Salmonella arizonae* (2); *Salmonella salamae* (2); *Campylobacter fetus* (1); *Proteus mirabilis* (1); *Salmonella derby* (1); *Salmonella dublin* (1); *Salmonella heidelberg* (1); *Candida famata* (1); *Zygomycetes sp* (1).

3.2. Distribution of Identified Organisms

Among the bacteria isolated from stool samples, 28 gram-negative bacillus species were identified during the study period. The most represented in the stool samples were: *Shigella flexneri* (1.16%), *Vibrio cholerae* (1.14%), *Salmonella sp*. (0.74%), *Escherichia coli* (0.62%), *Campylobacter sp.* (0.15%), *Shigella sonnei* (0.13%), *Shigella sp.* (0.11%), *Shigella boydii* (*0.*11%), *Salmonella enteritidis* (0.09%), *Salmonella typhimurium* (0.09%), *Campylobacter jejuni* (0.08%), *Shigella dysenteriae A2* (0.04%), *Salmonella typhi* (0.03%), *Campylobacter coli* (0.03%), *Campylobacter lari* (0.03%), *Salmonella hadar* (0.03%*)* and *Shigella dysenteriae A1* (0.03%). The other species isolated in stool samples in the study are presented in Table 2. Concerning fungi, *Candida albicans* (2.63%) was the most represented followed by other strains of Candida (*Candida sp.* with a prevalence of 0.85%). The other fungal species isolated in the study are presented in Table 2.

3.3. Association of Identified Organisms with Age and Sex

Pearson's Chi-2 test and Fisher's exact Chi-2 test were used to assess the association between single germs and sex. The results of this test showed that a statistically significant association exists between gender and the identified germs ($p<0.001$). The germs for which the difference in distribution was significant were: *Shigella boydii* ($p = 0.04886$); *Shigella dysenteriae A1* ($p = 0.01918$); *Shigella sonnei* ($p = 0.0414$) and *Vibrio cholerae* ($p<0.001$). The logistic regression model showed that the genus is a risk factor for *Shigella dysenteriae A1* infection with an OR significantly different from 1 (OR=5.17, CI=1.07-49.23). This would mean that *Shigella dysenteriae A1* infection would be 5 times higher in women than in men. On the other hand, *Vibrio cholerae* infection was a protective factor against gender although the OR value was not significantly different from 1 (OR=0.69, CI=0.56-0.86). A distributional difference between *Shigella flexneri* infection and genus was also observed although it was not significant ($p = 0.05032$). For the fungi isolated in this study, no statistically significant association with genus was observed. Details on the other association patterns can be found in Table 3.

The age of the participants was also associated with the germs isolated in this research with a statistically significant distribution between bacterial and fungal species with different age groups ($p<0.0001$). The results in Table 4 showed that children (<20 years of age) were the most contaminated with a significantly higher distribution of *Campylobacter jejuni, Campylobacter lari, Campylobacter sp., Escherichia coli*, *Salmonella hadar, Salmonella sp., Shigella flexneri*, *Shigella sonnei* and *Candida albicans*, in this age group compared to others. *Vibrio cholerae* species, on the other hand, was more represented in adults (patients 20-39 years old and 40-59 years old) with a significant distribution of this species across age groups ($p<0.0001$). *Campylobacter coli*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae A1* and *A2*, *Shigella sp.*, and *Candida sp.* were also well represented in children but differences in the distribution of these germs across age groups were not statistically significant ($p>0.05$).

Table 3. Distribution of the 19 most common organisms isolated according to sex

Isolated organism	Sex		Total (n=40.339) No. (%)	OR (95%-CI)	P-value
	Men (n=20.295) No. (%)	Women (n=18.982) No. (%)			
Bacteria					
Campylobacter coli	4 (0.0)	6 (0.0)	10 (0.0)	1.71 (0.40-8.29)	0.5294*
Campylobacter jejuni	20 (0.1)	13 (0.1)	33 (0.1)	0.74 (0.34-1.57)	0.4008
Campylobacter lari	6 (0.0)	6 (0.0)	12 (0.0)	1.14 (0.31-4.29)	0.8158
Campylobacter sp	33 (0.2)	24 (0.1)	57 (0.1)	0.83 (0.47-1.45)	0.4875
Escherichia coli	132 (0.7)	107 (0.6)	239 (0.6)	0.92 (0.70-1.21)	0.5442
Salmonella enteritidis	18 (0.1)	16 (0.1)	34 (0.1)	1.01 (0.48-2.12)	0.9619
Salmonella hadar	7 (0.0)	4 (0.0)	11 (0.0)	0.65 (0.14-2.57)	0.4932
Salmonella sp	151 (0.7)	140 (0.7)	291 (0.7)	1.07 (0.83-1.37)	0.6009
Salmonella typhi	6 (0.0)	7 (0.0)	13 (0.0)	1.33 (0.38-4.82)	0.6024
Salmonella typhimurium	17 (0.1)	19 (0.1)	36 (0.1)	1.28 (0.63-2.64)	0.4587
Shigella boydii	17 (0.1)	27 (0.1)	44 (0.1)	1.83 (0.96-3.60)	**0.04886**
Shigella dysenteriae A1	2 (0.0)	9 (0.0)	11 (0.0)	5.17 (1.07-49.23)	**0.01918**
Shigella dysenteriae A2	9 (0.0)	5 (0.0)	14 (0.0)	0.63 (0.17-2.11)	0.411
Shigella flexneri	225 (1.1)	233 (1.2)	458 (1.1)	1.21 (1.00-1.49)	**0.05032**
Shigella sonnei	20 (0.1)	31 (0.2)	51 (0.1)	1.79 (0.98-3.33)	**0.0414**
Shigella sp	22 (0.1)	23 (0.1)	45 (0.1)	1.20 (0.64-1.27)	0.5459
Vibrio cholerae	259 (1.3)	165 (0.9)	424 (1.1)	0.69 (0.56-0.86)	**<0.001**
Fungi					
Candida albicans	552 (2.7)	483 (2.5)	1035 (2.6)	1.00 (0.86-1.16)	0.9908
Candida sp	184 (0.9)	156 (0.8)	340 (0.8)	0.97 (0.77-1.22)	0.7634
Other organisms	13 (0.1)	10 (0.1)	23 (0.1)	0.88 (0.34-2.18)	0.7594
Overall prevalence	1697 (8.4)	1484 (7.8)	3181 (7.9)		**<0.001**

P-value: p-value of Pearson's Chi-squared test
*: P-value of Fisher's exact test
OR: Odd ratio
CI: Confidence interval.

Table 4. Distribution of the 19 most common organisms isolated according to age groups

Isolated organisms	Age groups (years) <20 (n=17.513) No. (%)	20-39 (n=8.167) No. (%)	40-59 (n=8.792) No. (%)	60-79 (n=4.839) No. (%)	≥80 (n=784) No. (%)	Total (n=40.339) No. (%)	P-value
Bacteria							
Campylobacter coli	11 (0.1)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	12 (0.0)	0.09963*
Campylobacter jejuni	34 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	34 (0.1)	**<0.0001***
Campylobacter lari	12 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (0.0)	**0.02131***
Campylobacter sp	53 (0.3)	0 (0.0)	0 (0.0)	5 (0.1)	1 (0.1)	59 (0.1)	**<0.0001***
Escherichia coli	248 (1.4)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	249 (0.6)	**<0.0001**
Salmonella enteritidis	16 (0.1)	8 (0.1)	8 (0.1)	3 (0.1)	1 (0.1)	36 (0.1)	0.727*
Salmonella hadar	4 (0.0)	0 (0.0)	4 (0.0)	3 (0.1)	0 (0.0)	11 (0.0)	**0.04066***
Salmonella sp	116 (0.7)	60 (0.7)	84 (1.0)	36 (0.7)	1 (0.1)	297 (0.7)	**<0.0001**
Salmonella typhi	7 (0.0)	2 (0.0)	2 (0.0)	2 (0.0)	0 (0.0)	13 (0.0)	0.6986*
Salmonella typhimurium	17 (0.1)	7 (0.1)	6 (0.1)	5 (0.1)	1 (0.1)	36 (0.1)	0.4229*
Shigella boydii	12 (0.1)	11 (0.1)	15 (0.2)	7 (0.1)	0 (0.0)	45 (0.1)	**0.008861***
Shigella dysenteriae A1	4 (0.0)	2 (0.0)	3 (0.0)	2 (0.0)	0 (0.0)	11 (0.0)	0.4715*
Shigella dysenteriae A2	6 (0.0)	2 (0.0)	5 (0.1)	2 (0.0)	0 (0.0)	15 (0.0)	0.5019*
Shigella flexneri	276 (1.6)	80 (1.0)	81 (0.9)	24 (0.5)	5 (0.6)	466 (1.2)	**<0.001**
Shigella sonnei	38 (0.2)	5 (0.1)	6 (0.1)	2 (0.0)	0 (0.0)	51 (0.1)	**0.01822***
Shigella sp	24 (0.1)	9 (0.1)	10 (0.1)	3 (0.1)	0 (0.0)	46 (0.1)	0.995*
Vibrio cholerae	43 (0.2)	209 (2.6)	144 (1.6)	42 (0.9)	3 (0.4)	441 (1.1)	**<0.0001**
Fungi							
Candida albicans	524 (3.0)	209 (2.6)	225 (2.6)	76 (1.6)	27 (3.4)	1061 (2.6)	**0.003297**
Candida sp.	164 (0.9)	68 (0.8)	74 (0.8)	30 (0.6)	6 (0.8)	342 (0.8)	0.7381*
Other organisms	11 (0.1)	5 (0.1)	4 (0.0)	3 (0.1)	1 (0.1)	24 (0.1)	0.4358*
Overall prevalence	1620 (9.3)	678 (8.3)	672 (7.6)	245 (5.1)	46 (5.9)	3284 (8.1)	**<0.0001**

P-value: p-value of Pearson's Chi-squared test
*: p-value of Fisher's exact test.

3.4. Drug Susceptibility and Resistance Profiles of Isolated Organisms during the Study

Approximately 40 antibiotics and 8 antifungals were used to determine the sensitivity and resistance profiles of the germs isolated in this study. The classes of antibiotics represented were: penicillin family antibiotics with penicillin G (PEN), ampicillin (AMP), amoxicillin (AMO), amoxicillin + clavulanic acid (AMC), ticarcillin (TIC), ticarcillin + clavulanic acid (TCC), piperacillin (PIC), piperacillin + tazobactam (TZP/PIT); the class of cephalosporins with cefatrizine (CFT), cefuroxime (CXM), cefoxitin (CXT), cefotaxime (CTX), ceftazidime (CAZ), cefixime (CFM), cefepime (FEP); the class of aminoglycosides with gentamicin (GEN), tobramicin (TOB), amikacin (AKN), netilmicin (NET), streptomycin (S); the class of quinolones with nalidixic acid (NAL), ofloxacin (OFL), pefloxacin (PEF), norfloxacin (NOR), ciprofloxacin (CIP); the class of sulfonamides with sulfonamid (SSS), trimethoprim + sulfamethoxazole or cotrimoxazole (SXT/TSU); the class of carbapenems with imipenem (IMI) and ertapenem (ETP); the class of monobactams with aztreonam (ATM/AZT); the class of cyclines with tetracycline (TET) and doxycycline (DOT); the class of macrolides and related with erythromycin (ERY); the class of peptides with colistin (COL) and polymyxin B (PB); the class of phenicols with chloramphenicol (CMP); the class of nitrofurans with nitrofurantoin (FUR) and various antibiotics with fosfomycin (FOS). For antifungals, the classes represented were azoles with miconazole (MIC), econazole (ECO), ketoconazole (KET), fluconazole (FLU), voriconazole (VRC), clotrimazole (CLO) and polyenes with amphotericin B (AMB) and nystatin (NYS).

For antibiotics of the penicillin family, high levels of resistance have been found for amoxicillin. The species most resistant to this antibiotic were: *Shigella flexneri* (83.3%) followed by *Salmonella typhi* (76.9%) and *Shigella dysenteriae A2* (73.3%). Significant levels of sensitivity were also observed for this antibiotic with *Salmonella hadar*, *Shigella sonnei* and *Salmonella sp. species* whose sensitivity levels were 100%, 82.4% and 76.8% respectively. The use of amoxicillin + clavulanic acid as another antibiotic revealed that only the *Shigella flexneri* species had a higher percentage of resistance (54.3%). The species most sensitive to this antibiotic were *Salmonella hadar* (100%), *Shigella sonnei* (90.2%) and *Salmonella enteritidis* (88.9%). Ticarcillin was also among the antibiotics for which high levels of resistance were observed. This is the case of *Shigella flexneri* with 81.0% resistance, *Salmonella typhi* with 76.9% resistance and *Shigella dysenteriae A2* with 73.3% resistance. Cephalosporins, inhibitors of bacterial envelope synthesis have shown for most antibiotics of this class very high levels of sensitivity (see Table 5). However, levels of resistance to cefatrizine have been observed in *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter sp*. (91.7%, 73.5%, 66.7% and 78.0% respectively). *Vibrio cholerae* was also one of the most resistant species to peptides with 82.1% resistance to colistin and 58.4% resistance to polymixin B.

Table 5. Susceptibility and Resistance rates for the 17 most common bacteria isolated from CPC during de study period

Antibiotics	Isolated bacteria (No.)								
	Campylobacter coli (n=12)	*Campylobacter jejuni* (n=34)	*Campylobacter lari* (n=12)	*Campylobacter sp* (n=59)	*Escherichia coli* (n=250)	*Salmonella enteritidis* (n=36)	*Salmonella hadar* (n=11)	*Salmonella sp* (n=298)	*Salmonella typhi* (n=13)
	%I/%R/%S								
PEN	NA	0/0/2.9	0/16.7/8.3	0/10.2/1.7	NA	NA	NA	NA	NA
AMP	NA	NA	0/0/8.3	NA	0/12.0/2.8	NA	NA	0/0.3/0.3	NA
AMO	0/58.3/41.7	5.9/50.0/29.4	8.3/66.7/16.7	8.5/66.1/11.9	0/52.4/12.4	0/11.1/83.3	0/0/100.0	1.7/17.8/76.8	0/76.9/23.1
AMC	8.3/16.7/25.0	11.8/8.8/50.0	0/25.0/75.0	8.5/11.9/67.8	9.6/38.0/31.6	0/5.6/88.9	0/0/100.0	2.3/12.8/82.2	23.1/30.8/46.2
TIC	NA	NA	NA	0/0/1.7	0.4/60.0/18.8	0/5.6/88.9	0/0/90.9	0.3/17.1/80.2	0/76.9/23.1
TCC	NA	NA	NA	NA	0.4/5.6/8.4	0/0/2.8	NA	0/2.3/2.7	NA
PIC	NA	NA	NA	NA	0/31.2/10.4	0/0/5.6	NA	0/3.7/5.0	0/7.7/0
TZP/PIT	NA	NA	NA	NA	2.8/1.2/18.4	NA	NA	1.0/1.0/1.0	NA
IMI	NA	NA	NA	NA	0/0.4/31.6	0/0/11.1	0/0/9.1	0.3/0/27.5	0/0/7.7
CFT	0/91.7/0	0/73.5/0	0/66.7/0	0/78.0/3.4	10.0/37.2/31.6	2.8/0/88.9	9.1/0/90.9	3.0/9.4/82.2	15.4/7.7/76.9
CXM	NA	NA	NA	NA	0/3.2/9.2	NA	NA	0/0/0.7	NA
CXT	NA	NA	NA	NA	0.4/3.2/40.0	0/0/50.0	0/0/45.5	0/2.0/52.3	0/0/69.2
CTX	0/33.3/16.7	2.9/32.4/20.6	0/33.3/25.0	1.7/15.3/10.2	0/15.2/64.4	0/0/94.4	0/0/100.0	0.3/6.4/89.6	0/0/100.0
CAZ	NA	NA	NA	NA	2.8/14.0/61.2	2.8/0/83.3	0/0/81.8	3.4/7.0/81.5	0/0/100.0
CFM	NA	NA	NA	NA	0/3.2/6.4	NA	NA	0/0/0.7	NA
FEP	NA	NA	NA	NA	1.2/2.8/5.6	0/0/5.6	NA	0/0.3/5.0	0/0/7.7
GEN	0/16.7/83.3	5.9/5.9/79.4	0/0/83.3	1.7/1.7/83.1	0.8/11.2/68.0	0/0/94.4	0/0/100.0	1.0/7.0/87.9	0/0/100.0
TOB	NA	0/0/2.9	NA	NA	0/8.8/42.0	0/0/5.6	NA	0/3.7/11.1	0/0/30.8
AKN	NA	NA	NA	NA	0.4/5.2/73.2	0/0/94.4	0/0/100.0	0.3/0.3/89.3	0/0/100.0
NET	NA	NA	NA	NA	0/2.0/10.4	0/0/5.6	NA	0/2.3/1.3	NA
S	NA	NA	NA	NA	NA	NA	NA	NA	NA
CMP	0/0/66.7	2.9/2.9/47.1	0/0/16.7	0/1.7/16.9	0/4.4/13.6	0/2.8/75.0	0/0/72.7	0.7/8.4/51.7	0/7.7/84.6
TET	0/25.0/8.3	0/26.5/2.9	0/25.0/0	0/35.6/11.9	0/30.4/9.2	13.9/5.6/55.6	0/63.6/18.2	4.0/20.5/38.6	0/53.8/15.4
DOT	NA	NA	NA	NA	NA	NA	NA	NA	NA
ERY	8.3/8.3/83.3	2.9/0/82.4	0/0/91.7	1.7/3.4/86.4	0/0/0.4	NA	NA	NA	NA
PB	NA	NA	NA	NA	NA	NA	NA	NA	NA
COL	NA	NA	NA	NA	NA	NA	NA	NA	NA
SSS	NA	NA	NA	NA	NA	NA	NA	0/0/0.7	0/0/7.7
TMP	NA	NA	NA	NA	NA	NA	NA	0/0/1.0	NA
FUR	NA	NA	NA	NA	0/0/1.2	NA	NA	NA	NA
NAL	8.3/50.0/41.7	0/58.8/35.3	0/100.0/0	5.1/64.4/18.6	1.2/20.0/40.0	0/11.1/77.8	0/81.8/18.2	7.0/16.1/73.2	0/15.4/84.6
OFL	NA	NA	NA	NA	0.4/13.6/33.6	0/0/5.6	NA	1.7/2.0/10.4	0/0/23.1
PEF	NA	0/2.9/0	NA	NA	0/0.4/9.6	0/0/58.3	0/0/54.5	0/2.3/23.8	0/0/15.4
NOR	NA	NA	NA	0/0/1.7	0/0/0.8	NA	NA	NA	NA
CIP	0/50.0/50.0	0/55.9/35.3	0/91.7/0	0/67.8/23.7	2.0/11.2/65.2	2.8/0/91.7	0/0/100.0	3.7/4.4/89.3	0/0/100.0
FOS	NA	NA	NA	NA	0/1.2/40.8	0/0/5.6	NA	0/0/7.4	0/0/7.7
TSU	NA	NA	NA	NA	0/64.0/14.4	0/2.8/91.7	0/45.5/54.5	0/22.8/72.5	0/76.9/23.1
AZT/ATM	0/0/50.0	0/0/50.0	0/0/33.3	0/1.7/20.3	0.4/1.6/7.6	0/0/44.4	0/0/45.5	2.3/5.0/25.5	0/15.4/30.8
ETP	NA	NA	NA	NA	0/0/0.4	NA	NA	NA	NA

PEN: Penicillin; AMP: Ampicillin; AMO: Amoxicillin; AMC: Amoxicillin + clavulanic acid; TIC: Ticarcillin; TCC: Ticarcillin + clavulanic acid; PIC: Piperacillin; TZP/PIT: Piperacillin + Tazobactam; IMI: Imipenem; CFT: Cefatrizine; CXM: Cephuroxime; CXT: Cephoxitin; CTX: Cephotaxime; CAZ: Ceftazidime; CFM: Cephixime; FEP: Cefepime; GEN: Gentamicin; TOB: Tobramicin; AKN: Amikacin; NET: Netilmicin; S: Streptomycin; CMP: Chloramphenicol; TET: Tetracycline; DOT: Doxycyclin; ERY: Erythromycin; PB: Polymyxin B; COL: Colistin; SSS: Sulfonamid; TMP: Trimethoprim-Sulfamethoxazole; FUR: Nitrofirantoïn; NAL: Nalidixic acid; OFL: Ofloxacin; PEF: Pefloxacin; NOR: Norfloxacin; CIP: Ciprofloxacin; FOS: Fosfomycin; TSU: Cotrimoxazole; AZT/ATM: Aztreonam; ETP: Ertapenem; I: Intermediate; S: Susceptibility; R: Resistance; NA: not available.

Table 5. Susceptibility and Resistance rates for the 17 most common bacteria isolated from CPC during de study period (Continued)

Antibiotics	Isolated bacteria (No.)							
	Salmonella typhimurium (n=36)	*Shigella boydii* (n=45)	*Shigella dysenteriae A1* (n=11)	*Shigella dysenteriae A2* (n=15)	*Shigella flexneri* (n=468)	*Shigella sonnei* (n=51)	*Shigella sp* (n=46)	*Vibrio cholerae* (n=459)
	%I/%R/%S							
PEN	NA	NA	NA	NA	NA	NA	NA	NA
AMP	NA	NA	0/0/9.1	NA	0/1.3/0.2	0/0/3.9	NA	NA
AMO	2.8/30.6/66.7	8.9/57.8/31.1	0/63.6/27.3	6.7/73.3/20.0	1.5/83.3/12.4	7.8/5.9/82.4	4.3/47.8/47.8	29.2/48.8/14.6
AMC	5.6/19.4/75.0	15.6/22.2/57.8	9.1/45.5/45.5	13.3/33.3/53.3	19.0/54.3/24.8	5.9/3.9/90.2	8.7/26.1/60.9	46.6/30.3/15.7
TIC	2.8/27.8/66.7	0/46.7/48.9	0/45.5/54.5	6.7/73.3/20.0	0.4/81.0/15.4	2.0/0/94.1	0/43.5/54.3	0.2/0.4/0.7
TCC	NA	NA	NA	NA	0/1.1/0.9	0/0/3.9	NA	NA
PIC	NA	0/0/2.2	0/0/9.1	NA	0/3.6/1.3	0/0/3.9	NA	NA
TZP/PIT	NA	NA	0/0/9.1	NA	0/0/2.8	0/0/3.9	NA	NA
IMI	0/0/36.1	0/0/17.8	0/0/18.2	0/0/6.7	0/0/22.6	0/0/23.5	0/0/30.4	NA
CFT	8.3/5.6/77.8	17.8/4.4/75.6	0/0/81.8	13.3/0/86.7	9.8/2.8/82.5	13.7/2.0/76.5	4.3/8.7/80.4	8.7/2.8/77.8
CXM	NA	NA	0/0/9.1	NA	0/0/1.5	NA	NA	NA
CXT	0/0/55.6	2.2/0/55.6	0/0/54.5	0/0/33.3	0.6/0.6/46.8	0/0/39.2	0/4.3/63.0	0/0/2.0
CTX	0/5.6/94.4	0/2.2/93.3	0/0/100.0	0/0/100.0	0.4/0.9/96.2	0/0/100.0	0/2.2/97.8	0.4/0/89.5
CAZ	8.3/2.8/80.6	0/4.4/88.9	0/0/100.0	0/0/100.0	1.9/0.6/92.1	2.0/0/88.2	0/2.2/97.8	0/0/0.2
CFM	NA	NA	0/0/9.1	NA	0/0/1.3	NA	NA	NA
FEP	NA	0/0/4.4	NA	NA	0.2/0.2/2.1	0/0/2.0	NA	NA
GEN	2.8/0/94.4	0/2.2/93.3	0/0/100.0	6.7/0/93.3	1.7/0.9/94.9	3.9/2.0/92.2	0/2.2/95.7	0.2/0/92.4
TOB	0/0/5.6	0/0/4.4	0/0/9.1	0/0/6.7	0/0/7.3	0/0/7.8	0/0/2.2	NA
AKN	0/0/94.4	0/0/91.1	0/0/81.8	0/0/73.3	0.6/0.2/94.0	0/2.0/98.0	0/0/97.8	0/0.2/1.3
NET	NA	NA	NA	NA	0/0/1.5	NA	NA	NA
S	0/2.8/0	NA	NA	NA	0/0/0.2	NA	NA	0/24.6/1.5
CMP	0/16.7/69.4	0/13.3/62.2	0/18.2/63.6	0/26.7/33.3	2.1/45.1/17.1	2.0/3.9/49.0	0/17.4/43.5	0/0/1.5
TET	8.3/19.4/52.8	0/64.4/8.9	0/63.6/18.2	0/53.3/6.7	0.2/64.3/2.8	0/60.8/2.0	0/52.2/15.2	0/0/1.7
DOT	NA	NA	NA	NA	0/0/0.2	NA	NA	0/0.7/91.1
ERY	NA	NA	NA	NA	0/0/0.2	NA	NA	2.6/0.2/88.4
PB	NA	NA	NA	NA	0/0/0.2	NA	NA	0/58.4/0.2
COL	NA	0/0/2.2	NA	NA	0/0/0.2	NA	NA	0/82.1/0
SSS	NA	NA	NA	NA	0/0.4/0.2	NA	NA	NA
TMP	NA	NA	NA	NA	NA	NA	NA	NA
FUR	NA	NA	NA	NA	NA	NA	NA	NA
NAL	0/8.3/91.7	0/6.7/91.1	0/0/90.9	0/0/93.3	0/1.1/94.7	0/2.0/94.1	0/6.5/91.3	0/92.4/0.2
OFL	0/2.8/8.3	0/0/8.9	0/0/27.3	NA	0.2/0/15.8	0/0/13.7	0/0/10.9	0/0/0.9
PEF	0/0/36.1	0/2.2/37.8	0/0/36.4	0/0/20.0	0/0.1/31.8	0/0/21.6	0/0/19.6	NA
NOR	NA	NA	NA	NA	NA	NA	NA	NA
CIP	0/2.8/97.2	0/2.2/95.6	0/0/100.0	0/6.7/93.3	0.4/0.6/96.2	0/0/98.0	2.2/2.2/95.7	1.7/0.2/87.6
FOS	NA	0/0/2.2	0/0/9.1	NA	0/0/5.1	0/0/3.9	NA	NA
TSU	0/19.4/75.0	0/88.9/6.7	0/90.9/9.1	0/100.0/0	0.2/84.2/11.1	0/96.1/3.9	0/82.6/15.2	0/77.3/11.1
AZT/ATM	0/2.8/30.6	8.9/4.4/40.0	18.2/0/45.5	13.3/6.7/6.7	5.8/2.1/39.1	5.9/3.9/27.5	4.3/0/32.6	0/0/1.1
ETP	NA	NA	NA	NA	0/0/0.4	NA	0/0/2.1	NA

PEN: Penicillin; AMP: Ampicillin; AMO: Amoxicillin; AMC: Amoxicillin + clavulanic acid; TIC: Ticarcillin; TCC: Ticarcillin + clavulanic acid; PIC: Piperacillin; TZP/PIT: Piperacillin + Tazobactam; IMI: Imipenem; CFT: Cefatrizine; CXM: Cephuroxime; CXT: Cephoxitin; CTX: Cephotaxime; CAZ: Ceftazidime; CFM: Cephixime; FEP: Cefepime; GEN: Gentamicin; TOB: Tobramicin; AKN: Amikacin; NET: Netilmicin; S: Streptomycin; CMP: Chloramphenicol; TET: Tetracycline; DOT: Doxycyclin; ERY: Erythromycin; PB: Polymyxin B; COL: Colistin; SSS: Sulfonamid; TMP: Trimethoprim-Sulfamethoxazole; FUR: Nitrofirantoïn; NAL: Nalidixic acid; OFL: Ofloxacin; PEF: Pefloxacin; NOR: Norfloxacin; CIP: Ciprofloxacin; FOS: Fosfomycin; TSU: Cotrimoxazole; AZT/ATM: Aztreonam; ETP: Ertapenem; I: Intermediate; S: Susceptibility; R: Resistance; NA: not available.

Table 6. Susceptibility and Resistance rates for the 2 most common fungi isolated from CPC during de study period

Isolated organism (No.)	%I/%R/%S							
	MIC	**ECO**	**KET**	**FLU**	**AMB**	**NYS**	**VRC**	**CLO**
Candida albicans (n=1062)	2.4/0.5/88.2	1.3/0.3/86.4	0.6/0.3/82.2	0/0/2.4	0.3/10.2/78.2	0.1/3.6/84.5	0.1/0.1/1.3	3.4/1.2/81.9
Candida sp (n=342)	6.7/0/83.3	4.4/0/83.0	2.3/0/87.1	0/0/2.9	1.2/28.1/62.9	0.3/3.8/84.8	0.3/0/3.2	10.5/3.5/75.4

MIC: Miconazole; ECO: Econazole; KET: Ketoconazole; FLU: Fluconazole; AMB: Amphotericin B; NYS: Nystatin; VRC: Voriconazole; CLO: Clotrimazole; NA: not available; I: Intermediate; S: Susceptibility; R: Resistance.

Among antibiotics that inhibit protein synthesis, the class of aminoglycosides was highly represented in terms of antibiotic susceptibility of germs. The antibiotic discs most represented in terms of sensitivity in this class were gentamicin for which the most sensitive germs were *Salmonella hadar* (100%), *Shigella dysenteriae A1* (100%) and *Salmonella typhi* (100%); and amikacin for which *Salmonella hadar* and *Salmonella typhi* were fully sensitive (Table 5). Phenicols were also represented in terms of sensitivity with Salmonella *typhi*, *Salmonella enteritidis* and *Salmonella hadar* being the most sensitive to chloramphenicol (84.6%, 75.0% and 72.7% respectively). Erythromycin was the only antibiotic represented in the macrolide and related class with high sensitivity levels for *Campylobacter coli* (83.3%), *Campylobacter jejuni* (82.4%), *Campylobacter lari* (91.7%), *Campylobacter sp.* (86.4%) and *Vibrio cholerae* (88.4%). In the cyclin class, most of the germs represented were resistant to tetracycline with 63.6% resistance for *Salmonella hadar*, 64.4% resistance for *Shigella boydii*, 63.6% resistance for *Shigella dysenteriae A1*, 64.3% resistance for *Shigella flexneri* and 60.8% resistance for *Shigella sonnei*. For doxycyclin, only *Vibrio cholerae* was the most represented in terms of susceptibility (91.1%).

The results in terms of sensitivity and resistance to antibiotics of the quinolone family, inhibitors of nucleic acid synthesis, showed that nalidixic acid and ciprofloxacin were the most represented antibiotics in terms of sensitivity. For nalidixic acid, the most sensitive germs were: *Salmonella typhimurium* (91.7%), *Shigella boydii* (91.1%), *Shigella dysenteriae A1* (90.9%), *Shigella dysenteriae A2* (93.3%), *Shigella flexneri* (94.7%), *Shigella sonnei* (94.1%) and *Shigella sp.* (92.4%). Resistance levels were also found for this antibiotic with *Campylobacter lari* (100%), *Vibrio cholerae* (92.4%) and *Salmonella hadar* (81.8%). For ciprofloxacin, only the *Campylobacter lari* species was the most resistant (91.7%), the other bacteria identified being mostly very sensitive with *Escherichia coli* (91.7%), *Salmonella hadar* (100%), *Salmonella sp.* (89.3%), *Salmonella typhi* (100%), *Salmonella typhimurium* (97.2%), *Shigella boydii* (95.6%), *Shigella dysenteriae A1* (100%), *Shigella dysenteriae A2* (93.3%), *Shigella flexneri* (96.2%), *Shigella sonnei* (98.0%), *Shigella sp.* (95.7%) and *Vibrio cholerae* (87.6%).

In the class of sulfonamides, folic acid inhibitors, the antibiotic most represented in terms of resistance was cotrimoxazole. Germs with high resistance to this antibiotic were: *Salmonella typhi* (76.9%), *Shigella boydii* (88.9%), *Shigella dysenteriae A1* (90.9%), *Shigella dysenteriae A2* (100%), *Shigella flexneri* (84.2%), *Shigella sonnei* (96.1%), *Shigella sp.* (82.6%) and *Vibrio cholerae* (77.3%). In terms of sensitivity to this antibiotic, *Salmonella enteritidis* was the most sensitive (91.7%) followed by *Salmonella typhimurium* (75.0%) and *Salmonella sp*. (72.5%).

The fungi isolated from stool samples in this research were susceptible to most of the antifungal agents used. For azole class antifungal agents, the sensitivity of *Candida albicans* was highest for miconazole (88.2%), followed by econazole (86.4%), ketoconazole (82.2%) and clotrimazole (81.9%). Other strains of Candida (*Candida sp.*) were more sensitive to ketoconazole (87.1%) followed by miconazole (83.3%), econazole (83.3%) and clotrimazole (75.4%). In the polyene class, *Candida albicans* was most sensitive to nystatin and then amphotericin B (78.2%). The other Candida strains were more sensitive to nystatin (84.8%), then to amphotericin B (62.9%) (Table 6).

4. Discussion

In this observational and retrospective study, the prevalence of gastrointestinal infections was 8.1% (3284 positive samples out of 40,339 samples tested). This result is different from those obtained in other studies [1,10,32], where the prevalence of intestinal infections was 49.6%, 9.9% and 12.6% respectively. In the study by Abera *et al.* [9], the prevalence of intestinal infections was lower than in the present study at 3.9%. In the study by Mahmoudi *et al. [33]*, the prevalence of gastroenteritis in children in Iran was higher than in this study 211/676 (31.2%) culture positive samples. This difference in prevalence of intestinal infections from one region to another or from one country to another could be explained by the environmental conditions, the location of the study and the laboratory methods used for the identification of bacterial species as underlined by Diriba *et al.* [34], who worked on the determination of the prevalence and resistance patterns of bacterial and parasitic species in food handlers at the University of Dilla in Ethiopia. This study also showed that male sex was the most represented with 1697 (4.2%) samples compared to 1484 (3.7%) samples for female sex for a sex ratio of 1.14. This result is similar to those obtained in other studies [3,4,16] where the male sex was the most represented in terms of contamination compared to the female sex. However, other studies including those of Aklilu *et al.* [11], by GebreSilasie *et al.* [7], by Dagnew *et al.* [8] and by Beyene and Tasew [1] where girls are more represented than boys in terms of gastrointestinal infections. In this study, this difference in the distribution of positive samples after culture by sex was statistically significant ($p<0.001$). The distribution of the age variable into groups showed that the most contaminated patients were less than 20 years old, ie 1620 (9.3%) positive samples for this age group. This result is different from that obtained by Ngalani *et al.* [21], where

the age groups most represented in terms of contamination by enteropathogenic bacteria were >50 years, 41-50 years and 31-40 years in HIV + patients and the age group 21-30 years in HIV-subjects. On the other hand, in the study of Ateudjieu *et al*. [3], carried out in Kousseri, in the Far North of Cameroon, children aged 0-5 years were the most affected by enteropathogenic bacteria associated with diarrhea. Several studies [2,6,16,19,35] have also obtained results similar to the present study showing that the youngest children, especially children, are the most affected by gastrointestinal infections. As pointed out by Gebreegziabher *et al.* [6] or else Mulatu *et al*. [2], this strong contamination observed in children in our context could be explained by the fact that the latter wash their hands less often after defecation compared to adults and even more, they often put their fingers or soiled objects in their mouth and play most often on the ground where they easily come into contact with feces.

Our study showed that all of the bacterial species isolated after culture belonged to the group of Gram-negative bacilli, in particular with the fungus *Candida albicans*, which was the most represented in stool samples. Among the Gram negative bacilli, the most represented were: *Shigella flexneri* (1.16%), *Vibrio cholerae* (1.14%), *Salmonella sp*. (0.74%), *Escherichia coli* (0.62%), *Campylobacter sp.* (0.15%), *Shigella sonnei* (0.13%), *Shigella sp*. (0.11%), *Shigella boydii* (0.11%), *Salmonella enteritidis* (0.09%), *Salmonella typhimurium* (0.09%), *Campylobacter jejuni* (0.08%), *Shigella dysenteriae A2* (0.04%), *Salmonella typhi* (0.03%), *Campylobacter coli* (0.03%), *Campylobacter lari* (0.03%), *Salmonella hadar* (0.03%) and *Shigella dysenteriae A1* (0.03%). These results are close to those of Ngalani *et al*. [21] and Ateudjieu *et al.* [3], studies carried out in Cameroon where the species *Escherichia coli*, *Salmonella spp*., *Shigella spp*., *Vibrio spp.* were frequently encountered in gastrointestinal infections, especially diarrhea in children. Other studies, notably the one conducted in China by Wei *et al.* [36], showed that the species *Salmonella typhimurium* and *Salmonella enteritidis* were associated with diarrhea, as in the present study. In the study by Sire *et al.* [37], in Senegal, the species *Shigella flexneri* was the most represented as shown by our study followed by *Shigella sonnei*, *Shigella boydii* and *Shigella dysenteriae*. In another study carried out in Iran by Mahmoudi *et al.* [33], *Salmonella spp*. and *Shigella spp*. were also the most represented in gastroenteritis. The strong presence of the Gram-negative bacilli *Shigella spp*., *Vibrio cholerae* and *Salmonella spp*. in stool samples in our context is a reflection of poor socio-economic development, an inadequate environment, inadequate hygienic conditions and difficulties in accessing drinking water. Regarding fungi, our study also found that *Candida albicans* was most abundant in stool samples followed by other strains of Candida. This result is close to the study by Awoyeni *et al.* [38] where *Candida albicans* was the predominant species in stool samples from HIV + patients with diarrhea or that of Ezeonu *et al.* [18] conducted in Nigeria where the same species was found more frequently in children <1 year to 12 years.

A statistically significant association between sex, age groups and organisms isolated from stool samples was found in this study ($p<0.001$ and $p<0.0001$ respectively) with the male sex being the most represented and the <20 years most contaminated. The profile of the germs identified by sex showed that the different distributions were significant for *Shigella boydii* ($p = 0.04886$), *Shigella dysenteriae A1* ($p = 0.01918$), *Shigella sonnei* ($p = 0.0414$) and *Vibrio cholerae* ($p<0.001$) with a 5 times higher risk of *Shigella dysenteriae A1* infection in females. Regarding age groups, the species in significant proportion in children were: *Campylobacter jejuni* ($p<0.0001$), *Campylobacter lari* ($p = 0.02131$), *Campylobacter sp.* ($p<0.0001$), *Escherichia coli* ($p<0.0001$), *Salmonella hadar* ($p = 0.04066$), *Salmonella sp*. ($p<0.0001$), *Shigella flexneri* ($p <0.001$), *Shigella sonnei* ($p = 0.01822$) and *Candida albicans* ($p = 0.003297$). These results agree with those obtained in several studies [3,20,33,39] where the species *Shigella spp*., *Salmonella spp*., *Escherichia coli* are frequently encountered in gastrointestinal infections in children and young people. In adults, the most abundant species were: *Shigella boydii* ($p = 0.008861$) in 40-59 year old and <20 year old; *Vibrio cholerae* ($p <0.0001$) in 20-39 years old and 40-59 years old.

Several families of antibiotics were used in this study in the antimicrobial susceptibility test and with regard to antifungals, only the classes of azoles and polyenes were used. *Shigella flexneri* and *Vibrio cholerae* were the most common species in stool samples. *Shigella flexneri*, significantly involved in diarrhea worldwide and particularly in developing countries, was multidrug resistant to antibiotics of the penicillin class with in particular 83.3% resistance to amoxicillin, 54.3% resistance to amoxicillin + clavulanic acid and 81% resistance to ticarcillin. In the phenicol family, resistance to chloramphenicol was 45.1%. In the cyclin class, *Shigella flexneri* showed 64.3% resistance to tetracycline and 84.2% resistance to cotrimoxazole from the sulfonamide family. These results are close to those of Yang *et al.* [35], study conducted in China over a period of 7 years (2005-2011) where *Shigella flexneri* was the most abundant species with higher resistance to tetracycline (90.9%), to cotrimoxazole (80.8%), ampicillin (93.2%) and nalidixic acid (96.4%) or those of Verma *et al.* [16], where 100% resistance was obtained for cotrimoxazole and nalidixic acid. In the study by Gu *et al*. [39], *Shigella flexneri* was the most represented species followed by *Shigella sonnei* as in the present study with a higher incidence in Africa and Asia compared to Europe and America. The highest resistance was found for nalidixic acid and ciprofloxacin in contrast to high sensitivity (96.2%) for this antibiotic in the present study. Further research by Kahsay and Muthupandian [40] also found that *Shigella flexneri*, which is widely represented in Africa and Asia, was very resistant to ampicillin, tetracycline, chloramphenicol, nalidixic acid and cotrimoxazole, results that these two researchers obtained from several articles published between 2001 and 2014. As for *Vibrio cholerae*, the second most represented species in this study, the greatest resistance was obtained for nalidixic acid (92.4%), cotrimoxazole (77.3 %), colistin (82.1%) and polymixin B (58.8%). These results are similar to the study conducted in the Far North Cameroon by Ateudjieu *et al.* [3], where the cases of *Vibrio spp*. were resistant to nalidixic acid (100%), cotrimoxazole (60%) and colistin (100%) or the

study by Verma *et al.* [16] in which the resistance of *Vibrio cholerae* was 100%.

After the previous species, *Salmonella sp*. And *Escherichia coli* were the 3rd and 4th most common species, respectively, in the stool samples of this study. The cases of *Salmonella sp*. were mostly sensitive to the families of antibiotics tested in this study. However, the highest resistance was obtained for cotrimoxazole (22.8%), tetracycline (20.5%), amoxicillin (17.8%), ticarcillin (17.1%), nalidixic acid (16.1%) and amoxicillin + clavulanic acid (12.8%). Our results are different from those of Gebreegziabher *et al*. [6] or the resistance percentages of *Salmonella sp*. were higher than those obtained in the present study with 31.6% resistance to nalidixic acid, 57.9% resistance to cotrimoxazole and 89.5% resistance to tetracycline. In the study by Mengist *et al.* [41], higher resistance values were also obtained for cotrimoxazole (37.5%) and tetracycline (62.5%) as well as that of Awol *et al*. where the resistance of *Salmonella sp*. was 80% for tetracycline and 40% for cotrimoxazole [42]. In the Far North of Cameroon, Ateudjieu *et al*. [3] also obtained high resistance rates for amoxicillin (71.43%), amoxicillin + clavulanic acid (71.43%), tetracycline (85.71%) and cotrimoxazole (71.42%). This difference in resistance percentages from one study to another could be explained by the differences in study periods or the study site. This study also showed that *Escherichia coli* was more resistant to ticarcillin (60%) and cotrimoxazole (64%). Similar results from the study by GebreSilasie *et al.* [7], and that of Zhang *et al*. [5] where the resistance of *Escherichia coli* (Diarrheagenic *Escherichia coli*) to cotrimoxazole was respectively 62.3% and 62.2%, but different from that of Ateudjieu *et al*. [3], where the resistance of *Escherichia coli* to cotrimoxazole was higher (83.33%). Moreover, the research of Verma *et al*. [16], also showed a higher rate of resistance of *Escherichia coli* to cotrimoxazole (91.9%) and to most of the antibiotics tested.

The cases of *Campylobacter sp*. and *Shigella sp*. were also multidrug resistant to several antibiotics tested. For *Campylobacter sp*. the highest resistance was recorded for cefatrizine (78%), ciprofloxacin (67.8%), amoxicillin (66.1%), nalidixic acid (64.4%) and tetracycline (35.6%). The cases of *Shigella sp*. were more resistant to cotrimoxazole (82.6%), tetracycline (52.2%), amoxicillin (47.8%), and ticarcillin (43.5%). These results are different from those of Kebede *et al.* [32], or the cases of *Campylobacter sp*. were less resistant to nalidixic acid (7.69%), ciprofloxacin (7.69%) and tetracycline (23.1%). For cases of *Shigella sp*. their study showed that the rate of resistance to cotrimoxazole was 66.7% and 100% for tetracycline. This difference could be explained by the immune status of the patients diagnosed in their study (HIV + subjects). In the study by Bafa *et al.* [43], 100% resistance to amoxicillin was observed in cases of *Shigella sp.* but that of Getie *et al.* [44], showed lower levels of resistance to amoxicillin (34.6%) and cotrimoxazole (38.5%) compared to those obtained in the present study.

The other Shigella species (*Shigella sonnei*, *Shigella boydii*, *Shigella dysenteriae A2* and *Shigella dysenteriae A1*) were very resistant to cotrimoxazole in our study with respective rates of 96.1%, 88.9%, 100% and 90.9%. These results are broadly similar to those of Kahsay and Muthupandian [40] who observed high resistance of *Shigella sonnei*, *Shigella boydii* and *Shigella dysenteriae* species to cotrimoxazole in most of the articles reviewed from 2001 to 2014; or even those of Panzhani *et al*. [45] who observed significant resistance for these three species to cotrimoxazole between 2001 and 2004. In addition, the study by Mahmoudi *et al.* [33] also revealed a high rate of resistance to cotrimoxazole (> 95%) for *Shigella sonnei*. Our study also showed that tetracycline was ineffective against the other *Shigella* species mentioned above with resistance rates of 60.8% for *Shigella sonnei*, 64.4% for *Shigella boydii*, 53.3% for *Shigella dysenteriae A2* and 63.6% for *Shigella dysenteriae A1*. Results different from those of Panzhani *et al*. [45], with high rates of resistance (>75%) to tetracycline for *Shigella sonnei* and *Shigella dysenteriae* but lower for *Shigella boydii*. In addition to cotrimoxazole and tetracycline which were ineffective against *Shigella* species, our study also showed high resistance for amoxicillin (57.8% for *Shigella boydii*, 73.3% for *Shigella dysenteriae* A2 and 63.6% for *Shigella dysenteriae A1*) and ticarcillin (45.5% for *Shigella dysenteriae A1*, 46.7% for *Shigella boydii* and 73.3% for *Shigella dysenteriae A2*).

Most of the *Salmonella* strains isolated from stool samples were also multidrug resistant to the antibiotics tested. For *Salmonella enteritidis*, the highest resistance was recorded for amoxicillin (11.1%), nalidixic acid (11.1%), amoxicillin + clavulanic acid (5.6%), ticarcillin (5.6%) and tetracycline (5.6%). *Salmonella typhimurium* was also more resistant to tetracycline (19.4%), cotrimoxazole (19.4%), amoxicillin + clavulanic acid (19.4%), ticarcillin (27.8%) and amoxicillin (30.6%). *Salmonella typhi* was more resistant to amoxicillin + clavulanic acid (30.8%), tetracycline (53.8%), amoxicillin (76.9%), ticarcillin (76.9%) and cotrimoxazole (76.9%). As for *Salmonella hadar*, the least represented of the *Salmonella* species, the highest resistance was obtained for cotrimoxazole (45.5%), tetracycline (63.6%) and nalidixic acid (81.8%). These results are in agreement with those of Li *et al*. [46], where the resistance of *Salmonella enteritidis* and *Salmonella typhimurium* to amoxicillin + clavulinate was low (5.6% and 19.4% respectively) as in the present study. However, the resistance of *Salmonella typhimurium* was higher for cotrimoxazole (75%) and tetracycline (40% for *Salmonella enteritidis* and 87% for *Salmonella typhimurium*). The study by Zhang *et al*. [5], carried out in China showed results different from those obtained in the present study with 28.57% resistance to amoxicillin + clavulanic acid against 0.00% for *Salmonella typhimurium*, 47.62% and 75% resistance to tetracycline respectively for *Salmonella enteritidis* and *Salmonella typhimurium*, 28.57% and 25% resistance to cotrimoxazole for the two preceding species. Wei *et al*. [36], in their study carried out in children victims of diarrhea obtained different results from those of the present study with 11.5% and 11.0% resistance to amoxicillin + clavulanic acid respectively for *Salmonella typhimurium* and *Salmonella enteritidis*, 79.7% and 21% resistance to tetracycline respectively for the two preceding species and 45.3% resistance to cotrimoxazole for *Salmonella typhimurium*.

The three species *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* showed, like the other organisms isolated from stool samples, multiple resistance to several antibiotics tested. The first species *Campylobacter jejuni* was more resistant to amoxicillin (50%), cefatrizine (73.5%), cefotaxime (32.4%), tetracycline (26.5%), nalidixic acid (58.8%) and ciprofloxacin (55.9%). For *Campylobacter coli*, the greatest resistance was obtained for the same antibiotics with respective resistance rates of 58.3%, 91.7%, 33.3%, 25%, 50% and 50%. As for *Campylobacter lari*, the resistance rate was higher for nalidixic acid (100%) followed by ciprofloxacin (91.7%), amoxicillin (66.7%), cefatrizine (66.7%), cefotaxime (33.3%) and tetracycline (25%). These results are different from those of Mulatu *et al.* [2], where *Campylobacter* species were less resistant to nalidixic acid (20%) and more resistant to amoxicillin (80%).

The fungi isolated from the stool samples in this research were sensitive to most of the antifungals used. For antifungals of the azole class, the sensitivity of *Candida albicans* was greater for miconazole (88.2%), followed by econazole (86.4%), ketoconazole (82.2%) and clotrimazole (81.9%). As for the other strains of Candida (*Candida sp*.), They were more sensitive to ketoconazole (87.1%) followed by miconazole (83.3%), econazole (83.3%) and clotrimazole (75.4%). In the polyenes class, *Candida albicans* was more sensitive to nystatin (84.5%) then to amphotericin B (78.2%).

In view of the results of the present study, the prevalence of gastrointestinal infections in the Cameroonian context is high and faced with these infections, which until now constitute a public health problem, it remains essential to take measures to control the routes of contamination and systematically reduce the risk of infection in children. First, it would be important to extend this research to other regions of the country in order to identify the germs most involved in these infections and to see how they behave with regard to the antibiotics routinely used in our context. Then resort to molecular biology in order to map the resistance genes of these germs in our context and research the factors associated with the emergence of these genes and finally expand awareness programs on the use of antibiotics in families by targeting in particular the youngest who remain the most vulnerable.

5. Conclusion

The main findings of this study showed that intestinal infections caused by bacterial and fungal species were high in children under 20 years in the Cameroonian context. This study also showed that the risk of infection was also associated with age and gender with men who are most infected. The alarming finding of antibiotic resistance observed in this study should lead physician to prescribe these drugs rationally, preferably based on data from an antimicrobial susceptibility test. Thus, it is more than necessary to establish regular monitoring of antibiotic resistance, which must be generalized at all health centers in order to define therapeutic and prophylactic strategies adapted to the current context. The application of these preventive measures can only be conceived within the framework of a program which interests all health actors.

List of Abbreviations

CPC	Centre Pasteur of Cameroon
CDC	Center of Diseases and Control
NTS	Non-Typhoidal Salmonella
DEC	Diarrheagenic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
EAEC	enteroaggregative *Escherichia coli*
EHEC	enterohemoragic *Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
ESBL	Extended Spectrum β-Lactamase
PLHIV	People living with HIV
WHO	World Health Organization
HIV	Human Immunodeficiency Virus
MRSA	Methicillin-resistant *Staphylococcus aureus*
CLSI	Clinical and laboratory Standard Institute
AFST	Antifungal Susceptibility Testing
EUCAST	European Committee on Antimicrobial Susceptibility Testing

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Conflicts of Interest

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

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